The Influence of Hemp Seed Meal on Tenderness and Shelf Stability on Goat Steaks

by

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Abstract

Over the last decades, the increased adoption of sources for healthier, more tender red meat has become a growing trend. There is an increasing demand for healthier protein sources such as lean goat meat. Just as important as providing a new protein source, it is equally as important to produce a fresh and safe product. However, the ability to provide lean meat proteins to the consumer in the freshest, safest manner is equally as important. Additionally, it is important to understand the influence an animal's diet on meat. Two studies were conducted evaluating the influence of hemp seed meal (HSM) as a feed ingredient with inclusion rate of 0, 10, 20, and 30 percent for goats and the subsequent impact on both fresh and cooked meat quality characteristics. Study one evaluated goat steaks stored in simulated retail conditions using vacuum packaging technology for 21 days in a refrigerated case. Carcass characteristics were not altered (p > 0.05) with inclusion of HSM in the diet. However, throughout the 21-day study, fresh characteristics of goat steaks were negatively altered (p < 0.05). Study two assess the effects of HSM supplementation on the cooked cook, cook time, cooked instrumental color and tenderness of goat steaks. In the second study, HSM did not alter (p > 0.05) cooked color or instrumental tenderness of goat steaks. The results from these studies suggest that hemp seed meal does have an overall effect on overall tenderness of the product. Overall, these studies found that HSM was a nutrient dense feed additive that did not impact the tenderness or growth of the goats. Additionally, results showed that as the inclusion rate increased it improved the cook loss as well as caused a slight change in instrumental color.

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The literature review is formatted to fit the style and guidelines for the peer-reviewed journal Meat Science

CHAPTER I

Introduction

Consumers remain focused on identifying healthy sources of protein that can meet their demands for convenience and palatability. Beef is a great nutritionally dense source of protein however, another healthy and currently underutilized option is goat meat (McWhinney & Garza, 2018). The annual consumption of goat and sheep is estimated at 0.60 kg per capita compared to the 37.31 kg per capita of beef (Ritchie, 2017). There are advantages to eating goat meat; for example, goat meat has a healthier fatty acid profile when compared to beef and lamb while maintaining a comparable protein amount of 20.6 to 22.3 percent (Malekian et al., 2014; Pophiwa et al., 2020b; Sheridan et al., 2003). Goat meat is low in overall fat content and saturated fatty acids compared to other meat proteins, while also being high in unsaturated fatty acids like linoleic and oleic acids (Malekian et al., 2014).

Due to the expanding number of people immigrating to the United States, the amount of goat meat being consumed has drastically increased over the last several decades (Kannan et al., 2014). According to the Observatory of Economic Complexity, in 2020 the United States imported nearly 945 million dollars of goat and sheep meat. The United States has become the 2nd largest importer of goat and sheep meat globally (Gaulier & Zignago, 2010; *USDA*, 2023.). Although goat meat may be considered a healthy option, studies have concluded that consumers avoid this protein due to toughness, stringiness, and strong flavor attributes (Webb et al., 2005). Moreover, recent research in South Africa concluded the preference for goat meat is relatively low due to factors such as odor, color, and toughness (Pophiwa et al. 2017). Similar to other proteins like lamb and poultry, the aroma and lack of tenderness may cause goat meat to have a

negative acceptance among consumers. There is limited literature that elaborates on factors that contribute to the variation in goat meat. However, literature has stated that palatability traits such as tenderness, flavor, and even color are attributes that can be affected by countless factors throughout the many phases of goat production. Of particular interest is the impact of an animal's diet on the tenderness of goat meat (Leick et al., 2012; Wu et al., 2014). It is possible that a ration could be developed to help improve growth and tenderness.

Determining the factors that can negatively affect goat meat quality could result in substantially increasing the sales volume of goat meat. Identifying and altering the steps prior to goat carcass fabrication may assist in producing a more consistent retail consumer product. Through different studies it has been concluded that the animals' diet and the chilling rate of goat carcasses may affect the overall tenderness and quality characteristics of goat meat (Pophiwa et al., 2020; Leick et al., 2012). Understanding factors that can influence the overall characteristics of goat meat can help the industry produce a more consistent product.

Goat Carcass Characteristics and Impacts of Diet on Growth and Carcass Characteristics

Eating a good nutritional diet aids in maintaining a long, healthy life (*CDC*, 2022). A growing trend today is finding a healthier source of protein while also meeting all of the nutritional attributes (van Wyk et al., 2022). Beef is an excellent source of protein, but consumers are concerned about the environmental impacts and amount of resources needed to raise cattle (Sanchez-Sabate & Sabaté, 2019). For people who want to eat and be a part of the production of red meat, goats are a potentially profitable option (Pophiwa et al., 2020b). Goat meat has been considered a staple in the human diet for centuries (Webb et al., 2005). The

world's goat population can be found predominately in southeast Asia and Africa (Dhanda, et al., 2003).

Goats can survive in regions that are nutritionally deficient and possess tolerance to heat stress. Whereas other ruminants have greater nutritional demands and succumb to variation in temperatures (Amankwah et al., 2012; Dhanda et al., 2003; El Khidir et al., 1998; Silanikove, 2000). Although goats have this advantage over other ruminants, they also have their own set of drawbacks. Goats tend to perform poorly compared to other species. This has typically attributed to poor management such as insufficient facilities and the lack of labor which is coupled with an increase in diseases (Jones, 2004; Tahuk & Bira, 2020). While goats can survive on unconventional diets, researching methods to incorporate additional feed ingredients into a goat's diet could improve the growth and the carcass characteristics of goats.

Over the years, studies have proven that nutrition plays a vital role in the growth and development of an animal. In a study conducted by Joemat et. al (2003), nutritional restrictions and levels of concentrated supplementation during realimentation is altered among Spanish doelings and Boer \times Spanish doelings. Additionally, a longer realimentation rather than restriction achieves similar average daily gains compared to doelings continuously on a moderate nutritional plane.

Goats can have reduced growth rates and less fat content compared to sheep (Colomer-Rocher et al., 1992; Van Niekerk & Casey, 1988). The average growth rate of a goat is 176 grams per day when goats live in a tropical setting and 200 grams per day when goats live in a typical setting, such as Mediterranean climates (McGregor, 1985; Van Niekerk & Casey, 1988). Additionally, low planes of nutrition, poor management, or inbreeding may cause the goat to grow at a reduced rate often resulting in leaner carcasses (McGregor, 1985; Van Niekerk &

Casey, 1988). It has also been reported that late maturing animals tend to be leaner compared to early maturing animals at comparable harvest weights (Dhanda et al., 2003). Additionally, as an animal matures the bone to muscle ratio and dressing percent will increase (Dhanda et al., 2003). Dressing percentages of goat carcasses can range between 44 and 55% (Naude and Hofmeyr, 1981; Casey et al., 2003). In addition to dressing percentage, studies have concluded that the fatty acid composition of the animal can influence the body weight. Previous studies have reported that male goats can have greater levels of C10, C12 and C15 fatty acids compared to wethers (Mahgoub et al., 2002). Furthermore, intact males can have greater levels of C14 compared to castrated kids (Banskalieva et al., 2000). With greater concentrations of fatty acids goat meat can have an increased amount of oleic (C18:1), palmitic (C16:0), and stearic acid (C18:0) when using fatty acid methyl ester quantification techniques (Beserra et al., 2004).

Over the last few decades, studies have been conducted to capture a baseline for meat goat carcass characteristics. Simela et al. (1999) conducted a study evaluating 52 male (milk-teeth to full teeth) and 18 mature female (6 to 8 teeth) Matebele goats purchased from regional producers. Gender increased side length, chest depth, thigh circumference, and eye muscle area when compared to male goats at various ages. It is plausible that Mateble male goats may reach marketable size within 12 months, whereas females require additional feeding time to achieve a marketable weight (Simela et al., 1999).

In another study Small East African goats were fed four levels of *ad libitum* corn bran and sunflower meal (0%; 33%; 66%; 100%) along with grass hay for 90 days (Safari, 2009). Results concluded that with increasing level of supplementation, hot and cold carcass weights, hind leg circumference and carcass fatness increased. Strikingly, there was no difference between the treatments for carcass length, chest depth, or hind leg length. These findings suggest

that energy intake rather than physical fill was the main contributor influencing the dry matter intact from the diets and feeding goats 66% concentrate would improve the weight gain through greater carcass fat deposition of these goats (Safari, 2009).

Animals' diet can affect meat quality such as tenderness and color of the meat. Mancini and Hunt (2005) have concluded that surface color changes in lightness and yellowness of fresh meat are connected to dietary effects on pre-harvest glycogen and marbling levels. These factors influenced by dietary intake of nutrients may ultimately be linked to intrinsic color traits, ultimate pH, oxygen consumption, or the metmyoglobin reducing activity within muscle (Mancini and Hunt, 2005).

Hemp seed meal

Cannabis sativa also commonly known as hemp or marijuana, has been cultivated since around 3450 BC, especially in Asian counties (El-Sohaimy et al., 2022). To differentiate between hemp and marijuana, the measurable amount of tetrahydrocannabinol (THC) is recorded. Legally, hemp seed meal must not contain more than 0.3 percent THC but has not been approved for use in feeding animals (*United States Congress*, 2018.). To fit within the confines of legal requirements, hempseed oil must be extracted from the plant which can be done through chemical or mechanical methods (Karlsson et al., 2012). Industry plants are bred to contain less than 1 percent THC within the plant but can produce more THC if grown in a stressful environment lacking water or one with poor soil conditions. Nutritionally, whole hemp seed prior to oil extraction consists of 18 to 23% protein, 25 to 30% oil, 30 to 40% fiber and 6 to 7% moisture (Bouloc et al., 2019; El-Sohaimy et al., 2022). Additionally, hemp protein contains nine essential fatty-acids such as leucine and lysine, along with phosphorus, potassium, magnesium, manganese, omega-3 and omega-6. Hemp seeds can be milled into hemp flour, which contains

25 to 30% fat, pressed into hemp seed cake containing 8 to 15% fat, or extracted to create a hemp seed meal that contains 0.4 to 10% fat (Karlsson et al., 2012; Semwogerere et al., 2020). Hemp seed also contains proteins that are essential for building and repairing tissue, as well as helping with bone and muscle growth and development (Wang et al., 2022). Recent studies have reported that when feeding hemp seed cakes to cattle, it does not alter overall quality of the muscle (Turner & Hessle, 2008).

In a recent study conducted by Antunović et al. (2021), carcass characteristics and instrumental color of the *m. semimembranosus* from lambs fed soybean meal compared to hemp seed cakes (HSC) did not improve. However, in a study conducted by goats fed 0% HSC had significantly lower lightness (L*) and yellowness (b*) values when compared to goats fed 2.5 %, 5.0%, 7.5%, or 10% HSC. Increasing storage time and concentration of HSC has caused lightness and yellowness values to increase, whereas redness values decreased (Semwogerere et al., 2023). HSC can be used in supporting goat diets, but caution should be considered when estimating inclusion rates to avoid negatively altering meat characteristics of goats.

Though there are limitations throughout the literature evaluating the impact of hemp seed meal on goat growth and meat qualities, there have been a few studies that compare hemp seed meal to its impact on other ruminants. Investigating hemp seed meal on large ruminants such as cattle have further identified nutritional, and carcass impacts of this by-product feed ingredient. In a study conducted by Turner and Hessle (2008), Swedish red steers were fed for 177 days a 45:55 dry matter grass silage containing rolled barley and a protein supplement of either soybean meal or HSC. Results suggest that meat quality was not altered regardless of SBM or HSC inclusion. In a study led by Gibb et al. (2005), sixty steers fed a barley-based finishing diet for 166 days containing 0, 9 or 14% hemp seed was conducted. The effect of hemp seed inclusion

rate linearly increased proportions of the fatty acids: C18:0, C18:3 and C18:1 *trans*- 9 in pars costalis diaphragmatic. Hemp seed did not affect the rate or efficiency of gain, carcass weight, dressing percentage, back fat thickness, ribeye area, quality grade, or meat yield. Although hemp seed does not seem to influence the carcass characteristics of large ruminants, previous studies suggest that the inclusion of hemp seed meal into a small ruminant diet such as a lamb may be an excellent source of rumen undegradable protein and used as a replacement for canola meal without causing detrimental effects on lamb nutritional standards (Bailoni et al., 2021).

Water Holding Capacity and Cook Loss in Meat

The ability of meat to retain water within the myofibrils is of great concern within industry. Water holding capacity is known as the fresh meats ability to retain water during cutting, transporting, storage and cooking (Hughes et al., 2014). Within fresh meat, the water holding capacity can be determined by measuring the purge loss collected from the meat (Kerry, 2009). If a product has poor water holding capacity it can cause a significant loss of weight from the carcass, cuts or processed meats as well as have a negative appearance of the meat color (Hughes et al., 2014).

Bouton and Harris (1972) have reported that as the cooking temperature increases, the cook loss decreases. Greater cooking loss within meat samples occurred at 50 °C compared to meat samples cooked at 60 °C. They also found that the amount of free liquid was at a maximum for the samples at 50 °C compared to those at 60 °C. Similar results were recorded by Combes et al., (2004). This is study when rabbit meat was cooked it was found that the cook loss increased as the temperature increased. At 80 °C there was a 31.3 percent cooking loss and continued to remain shelf stable between 80 °C and 90 °C within the samples.

Instrumental Fresh Color

Surface color is a major factor when a consumer is determining to purchase a meat product (Suman & Joseph, 2013; Turan & Şimşek, 2021). Often times consumers have a misconstrued conception that the discoloration of meat is due to the lack of freshness (Ben Abdallah et al., 1999). Recent studies have identified that roughly 2.6 percent of beef is discarded annually due to discoloration at the retail counter. Losses of fresh meat equate to over 3.73 billion dollars or 194.70 million kg worth of meat being discarded annually (Ramanathan et al., 2022). There are several factors that influence surface color of meat during pre- or postharvest conditions. Factors such as species, age, diet and muscle pH have all been investigated and reported to influence surface color of meat (MacDougall, 1982). Maintaining and improving the stability of meat color could prove to be advantageous when it comes to increasing meat purchasing decisions by consumers. Additionally, identifying methods to extend storage periods and limit surface color deterioration may also contribute to reducing the amount of meat lost throughout the world (Turan & Simsek, 2021).

Surface color is considered the key indicator of freshness and perceived quality of meat and is often the sole limiting factor to the storage period and consumer acceptance. Customers often seek red meat that has a bright cherry red color as their indicator of a fresh beef product and discard any meat products lacking these colors. The red meat color is due to the concentration of myoglobin and its redox form. When in the redox form, it allows oxymyoglobin to oxidize resulting in a surface color change causing a discoloration in the meat (Suman & Joseph, 2013; Turan & Şimşek, 2021). However, it has been reported that when comparing goat meat to beef, goat meat has a greater redness value (Stajic et al., 2011). More research needs to

be done into consumers' perception of meat, so we can better understand if dark meat influences buying decisions.

Additional studies have also confirmed that visual color can be dependent on the meat pH. Studies have found that when the pH of meat increases visual color becomes more intense and the lightness values vary depending on the muscle (Hughes et al. 2019). The lightness values tell the luminosity values, which correlative with the amount of light scattering within the meat. This correlation is due to the amount of free water within the meat and the degree of protein damage. When the pH approaches the isoelectric points of the various water binding muscle proteins, such as lactic acid, the free water increases allowing more light to scatter, making the overall appearance of the muscle to be lighter. There have been limited studies in meat color in goat; however, a study conducted by Kannan et al., (2001) demonstrated that surface color changes occurred within the first 24-hours postmortem within steaks from the Chevon goat. Over the 12- day study values for redness declined, but lipid oxidation and metmyoglobin percentages increased. Additionally, they reported that when evaluating the shoulder cuts, there was a darker red color which could be due to the elevated pH. It is likely that over time the goat steaks became darker as a result of surface oxidation.

Further studies have been conducted to evaluate the impact of various terrains and the available forages within the area as well as various weights at harvest and the impacts on fresh instrumental color. In a study conducted by Ivanovic et al. (2016), investigators harvested 120 goats from the rural mountain, hilly, or plain region of Serbia. Goat meat was lighter and redder from the plan region than goat meat from the mountain or hilly regions. Research has proven that the goat meat differences in surface color may be attributed to the bevy of factors that influence meat surface color such as breed, age, sex, cut of meat, myoglobin quantity, and even

intramuscular fat. Interestingly, Pratiwi et al. (2007), reported that slaughter weight of a goat can have an influence on surface color factors. Heavier slaughter weights were associated with older goats suggesting that meat color concentrations of myoglobin are greater in older animals. Factors like age, sex, ultimate muscle pH and breed can influence surface color of meat. Typically, the pH of postmortem skeletal muscle in goat is around 5.4. In addition to this, the pH of meat may be influenced by biochemical events that happen pre- and postmortem. This will cause the structural components within the muscle cells and their associated connective tissue to change allowing for fluid relocation (Hughes et al., 2014). When the pH of the meat begins to decrease it causes the meat to become brighter and seemingly more wet on the surface (Abril et al., 2001; Lawrie & Ledward, 2014; Suman et al., 2016). Ultimate muscle pH can alter the volume of moisture that can be retained within the muscle regardless of external forces such as cutting, aging, or cooking. Turan and Şimşek (2021), discovered that the packaging method, black mulberry water and storage duration altered ultimate pH values in fresh ground beef patties. Fresh meat is a unique item that can altered through antemortem, or postmortem activities and any alternations can either improve or deteriorate characteristics that influence consumer purchases. As the previous studies have stated, factors such as age, breed, sex, packaging method, and pH have influenced the surface color of meat products.

Instrumental Cooked Color in Meat

The uniformity of cooked meat color is an important determinant of consume acceptability of the product. Cooked meat color will appear dull brown internally and can be attributed to the heat-induced denaturation of myoglobin. several factors that may influence the cooked color of a product before or after cooking can be linked to the multitude of endogenous and exogenous factors associated with fresh meat properties. Endogenous factors include

ultimate pH, the fiber type of the muscle, species, and redox state, whereas the exogenous factors may include packaging materials, ingredients, and storage temperature or duration (Seyfert et al., 2004; Suman et al., 2004, 2009, 2016). These interactive factors can be critical influences on consumers acceptability of wholesome meat products that lack consistency of internal cooked color.

Internal cooked color should not be used as an indicator of doneness as previous research has identified that myoglobin's redox state prior to cooking can influence internal cooked meat color (Mancini & Hunt, 2005). Often consumers perceive internal cooked color to be a determinant for degree of doneness, but due to premature browning cooked color can mislead the consumer to believe that a hotter internal temperature has been achieved (Geileskey et al., 1998). This can mislead consumers and ultimately result in a foodborne illness. While this is an issue in beef, there is very limited literature regarding these issues in goat meat. However, Liu et al., (2013) conducted a study to evaluate how various cooking temperatures affect the internal instrumental color of goat semimembranosus steaks. They reported that steaks cooked to greater internal temperatures had increased lightness values. They hypothesized this was due to an increase in light scattering caused by protein denaturation. They also found that the redness values decreased when the cooking temperature was between 50 and 80 °C and remained low values when the temperature was above 80 °C. They believe this is due to the imbalance of hemochrome and hemichrome caused by the cooking process. Given this, it is important to educate the consumers that cooked goat meat may have a different internal cooked color an expected.

In the cooking process, myoglobin is denatured the heme groups to be exposed to the environment, this will cause the dull-brown color in meat The application of heat and exposure

to the environment causes the globin pigment to coagulate and becomes insoluble in water or buffer (Suman et al., 2016). In a study conducted by Geileskey et al. (1998), investigators found that the myoglobin was less stable to heat at the same pH and ionic strength as the meat. It has also been suggested that when there is a higher pH in the meat, the product should remain in a red or pink state during and after the cooking process (Geileskey et al., 1998; Mancini et al., 2005).

Tenderness

For consumers, the tenderness of a product will affect their perception of meat quality (Weston et al., 2002). Consumers are expecting meat products to be wholesome, fresh, lean, balanced nutritionally, and to exhibit adequate juiciness, flavor and uniform tenderness (Hoffman & Wiklund, 2006). Consistency of tenderness for a meat product strongly influences consumer satisfaction and leads to the possibility of repeat a meat purchase (Bolumar & Toepfl, 2016). Meat tenderness can simply be defined as the measurement of the amount of effort required to bite through the meat (Purchas, 2014).

However, overall tenderness is complex and is driven by different biochemical properties of the meat. The first is the degree of contraction within the muscle sarcomeres. Second being the integrity or degradation of the myofibrillar structure and finally the amount of connective tissue (Bolumar & Claus, 2017; Weston et al., 2002). In a study conducted by Dhanda et al, (2003), 110 male goat kids from six different goat genotypes were harvested to determine instrumental tenderness values. Variation existed among genotypes as the shear force values ranged from 3.7 to 4.6 kg/cm². They discovered that Boer x Feral crosses resulted in the lowest shear force values whereas, Saanen x Feral crosses concluded in the highest tenderness values. They also concluded that there was a decrease in the overall muscle tenderness as the age and body weight of the animals increased. These results could be due to the crosslinking of the collagen fibers that occurs as an animal ages. An additional study conducted by Bakhsh et al., (2019), investigated the muscle composition, intramuscular connective tissue and tenderness of goat steaks. They reported that throughout the 21-days of this study, WBSF values decreased, due to the weakening of the myofibrillar structures. Additionally, they found that as the aging time increased, the sarcomere length and myofibrillary fragmentation index also increased.

When a muscle contracts, it is due to the myosin attaching to the actin and moving along the muscle. When the muscle is being converted into meat, permanent actomyosin will form. As the glucose supply begins to deplete within the muscle, the degree of contraction within the muscle when the actin and myosin adhere to one another will affect the tenderness of the muscle when full rigor has been completed (Li et al., 2012). However, in a study performed by Hopkins and Thompson (2002), they found no evidence that the actomyosin had an overall effect on the tenderness of the meat.

Collagen has been linked to the overall tenderness of meat. Collagen is a connective tissue fiber that contributes to the variations between the texture and tenderness of meat. As an animal matures, the collagen fiber within the muscles will begin to bond together and crosslink to help provide strength and structure for the animal. When this happens and the meat is applied to heat, the collagen will shrink and tighten around the muscle fibers. This will result in cook loss ultimately resulting in a less tender product (Weston et al., 2002). However, in a study performed by Purslow (2018), they found that when meat is cooked above 65 °C there is no evidence that collagen shrinkage causes meat shrinkage and cook loss. Additionally, Martens et

al. (1982) found that as the actin and myosin denatured, it resulted in the toughening of the meat, whereas when the collagen denatured causes a decrease in firmness.

Sentandreu et al. (2002) stated that postmortem meat tenderness improved due to the softening of myofibrillar structures by the endogenous proteolytic enzymes. Studies have shown that breed, stress, rapid chilling and carcass handling all have lasting effects on the toughness regarding the meat tenderness (Pophiwa et al., 2017, 2020a; Van Niekerk & Casey, 1988; van Wyk et al., 2022; Webb et al., 2005; Weston et al., 2002). In a study performed by Locker in 1959, it was reported that after rigor mortis, the length of the sarcomere ranged between 0.7 to 3.7 μ m in bovine muscles. Sarcomeres are comprised of the A band and I band, and the length of a sarcomere has lasting effects on the overall meat tenderness. When the sarcomere contracts, causing them to shorten, the overall meat tenderness declines. Studies have proven that if a carcass is chilled quickly, it can cause the sarcomere lengths to be reduced which will decrease the length of the sarcomere, causing a lack of tenderness (Joseph, 1996; Swatland, 1994).

The influence of storage temperature on meat can influence overall tenderness, as freezing and thawing case a disruption to muscle fibers and moisture retention. During freezing, ice crystals will form in various sizes and locations throughout the muscle fibers. Ice crystal formation can be a function of freezer temperature and the speed at which freezing/chilling occurs. Size of ice crystals distributed throughout the meat, may cause uneven denaturation of the meat during cooking (Ishiwatari et al., 2013). In a study performed by Rahelić et al. (1984), *Longissimus dorsi* steaks after freezing at -10 to -115 °C caused ice crystals in steaks at -10 °C within the extracellular space. However, steaks frozen at -20 °C resulted in ice crystal formation within the intracellular and extracellular spaces of the muscle. Additionally, when the temperature was between -33 °C and -115 °C, ice crystals would form intracellularly. Zhang &

Ertbjerg (2018), discovered that ice crystal formation caused myofibrillar proteins to denature during thawing. Interestingly, Zhang and Ertbjerg (2018) found that the freezing/thawing process did not impact overall tenderness of meat. Further studies have reported that the carcass chilling process can affect the overall tenderness of a muscle from the carcass. Joseph (1996), concluded that when a beef carcass is rapidly chilled, compared to conventionally chilled, overall tenderness of the steaks from the carcass increased. Traditional HACCP plans have a critical limit of less than or equal to 4 °C surface temperature within 24 hours postmortem, whereas in this study, the carcasses were rapidly chilled to -1 °C within 5 hours postmortem (Joseph, 1996; Savell, 2012). It is plausible that calcium triggers a proteolytic action within the muscle fibers resulting in a breakdown of the muscle fibers and improvement in tenderness. Which likewise suggest that a combination of proteolysis and crust freezing may help to produce more tender beef (Joseph, 1996).

An influence of overall tenderness in a meat product is the cooking process. When heat is applied to meat it causes denaturation throughout the meat to occur. The denaturation of collagen is dependent on the temperature and heating rate of the meat. Purslow (2018), reported that as cooking temperatures increased, collagen would denature. Roughly 5 percent of collagen denaturation can be achieved through low temperatures for a long-time heating (LTLT). In a similar study conducted by Li et al. (2019), comparing the effects of aging and LTLT can result in altered meat tenderness attributed to the weakening of connective tissues within the muscle fibers.

Shelf Life and Stability within Meat Products

In the retail marketplace, the variable shelf-life of a product is one of the greatest problems of meat sales today. The possibility of extending the shelf-life of meat and delaying oxidation within a meat product is of great importance in the industry (Possamai et al., 2018). Over the past several decades, shelf-life has had many definitions, but Delmore (2020), defines it as the amount of time between packaging of a product and its end use when the product is still acceptable for the user. A major contributor to storage time of a meat or food product is spoilage. Many chemical and physical reactions are ultimately responsible for meat spoilage. Packaging type, storage temperature, retail lighting, duration of storage, microbial growth and oxidation are a few of the main influencers that contribute to spoilage in a meat or food product (Dave & Ghaly, 2011).

A factor that contributes to storage of meat deterioration is lipid oxidation that is caused by the production of free radicals (Karami et al., 2011). Studies have reported that when feeding dietary antioxidant supplementation, such as vitamin E, it can improve surface color stability (Cannon et al., 1996; Gray et al., 1996; Karami et al., 2011; Leal et al., 2023; Possamai et al., 2018). Karami et al. (2011) investigated the effects of feeding vitamin E, turmeric, or *Andrographis paniculata*, to goats for 14 weeks. Results showed that after 14 days in refrigerated conditions, results showed that the antioxidant supplements of Vitamin E, turmeric and *Andrographis paniculate* had decreased TBARS values (p < 0.05) over the 14-day study. This suggest that feeding Vitamin E, turmeric and *Andrographis paniculate* improve the meat's oxidative stability values over an extended aging period.

In an article written by Alexandratos (2009), they predict that the Interim Report, which is responsible for reporting the progress, evaluation and overall financial situation, projection of growth 2050 is 95 kg per capita. With fresh meat products, the packaging type and duration of storage can hinder growth of spoilage organisms. Vacuum packaging is one of the most common methods of storing meat or food products for extended periods. Lorenzo and Gómez (2012) compared the spoilage rate of foal meat using modified atmosphere packaging, overwrapped packaging or vacuum packaging. Meat stored in vacuum packaging remained acceptable for the entirety of the storage time of 14-days. Vacuum packaging reduced the oxidation process of both lipids and proteins and increased the color stability throughout the storage time.

Microbiology of Meat Spoilage

A large proportion of food waste is attributed to microbial spoilage and the characteristics that alter the appearance of a meat or food product for the consumer. An estimated 25% of all foods produced globally are wasted due to microbial spoilage that occurs during post-harvest practices (Iulietto et al., 2015). Contamination of meat can occur in the supply chain with these spoilage microbes. Meat is an excellent substrate for bacteria because it contains all of the nutrients needed for their growth, such as moisture, acidity, temperature and oxygen. When a bacterial count grows to 7 Log CFU/g or greater, meat products are considered spoiled (Yim et al., 2019). The most common bacteria associated with meat spoilage are *Pseudomonas spp*, Enterobacteriaceae, *Brochothrix thermosphacta*, and Lactic Acid Bacteria. Bacterial growth of odors, off-flavor, discoloration, or slime formation across the surface of meat (Pellissery et al., 2020). Yim et al. (2019), compared the microbial growth of vacuum packaged and modified atmosphere packaged (MAP) beef. Populations of aerobic bacteria, Lactic Acid Bacteria and

Pseudomonas increased during transportation and distribution regardless of the packaging type. MAP packaging allowed meat storage with less total aerobic bacteria, Lactic Acid Bacteria and *Pseudomonas* than vacuum packaged meat. Packaging technologies such as MAP or vacuum packaging agrees with results from Sutherland et al. (1975), who concluded that spoilage occurred due to slime formation and off-odor associated with greater counts of *Pseudomonas* or discoloration and souring caused by lactic acid bacteria.

Packaging can enhance the storage time of meat products, and there are many alternative sources of packaging and preservation available. Turan and Şimşek (2021), determined the effects of lyophilized black mulberry water extract (BMWE) on lipid oxidation on beef patties using two different packaging treatments, such as aerobic packing or vacuum packaging. Thiobarbituric Acid Reactive Substance (TBARS) values were altered with packaging method and storage time. Additionally, TBARS values decreased in beef patties treated with BMWE, suggesting that BMWE could be used as a natural antioxidant to reduce lipid oxidation and extend the storage of beef patties.

Additionally in a study conducted by Sabow et al. (2016), they evaluated the refrigerated storage on microbial analysis of goat steaks from the Longissimus lumborum and Semitendinosus. After 14 days in refrigerated storage, results showed there was a significant difference (p < 0.05) when evaluating Total aerobic count, Lactic Acid Bacteria, *Enterobacteriaceae*, and *Pseudomonas* spp. However, samples did not reach values of log 7 CFU/g, which would consider them spoiled.

Duran and Kahve (2020), evaluated the microbial results and TBARS of beef strip loins stored in vacuum packaging with or without a chitosan coating. Results of total mesophilic aerobic bacteria in the vacuum packaged meat without chitosan was greater than that of the vacuum packaged with the chitosan coating. Technologies of packaging, antimicrobials, and storage temperatures can often contribute to limiting growth of spoilage organisms. Identifying mechanisms to alter the growth of spoilage organisms could improve the storage of meat products and reduce industry losses.

Conclusion

Satisfying consumers with their preference for healthy, tender, red meat while also producing a safe product is a challenge the meat industry must continue to resolve. Goats meet that demand for a healthier source of protein while also having the ability to live in terrain that is not suitable for other animals. They are able to survive of by-products other animals could not while still providing this lean source of protein. Understanding the new possibilities goat meat brings forth will allow for industry to incorporate this source of protein. Comprehending the elements that influence meat products and mitigating these variables will allow for the meat industry to produce safe and wholesome meat products. Understanding the influence of dietary supplementations fed to animals, such as hemp seed meal, has an effect on the overall quality of the product. Additional factors such as sanitation during pre- and post-harvest practices, storage temperatures for freezing and chilling and packaging technologies can all play a role production of quality meat products. Additional research should focus on new technologies that can further enhance meat production as well as the effects HSM has on meat products. Importantly, maintaining consumer education for best practices to achieve greater storage periods and safer handling practices can result in greater improvements within the meat industry.

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Chapter II

Influence of Hemp Seed Meal on Fresh Goat Meat Characteristics when Stored in Vacuum Packaging

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Abstract:

The objective of this study was to determine the influence of Hemp Seed Meal (HSM) on goat meat characteristics, such as ribeye area, backfat thickness and cooler shrink. Goats (n = 10/treatment) were allocated to a diet concentration (0, 10, 20, or 30 percent) of HSM, for 60 d prior to harvest. Carcass measurements were collected after chilling, and prior to fabricating carcasses into wholesale subprimals. Each subprimal of the shoulder and leg were cut into 2.54-cm thick steaks, vacuum packaged and assigned to laboratory methods: cook yield, instrumental color, lipid oxidation, microbial spoilage, and instrumental tenderness. HSM did not alter (p > 0.05) carcass characteristics, microbial plate counts, Thiobarbituric Acid Reactive Substance (TBARS) or cook loss. A decrease in tenderness (p < 0.05) was observed with greater concentrations of HSM supplementation in the diet. Instrumental surface color values for lightness (L*) indicated that steaks became lighter and redder (a*) as storage time (day) increased (p < 0.05). Results indicate that HSM may be an effective feed ingredient that does not alter carcass quality, yield, or storage characteristics of meat product for retail.

1. Introduction

Hemp comes from the herbaceous species *Cannabis sativa* and is one of the oldest plants cultivated in the world [1]. In 2018, the Hemp Farming Act was approved to allow for the production of hemp by agriculture producers but cannot contain more than 0.3 percent of THC [2]. Throughout the hemp industry, plants have been cultivated to contain less than 1 percent of THC, unless grown in a stressful environment [1,3]. During hemp seed milling, roughly 90 to 99.6% of the oil within the hemp seed is extracted resulting in a dense protein and fiber meal [4,5]. There is a lack of studies referencing HSM and meat quality, shelf stability, or textural influence of HSM on goat meat.

Healthier sources of protein that maintain nutritional attributes of high-quality meat have become a growing trend today [6]. There are many healthy options of meat proteins for consumers, but goat meat interest has risen [7]. Globally, goat meat is considered a highly nutritious source of red meat [7, 8, 9]. Goat meat is one of the most popular red meats in India and other regions, such as South-East Asia and Africa, but it is often found unfavorable in Western countries [8,10]. Goat meat is often perceived as exhibiting a tough and stringy texture, an unappealing color, in addition to a strong flavor and aroma [7,11]. Aside from the lack of popularity of goat meat in the United States, a study reported in 2014 that 43 million pounds of goat meat was imported [12]. Over the past decade, the demand for goat meat has increased due to an exponential growth in ethnic diversity within the United States [9,13]. In 2020, reports stated that the United States imported roughly 945 million dollars of sheep and goat meat [14].

Consumers remain steadfast in their beliefs that the most important attribute of goat meat during the last 20 years has been tenderness [15–17]. More specifically, goat meat can be less juicy and less tender than other types of meat protein [8]. Due to the lack of tenderness within goat meat, often consumers are discouraged from purchasing this source of protein [16]. Understanding the factors that are instrumental in affecting the quality of goat meat may aid in consumer acceptability of the product [8,16]. Studies have reported that diet and physiological age may have lasting effects on the color stability and tenderness of goat meat [13]. Therefore, the objective of this study was to determine the influence and changes hemp seed meal had on goat meat characteristics such as fresh color, storage stability, and cooked qualities.

2. Materials and Methods

2.1 Raw Materials

Bucks (n = 39) were randomly allocated to one of four feeding treatments (n = 9 - 10) for 60 days. Treatments consisted of 0, 10, 20 or 30% HSM supplementation within a standard diet (Table 1). Goats were fed and cared for by Tuskegee University (Tuskegee, AL) according to procedures outlined in the institutional animal care and use guidelines (PRN R07-2019-5). Due to health complications one goat was removed from this study. Diets offered to each goat daily consisted of 50% concentrates and 50% hay. Using the USDA humane slaughter standards under simulated commercial conditions, goats were harvested at the Auburn University Lambert-Powell Meat Laboratory. Carcasses were chilled after harvest procedures at 2.0 °C \pm 1.0 °C for 24 hours. Following chilling, carcasses were split between the 12th and 13th ribs, and the exposed loineye area was allowed to bloom for 15 minutes prior to collection of carcass measurements by university trained personnel. Carcasses were fabricated according to USDA institutional meat purchase specifications (roasting, barbeque, hotel, food service) for fresh goat [18]. At the time of fabrication, subprimals from the leg, loin, rack, and shoulder were weighed (PB3002-S, Mettler Toledo, Columbus, OH, USA) and recorded. Subprimals were individually identified vacuum packaged (Cryovac Bone Guard Barrier Bag, Sealed Air, Charlotte, NC, USA) stored in the absence of light for 7 days at 4°C. At the conclusion of dark storage, subprimal legs and shoulders were removed from packaging and cut into 2.54-cm thick steaks using a BIRO bandsaw (Model 334, Biro Manufacturing Company, Marblehead, OH, USA).

2.3 Packaging and Display Conditions

Sliced leg and shoulder steaks were vacuum packaged using a Reiser roll-stock form and film vacuum packaging machine (Optimus OL0924, Variovac, Zarrentin, Germany). Steaks were

packaged in a forming film with an oxygen transmission rate of 0.4 cc/sq. m²/24 h/atm and a nonforming film with an oxygen transmission rate of 1.5 cc/sq. m²/24 h/atm. Packages of steaks were individually identified, randomly assigned to an analysis method and placed on shelves of a lighted, refrigerated display case (Model TOM – label 60 DXB-N, Turbo Air Inc., Long Beach, CA, USA) for 21 days. Steaks were stored in simulated retail refrigerated cases operating at 3.0 °C \pm 1.5 °C with four 45 minute defrost periods every 24 hours. Case temperatures were monitored using temperature data loggers (Model-TD2f, Thermoworks, American Fork, UT, USA). Steaks were distributed throughout each case and rotated daily to simulate shopping conditions. Packages of steaks were removed from refrigerated storage on days 0, 7, 14, and 21 for laboratory analysis. 2.4 Instrumental Color

Instrumental color was measured using a HunterLab MiniScan EZ colorimeter, Model 45/0 LAV (Hunter Associates Laboratory Inc., Reston, WV, USA). Prior to instrumental color readings, the colorimeter was standardized using black and white tiles. Surface color values were obtained by instrument reading through the unopened package using illuminant A, a 10° observer, and an aperture of 31.8 mm. The color value of each package was measured in three different locations to record the lightness (L*), redness (a*) and yellowness (b*). Chroma was used as a measure of brightness or dullness, and was calculated using ($\sqrt{(b^*)^2+(a^*)^2}$). Hue angle illustrates a shift in color from redness to yellowness, was calculated using [tan-1(b*/a)]. Red:Brown (RTB) was calculated using the equation (630mm/580mm). Relative values for deoxymyoglobin, metmyoglobin and oxymyoglobin were determined using the formulas provided by AMSA Meat Color Measurement Guidelines [19]. Deoxymyoglobin (DMb) was determined using the equation (%DMb = {2.375*[1-(A473-A730)/(A525-A730)]*100}. Metmyoglobin (MMb) was calculated using the equation $(\% MMb = \{1.395 - [(A572 - A730)/(A525A730)]\}*100)$. Lastly, oxymyoglobin (OMb) was calculated using the equation [% OMb = 100 - (% MMb - % DMb)].

2.5 Purge Loss and pH

Weight of each package was measured on an analytic balance (PB3002-S, Metter Toledo, Columbus, OH, USA) and recorded. Once the weight was recorded, each steak was removed from the package, patted dry using a paper towel and weighed. Purge loss was calculated using the following formula: [(initial weight – final weight) / initial weight * 100]. Muscle pH of each sample was taken in two random locations by inserting the pH probe into the geometric center of the steak. Using a glass electrode pH meter (Model-HI99163, Hanna Instruments, Woonsocket, RI, USA) where these values were averaged together to obtain the reading for the steak. Prior to the pH being taken, the pH probe was calibrated (pH 4.0 and 7.0) using a 2-point standard buffer (Thermo-Fisher Scientific, Chelmsford, MA, USA).

2.6 Thiobarbituric Acid Reactive Substance (TBARS)

Following previously published guidelines [20], steaks were then cut and weighed into approximately 2 g and placed into plastic centrifuge tubes. Two samples were collected from each steak. eight mL of phosphate buffer (50mM, pH of 7.0 at 4 °C) containing 0.1 percent EDTA and 0.1 percent n-propyl gallate as well as 2mL of trichloroacetic acid (Sigma-Aldrich, Saint Louis, MO, USA) were added to each tube. Samples were homogenized for approximately 45 seconds and filtered through Whatmann No. 1 filter paper. Samples of the clear aliquot were transferred in duplicate by pipetting 2 mL aliquot into 10 mL borosilicate tubes. After pipette transfer of supernatant, 2 mL of 0.02 M 2-thiobarbituric acid reagent (Sigma-Aldrich, Saint Louis, MO, USA) was added to each tube. Tubes were then boiled for 20 minutes in a 100 °C water bath. After boiling, the tubes were placed into an ice bath for 15 minutes. The absorbance was then measured

at 533nm using a spectrophotometer (Turner Model – SM110245, Barnstead International, Dubuque, IA, USA). Absorbance values were multiplied using a factor of 12.21 to obtain the TBARS values (mg malonaldehyde/kg of meat).

2.7 Microbiology

Using standard method outlines in the USDA Bacteriological Analytical Manual [21], two 10 g samples were removed from each steak every day of testing and placed in a 3M sample bag with Sterile Filter (3M Corp., St. Paul, MN, USA) with 50 mL of 3M Butterfield Buffer (3M Corp., St. Paul, MN, USA). Filter bags were stomached by hand for 2 minutes, and the remaining solution was diluted using a five-time serial dilution system. Dilutions were plated on duplicate corresponding Petrifilm® (3M Corp., St. Paul, MN, USA) aerobic plate count (APC) plates. Plates were incubated at 35 °C in an incubation chamber (Model IB-05G, Lab Companion, Yuseong-gu, Daejeon, Republic of Korea), counted after 48 hours, and recorded as colony-forming units per gram (CFU/g).

2.8 Warner-Bratzler Shear Force and Cook Loss

Prior to cooking, each steak was removed from their respective package, blotted dry with a paper towel, and weighed (PB3002-S, Metter Toledo, Columbus, OH, USA). Each steak was placed on an aluminum wire roasting rack and the convection oven (Model-VC5ED, Vulcan, Baltimore, MD, USA) was preheated to 177 °C. Steaks were cooked until the internal temperature reached 71 °C, removed from the oven and allowed to cool to room temperature [22]. Upon each steak reaching room temperature, cooked steak weights were recorded. Cook loss was calculated using the following equation: [(initial weight – final weight) ÷ (initial weight) * 100] [23]. Six cores were obtained parallel to the muscle fibers from each steak and sheared using the Warner-Bratzler Shear Force attachment on a TA XTPlus texture analyzer (TA-XTT2i Texture Analyzer,

Stable Micro Systems, Scarsdale, New York, USA) at a crosshead speed of 200 mm/min and a loadcell of 5 kg [24]. Peak force in newtons (N) for each core was obtained and recorded [25]. *2.9 Statistical Analysis*

Data were analyzed with SAS (ver. 9.4, SAS Institute Inc. Cary) using the GLIMMIX procedures, with treatment and day as the fixed effects and replication as a random effect for surface color, pH, microbiology, lipid oxidation, WBSF, and cook loss. For carcass traits and subprimal weights, treatment served as the lone fixed effect. Least square means were computed and the significant (p < 0.05) F-value were separated using the pair-wise *t*-test (PDIFF option).

3. Results and Discussion

3.1 Carcass Data

Carcass measurements did not differ (p > 0.05) across HSM inclusion rates during the feeding period (Table 1). Regardless of inclusion rate, the backfat, carcass weight, cooler shrink, and ribeye area did not change due to the HSM use. Additionally, weight of primals (shoulder, rack, loin, and leg) did not differ (p > 0.05) with increasing HSM inclusion rates (Table 2). Current results agree with previous studies evaluating HSM within animal diets [26, 27, 28]. One study evaluating carcass characteristics of cattle fed hemp seed cakes reported no changes in carcass characteristics such as carcass weights, dressing percentage, ribeye area, or backfat thickness with the use of hemp seed cake [28]. It has been suggested that these differences in carcass characteristics when feeding HSM are due to a lack of essential amino acids such as lysine, which can limit growth and have been recorded at low levels within by-product ingredients such as hemp [26]. Additionally, when feeding peanut skins to lambs, there were no significant differences in cold carcass weights of lamb carcasses regardless of the at a peanut inclusion rate. [27]. Feeding peanut skins, which are high in protein, can increase ribeye area of lambs when fed at 20 percent of the total diet dry matter but not exceeding 40 percent inclusion peanut skins of the lamb diet [28].

3.2 Instrumental Fresh Color

Instrumental surface color values were measured over a simulated storage period of 21 days. There was no interaction (p > 0.05) for HSM × day of display on packaged goat steaks. However, there were differences in color by day of simulated storage (Table 3). Surface color of goat steaks became lighter (L*) as the duration of storage increased (p < 0.05). Redness values (a*) and RTB decreased (p < 0.05), whereas hue angle increased (p < 0.05) which is indicative of the surface color of goat steaks became less vivid (C*) as storage time during simulated display increased (p < 0.05), suggesting surface color was altered during the storage period.

In addition to the impact of storage length, HSM inclusion rate in the diet altered the surface color of goat steaks (Table 4). Concentrations of HSM resulted in a darker (L*) surface color (p < 0.05). Additionally, the redness (a*) and yellowness (b*) values decreased (p < 0.05) as the concentration of HSM increased (Table 4). Whereas steaks became less (p < 0.05) vivid (C*) as the percentage of HSM increased. These factors were all dependent and consistent throughout the study.

Surface color results of goat steaks in the current study tend to agree with other references in lambs [9, 29 - 31]. In a similar study it was concluded that L*, a*, and b* values increased in lamb meat stored over a 28-day simulated retail display period [30]. Studies have suggested an increase in L* values could be due to the changes in the meat structure via protein denaturation which allows for greater light dispersion [30]. It has been reported that a shift in redness could be responsible for increasing values of yellowness (b*) in goat steaks [30]. Additional studies have

indicated that red to brown and chroma (C^*) values tend to decline as storage time increases when looking at [32-33].

Previous research showed that goats fed 75 percent supplemental peanut skins, which is high in protein and fiber, within the diet had greater yellowness values than goats fed 0 to 25 percent supplemental peanut skins [31]. Additional studies have concluded that feeding supplements, hay and concentrates, alfalfa grazing, or alfalfa grazing with hay and concentrates to lambs for 74 days, it did not alter surface color values [34]. Nevertheless, surface color of goat steaks in the current study deteriorated as storage time and concentration of HSM in the diet increased.

3.3 Purge Loss and pH

There was no significant (p = 0.88) difference in the purge loss (data not reported) of packaged goat steaks throughout this study. Additionally, there was no interaction (p = 0.67) between the concentration of HSM in the diet and the day of display when evaluating the pH. Duration of storage resulted in pH values declining (p < 0.05) within 7 days of storage (Figure 1A). In addition, pH values were greater (p < 0.05) with increasing concentration of HSM in the diet (Figure 1B). Postmortem pH values of goat steaks were an average of 5.91 is slightly higher than normal postmortem pH meat samples, which is approximately 5.55 for red meat [35].

At the time of goat harvest and prior to chilling, each carcass was rinsed with a USDA FSIS approved organic acid (2% lactic acid) [36]. Organic acids in combination with vacuum packaging may contribute to a decline in postmortem muscle pH [37-38]. A change in muscle pH when using various packaging methods during storage could cause a decrease in the enzymatic breakdown of meat proteins altering pH values [30]. Current results are inconsistent with previous studies reporting that pH values measured on goat rib meat. Postmortem pH remained the same throughout the duration of the 7-day study [30].

Another study observed that various diet supplementation of tannin-rich peanut skins, which are high in protein, influenced goat carcass and meat quality [31]. However, peanut skin supplementation did not alter ultimate pH among dietary treatments of peanut skins [31]. Additional research evaluating stress in lambs before harvest could increase meat pH. Lambs subjected to underfeeding, shearing twenty-four hours prior to harvest, and washing before harvest caused more stress, increasing the pH levels of the meat [39]. Results suggest that pH in the *M. triceps brachii, supraspinatus,* and *infraspinatus* is more affected by stress prior to harvest than the *m. longissimus dorsi* [39].

3.4 Cook Loss

There was no interaction between the treatment \times day of simulated display. Nevertheless, cook loss percentages increased (p < 0.05) as duration of storage increased (Figure 2A). However, cook loss did not differ (p > 0.05) regardless of treatment (Figure 2B).

Results agree with previous studies reporting that cooking loss is related to water holding capacity by protein integrity [40]. Goats fed various levels of tannin-rich peanut skins identified a correlation between muscle pH and cook loss [31]. Previous studies have concluded that meat cooked at lower temperatures, limits cook loss and improves tenderness [41]. Increases in cook loss can be dependent upon muscle pH, so when protein denaturation occurs, muscle pH can begin to decline due to increasing concentrations of lactic acid altering water retention and increasing cook loss [42 - 44].

3.5 Lipid Oxidation

There was no interaction (p = 0.322) of treatment × day of display for lipid oxidation values. Lipid oxidation of packaged goat steaks increased (p < 0.05) throughout the duration of the study (Figure 3A). Though samples remained within the acceptable levels of lipid oxidation. This increase in lipid oxidation agree with previous studies that evaluated vacuum stored meat products [46]. When evaluating the lipid oxidation of steaks vacuum packaged and stored in simulated retail conditions for 25 days, authors found there was an increase in lipid oxidation [46]. When comparing the treatments to lipid oxidation there was no significant difference (p = 0.2090; Figure 3B).

Current results contradict previous studies particularly when feeding goats various levels of peanut skins, which caused lipid oxidation to increase [31]. Lipid oxidation of the loin was possibly not affected due to variation in the fatty acid composition, especially unsaturated fatty acids [31]. Factors such as light, temperature, humidity, and muscle pH can influence the degree of lipid oxidation within meat [31]. Numerous studies have reported that TBAR values will increase as the duration of storage time increases [29, 44, 47-48]. Furthermore, studies have suggested that lipid oxidation is the limiting factor for quality and acceptability within meat and meat products [48]. Previous studies have indicated that when TBAR values increase over 2.0 mg/kg of malonaldehyde the product would be deemed unacceptable [49]. Vacuum packaging has been shown to lower rancidity rates, but with 0 to 2% oxygen within the packaging lipid oxidation can occur [29]. These results indicate that the various amounts of HSM within the diets had no effect on the oxidation rate of these samples.

3.6 Microbiology

Throughout this study microbial growth was measured every 7 days to identify the spoilage organism growth rate of vacuum packaged goat steaks. Results indicate that during the storage period, microbial population increased (p < 0.001) with increasing storage time (Figure 4A). However, there were no differences (p = 0.2315) in spoilage organism growth caused by HSM diet

inclusion rate (Figure 4B). Specifically, the microbial spoilage increases throughout the duration of the study did not approach a threshold of spoilage that was greater than 7 log CFU/g [50].

Results are consistent with those reported in recent studies [29, 50-52]. Packaging conditions play a pivotal role on microbial growth in lamb steaks, with the greatest growth of spoilage organisms occurring by day 14 of storage [29]. Additional studies have identified that when using vacuum packaging for lamb steaks there is often greater microbial growth when evaluating total viable counts, lactic acid bacteria, or Enterobacteriaceae [29]. Spoilage in meat can be attributed to bacteria utilizing carbohydrates as a carbon source, resulting in the production of unique odors and flavors [51]. Numerous factors may affect the storage life of a meat product, such as the packaging, storage temperature, presence of fat, salt, nitrites, and overall pH [52]. Results from this study suggest that HSM does not hinder the microbial growth of packaged goat steaks during simulated storage conditions.

3.7 Warner-Bratzler Shear Force

There was no interaction (p = 0.53) for HSM and the day of simulated display. However, our study concluded that WBSF values decreased (p < 0.001) with increasing storage time (Figure 5A) and HSM concentration (Figure 5B). Our results agree with previous tenderness results that storage time postmortem and feeding ingredients can alter instrumental tenderness values. Previous literature has suggested that WBSF will decrease as intramuscular (marbling) and subcutaneous fat (carcass) amounts increase [9, 53]. Additionally, tenderness values can be dependent on other factors such as antemortem animal activities, postmortem storage methodologies, anatomical muscle location, and cookery methods [53]. Studies have concluded that cooking temperature can be one of the most influencers on overall tenderness of steaks [53].

In a recent study, *Longissimus dorsi* muscles from intact male goats were classified on muscle pH and muscles aged for 28 days concluded that despite initial pH, tenderness values decreased daily throughout the duration of study [56]. Dissimilar results have reported that feeding bucks one of three dietary treatments of hay, concentrate, or hay and concentrate, does not overall tenderness [53]. Denaturation of connective tissue occurs below 60 °C and the denaturation of myofibrillar components is altered at temperatures between 65 and 80 °C [23]. Denaturation of the muscle fiber components can increase the strength of the perimysium as well as in the single muscle fibers [23]. It is possible that HSM is altering the muscle fiber components prior to cooking, but additional research is needed to identify the mechanism at the muscle fiber level.

5. Conclusions

To date, there is very limited information regarding the influence of dietary supplementation with HSM on muscle quality characteristics in meat goats. Current results provide a brief illustration on the influence of HSM as a by-product within the diet that has no impact on carcass characteristics up to 30% inclusion rate when fed for a 60-day period, it also did not cause a negative effect on the carcass characteristics. However, the use of HSM did alter instrumental surface color, postmortem pH, and instrumental tenderness. Additionally, results displayed that the day of retail simulation does have an effect on the lipid oxidation, instrumental surface color, instrumental tenderness, postmortem pH and microbial analysis. It would be advantageous for future research to evaluate the overall influence of HSM on sensory taste profiles of retail cuts from the goat carcass to further illicit any detrimental impacts of HSM as a by-product feed ingredient. **Author Contributions:** Conceptualization, J.T.S., V.E.Z., F.W.A., N.K.G.; methodology, V.E.Z. validation, V.E.Z., K.E.C., W.S.R., N.C.K.; formal analysis, J.T.S.; investigation, V.E.Z., K.E.C., W.E.S., N.C.K.; resources, J.T.S, N.K.G.; data curation, V.E.Z.; writing—original draft preparation, V.E.Z.; writing—review and editing, V.E.Z, J.T.S.; supervision, J.T.S.; funding acquisition, J.T.S, N.K.G. All authors have read and agreed to the published version of the manuscript.

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Table 1: Diets of goats fed various levels of Hempseed Meal							
Feedstuffs		Hempseed meal s	upplementation (9	on (%)			
	0%	10%	20%	30%			
Hempseed meal	0	10	20	30			
Cotton Seed Hulls	20	20	25	16			
Alfalfa meal	30	25	20	23.2			
Cracked Corn	31.6	30.9	27.2	27.3			
Soybean Meal	15.9	10.6	4.3	0			
Cane molasses	2.5	2.5	2.5	2.5			
Premix ^{\$}	1	1	1	1			
Total	100	100	100	100			

	Treatment $(\%)^1$				
	0	10	20	30	SEM*
Backfat Fat	0.06	0.04	0.03	0.04	0.013
Cold Carcass Weight	60.90	59.10	56.50	56.33	2.36
Cooler Shrink	1.02	1.01	1.02	1.02	0.008
Ribeye Area	5.12	4.99	5.29	5.13	0.234

Table 2. The effects of HSM supplementation on carcass characteristics of meat goats

¹Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added meal. Mean values within a row lacking common superscripts differ (p ≤ 0.05). *SEM, Standard error of mean.

	Treatment $(\%)^1$					
	0	10	20	30	SEM	
Shoulder	6.79	6.56	6.28	6.25	0.247	
Rack	3.02	3.13	2.85	2.79	0.127	
Loin	2.86	2.79	2.75	2.54	0.157	
Leg	6.19	5.99	6.02	5.91	0.193	

Table 3. The effects of HSM supplementation on subprimal weights of meat goats.

¹Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM. Mean values within a row lacking common superscripts differ ($p \le 0.05$). *SEM, Standard error of mean.

	Day				
Instrumental					
Color^1	0	7	14	21	SEM*
L*	40.72 ^c	42.84 ^b	44.77 ^a	44.70 ^a	0.360
a*	17.69 ^a	16.64 ^b	16.58 ^b	16.78 ^b	0.152
b*	7.08^{ab}	7.61 ^b	7.56 ^b	8.00^{a}	0.121
C*	19.35 ^a	18.33 ^b	18.24 ^b	18.62 ^b	0.177
Hue (°)	23.77 ^b	24.45 ^b	24.37 ^b	25.43 ^a	0.252
RTB	3.02 ^a	2.69 ^b	2.59 ^c	2.64 ^{bc}	0.025

Table 4. Day of display for instrumental surface color on goat steaks

¹Instrumental color: L* values are a measure of darkness to lightness (larger values indicate a lighter color); a* values are a measurement of redness (larger values indicate a redder color); b* values are a measure of yellowness (larger value indicates a more yellow color). C* (Chroma) is the measurement of total color (larger values indicate a more vivid color). Hue (°) angle represents a change from the true red axis (larger numbers indicate a greater shift from red to yellow). RTB calculated 630 nm ÷ 580 nm. This represents the change in color from red to brown (larger values indicate a redder color). ^{a-c} Mean values within the row and treatment lacking common superscripts differ (p ≤ 0.05). *SEM, Standard error of mean.

Treatment (%) ¹							
Instrumental	01	10 ²	20 ³	304	SEM*		
Color ²							
L*	43.62 ^{ab}	44.32ª	42.71 ^{bc}	42.38 ^c	0.360		
a*	17.28 ^a	16.83 ^b	16.87 ^{ab}	16.70 ^b	0.152		
b*	8.05 ^a	7.81 ^{ab}	7.54^{b}	7.57 ^b	0.121		
C*	19.10 ^a	18.57 ^b	18.50 ^b	18.36 ^b	0.177		
Hue (°)	24.89 ^a	24.84ª	24.01 ^b	24.28^{ab}	0.251		
RTB	2.75 ^a	2.68 ^b	2.76ª	2.75 ^{ab}	0.025		

Table 5. The influence of treatment on instrumental surface color of goat steaks

¹ Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM. ²Instrumental color: L* values are a measure of darkness to lightness (larger values indicate a lighter color); a* values are a measure of redness (larger values indicate a redder color); b* values are a measure of yellowness (larger value indicates a more yellow color) C* (Chroma) is the measurement of total color (larger values indicate a more vivid color). Hue (°) angle represents the change from the true red axis (larger numbers indicate a greater shift from red to yellow). RTB calculated 630 nm ÷ 580 nm. This represents the change in color from red to brown (larger values indicate a redder color). ^{a-c} Mean values within the row common superscripts differ (p ≤ 0.05). *SEM, Standard error of mean.

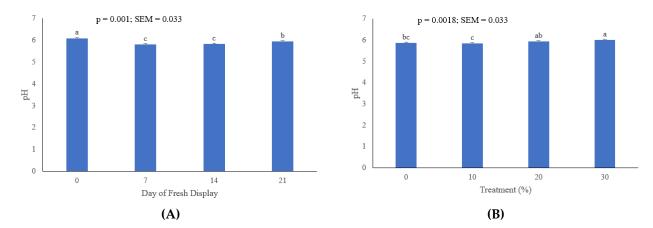


Figure 1: The influence of display day (A) or diet inclusion rate (B) of hemp seed meal on postmortem fresh muscle pH of goat steaks. Bars lacking common letters differ (p < 0.05).¹ Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM.

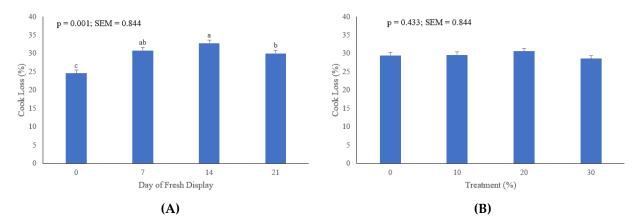


Figure 2: The influence of display day (A) or (B) diet inclusion rate on cook loss of goat steaks. Bars lacking common letters differ (p < 0.05). ¹ Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM.

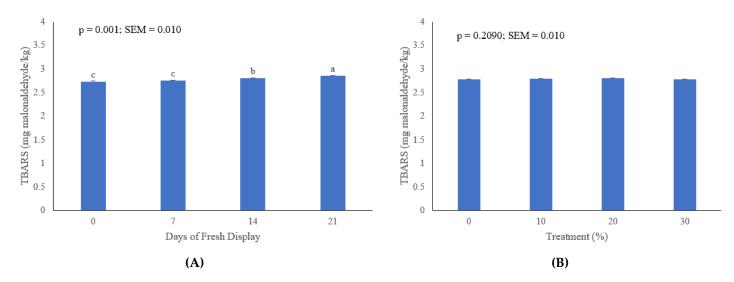


Figure 3: Day of display **(A)** or diet inclusion (B) rate of hemp seed meal on 2-Thiobarbituric Acid Reactive Substances (TBARS) of goat steaks during simulated retail display. Bars lacking common letters differ (p < 0.05). ¹ Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM.

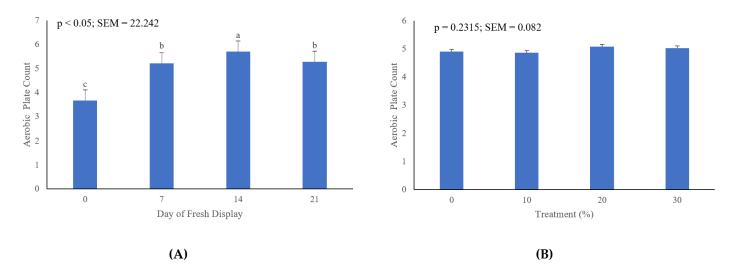


Figure 4: Day of display **(A)** or diet inclusion **(B)** rate of hemp seed meal on microbial spoilage of goat steaks. Bars lacking common letters differ (p < 0.05). ¹ Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM.

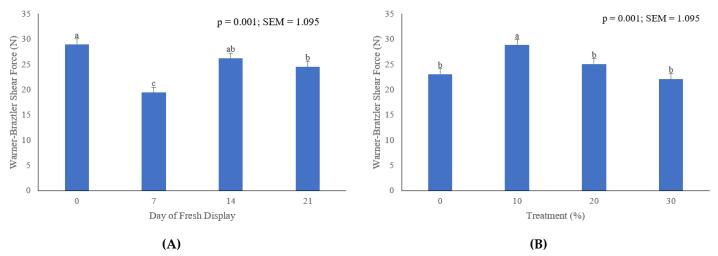


Figure 5: Day of display **(A)** or diet **(B)** inclusion rate of hemp seed meal on Warner-Bratzler shear force of goat steaks. Bars lacking common letters differ (p < 0.05).¹ Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM.

Chapter III

Hemp Seed Meal Influence on Goat Meat Tenderness

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Abstract

This study was conducted to identify alternative feed ingredients for use in production agriculture that can lead to greater animal efficiencies. The objective for this study was to determine the effect of hemp seed meal (HSM) supplementation rate on cooked characteristics of goat steaks. Goats (n = 39) were assigned to one of four diet concentrations (0, 10, 20, 30%) of HSM, and were harvested at the Auburn University Lambert-Powell Meat Laboratory after a 60-day feeding period. Paired racks and loins were fabricated, vacuum sealed, and stored in the absence of light at -23 °C until cooked analysis could be completed. Racks and loins were then sliced frozen into 2.54-cm thick steaks and thawed overnight prior to analysis. Steaks were randomly assigned to instrumental color or Warner-Bratzler Shear Force (WBSF). It was found that cook loss and cooking time decreased (p < 0.05) with increasing diet concentration of HSM. However, there were no differences (p > 0.05) in cooked color or WBSF regardless of diet concentration. Results indicate that feeding various rates of HSM as part of a finishing ration effect overall tenderness (p < 0.05) and cook loss (p < 0.05) of the meat product. Dietary inclusion rates of less 20 percent HSM can alter cooked characteristics of goat steaks.

Keywords, cooked color, cook loss, cook time, goats, hemp seed meal, Warner-Bratzler Shear Force

1. Introduction

Healthy alternatives for protein are becoming a growing trend, and goat meat is a great source of lean red meat. Most goat meat consumers can be found in South-East Asia, Africa, India and other developing countries as it is often considered a less favorable protein in western countries [1–4]. Recent studies have concluded that goat meat is inconsistent in tenderness and juiciness causing consumers to avoid this source of protein [3,5]. Variation in meat quality may be best described by changes in physiological age, diet, cooking methods or cooking time [6–8].

It has been reported that in lamb meat, when aged postmortem for greater durations and cooked at a lower temperature, overall tenderness improves compared to lamb steaks with no aging or cooked at higher temperatures [9]. Additionally, studies have indicated that various cooking methods, muscles and cooking temperatures can affect the overall tenderness of beef [10]. Using a belt driven grill for cooking the beef *Gluteus medius* can result in a more tender steak when compared to other muscles, cooking methods, or cooking temperatures [10]. These studies suggest that countless factors such as postmortem aging, cooking method, cooking temperature, and muscle can affect overall tenderness of a product. Furthermore, recent studies have shown that feeding various supplements, such as linseed or quinoa to lambs altered tenderness and proteolytic patterns in the *Longissimus lumborum* muscle of lambs [11].

Over the last few decades, the hemp plant, known as *Cannabis sativa*, has been rediscovered [12]. Originating from Asia, it has now become a cultivated plant throughout the world [13]. The increased interest in this plant is due to its uses such as oil extraction from the seeds, fiber extraction from the stems and drug extraction from the flowers and leaves [14–16]. Studies have concluded that the global market value for low THC hemp is around 100 to 200 million US dollars, and the North American market is increasing at a rate of 8 to 10 million dollars per year [17].

Regardless of the use for this plant, it has been suggested that hemp seed by-products could serve as a potential feed ingredient for animals in the future [14, 18]. Studies have concluded that hemp seed is a great source of protein, with an average of $24.8 \pm 2.0\%$ for dry matter [13]. Hemp seed consists of 20 to 25% protein, 20 to 30% carbohydrates, 25 to 35% oil, 10 to 15% insoluble fibre in addition to an abundance of minerals [17]. Hemp seed meal has the potential to be a great feed source. The Hemp Farming Act Bill of 2018 approved the production of hemp but not as a feed ingredient for animals, if approved for feeding, hemp produced cannot contain more than 0.3 % THC [19]. If allowed for feeding, hemp must be milled, pressed, and extracted to remove oils, which contain THC [13, 18]. The objective of this study was to evaluate the influence of hemp seed meal on cooked meat quality characteristics.

2. Materials and Methods

2.1 Raw Materials

Spanish × Boer bucks (n = 39) were randomly allocated to one of four feeding treatments (n = 9 to 10) for 60 days. Goats were fed and cared for by Tuskegee University (Tuskegee, AL) according to procedures outlined in the institutional animal care and use guidelines (PRN R07-2019-5). Treatments of 0, 10, 20 or 30 percent HSM supplementation within a standard diet consisting of 50% concentrates and 50% mixed hay with ad libitum access to water throughout the experiment (Table 1). Under USDA humane slaughter standards goats were harvested at the Auburn University Lambert-Powell Meat Laboratory, weighed then chilled at 2.0 °C ± 1.0 °C for 24 hours. Carcasses were fabricated into subprimal pieces, vacuum packaged in a bone-guard bag (Cryovac Bone Guard Barrier Bag, Sealed Air, Charlotte, NC, USA) and stored in the absence of light for 7 days at 4 °C. After being stored in the dark, subprimals were frozen at -23 °C until analysis could be completed. Subprimals were cut into 2.54-cm thick steaks using a

BIRO bandsaw (Model 334, Biro Manufacturing Company, Marblehead, OH, USA), identified and vacuum packaged in a bone-guard bag (Cryovac Bone Guard Barrier Bag, Sealed Air, Charlotte, NC, USA). Prior to analysis, steaks were thawed in a refrigerated case (Model TOM – label 60 DXB-N, Turbo Air Inc., Long Beach, CA, USA) for 12 hours.

2.2 Cook Loss and Cook Time

Steaks (n = 10/treatment) were used to determine cook loss. Each steak was dried with a paper towel and weighed on an analytic balance (PB3002-S, Mettler Toledo, Columbus, OH, USA). Once the initial weight was obtained, steaks were then placed on an aluminum rack baking sheet. Thermoworks Penetration probes (Q-Series Type K 2.5-inch Penetration Probe, American Fork, UT) were inserted into the geometric center of each steak. Steaks were placed in a convection oven (VC5ED, Vulcan, Baltimore, MD, USA) pre-heated to 176 °C. Cook time was recorded using a hand-held digital stopwatch and cook time completed when steaks reached an internal temperature of 70 °C. After cooking, steaks were placed on a cooling rack and cooled to room temperature prior to weighing and recording cooked weight. Cook loss was calculated using the equation: [(initial weight – final weight) / (initial weight) * 100] as previously described [20].

2.3 Instrumental Cooked Color

After the cooking process was completed, instrumental color was measured once the steaks reached room temperature. Steaks were sliced horizontally to allow for enough surface area to take a reading. Readings were measured using a HunterLab MiniScan EZ colorimeter, Model 45/0 LAV (Hunter Associates Laboratory Inc., Reston, WV, USA) using an illuminant A, an aperture of 31.8 mm, and a 10° observer. Prior to the surface readings being taken, the colorimeter was standardized using black and white tiles. The lightness (L*), redness (a*), and yellowness (b*) of these readings were calculated using the guidelines from AMSA Meat Color Measurement Guidelines [21].

Additionally, Chroma (CHMA) was calculated using the equation: $\sqrt{a^{*2} + b^{*2}}$, which illustrates the saturation index. A calculated larger value is indicative of greater surface vividness. Hue angle was also calculated using the equation: $\tan^{-1}(b^*/a^*)$. Hue angle is used to calculate the shift in surface color from red to yellow [21].

2.4 Warner-Bratzler Shear Force

Shear force was measured after cooking, cooling, and coring steaks using previously outlined procedures [2]. Six cores from each steak were removed parallel to muscle fibers with a hand-held coring device. Cores were sheared using a TAXTPlus texture analyzer (TA-XTT2i Texture Analyzer, Stable Micro Systems, Scarsdale, New York, USA) with the Warner-Bratzler shear force blade at an extension rate of 200 mm/min and a load cell of 5 kg [22]. Peak force of each core was obtained and an average of the six cores from each steak was expressed in Newtons (N) and calculated for statistical analysis [19, 22].

2.5 Statical Analysis

Data was analyzed with SAS (version 9.4, SAS Institute Inc. Cary, NC, USA) using the GLIMMIX procedure. Fixed effects were HSM treatment and cut. The replications were surface instrumental color, cook loss, and cook time. Least square means were computed and the significant (p < 0.05) F- value was observed. Finally, least square means were separated using the pair-wise *t*-test (PDIFF option).

3. Results and Discussion

3.1. Cook Loss

Cook loss was measured to identify the amount of moisture lost from the goat steaks during the cooking process. Cook loss percentages were greater (p < 0.05) in steaks when HSM was at or below 20 percent inclusion of the diet (Figure 1). At the greatest diet inclusion rate of HSM, cook

loss was the lowest (p < 0.05). It is plausible that HSM may play a pivotal role in moisture retention during cooking. Interestingly, current results agree with previous research investigating the effects of hemp seed meal on meat quality of quail [24, 25]. Previous results indicated that increasing HSM to concentrations greater than 30 percent in the diet can alter cooking loss in quail [24]. While species differences and the nutritional needs of these animals may differ, it is expected muscle biochemistry to be similar [26]. On the contrary, additional research reported no differences when feeding Kiko goats varying concentrations of peanut skins at 25 percent or more of the diet formulation [27]. A feeding study evaluating treated wheat straw to Small East African goats reported cooking loss in the *Psoas major* did not differ [25]. It has been noted that cook loss may be attributed to a multitude of factors such as contraction of muscle fibers and intramuscular connective tissue [7, 28]. Previous research has concluded that ultimate muscle pH does not impact cooking loss, as postmortem muscle pH did not influence water holding capacity of the meat [29]. Additionally, intramuscular connective tissue may also impart changes in cook loss due to the cooking temperatures causing contraction of muscle fibers and connective tissue whereby trapping moisture within the muscle fiber [25, 28, 30].

3.2 Cook Time

Cooking time declined (p = 0.008) when HSM concentrations increased over 20 percent within the diet (Figure 2). It is plausible that cooking time and cook loss of goat steaks are linked to the available free moisture within the muscle. Percentages of free, bound, and immobilized water were not measured during the current study. However, current results for a decline in cooking time and cook loss when HSM supplementation exceeds 20% suggest water percentages in the meat may be altered.

While there is very limited literature on how animal diet affects cooking time. Previous studies have reported how feed ingredients or individual diet components do not influence cook loss. Specifically, it has been reported that pigs finished with ionophores such as narasin, did not alter the cooking time of pork chops [31]. Several studies have suggested that cooking time can influence overall tenderness of a product [32 - 34]. Cooking time has been correlated to cook loss [36], which agrees with the results of cooking time and cook loss in the current study.

3.3 Instrumental Cooked Color

Instrumental color was measured throughout the cooking process. The effect of dietary hemp seed meal treatment on the internal cooked color are compared in Table 2. Regardless of the inclusion rate, lightness, yellowness, redness, Hue, Chroma, and red to brown values were not altered by HSM diet inclusion. Literature of various feeding treatments impacting cooked instrumental color of goat meat is limited. Studies have reported that when ground goat patties were mixed with 10 ml of either kinnow rind power (KPR), pomegranate rind powder (PRP) or pomegranate seed powder (PSP), the lightness, redness and yellowness values were altered [36]. It has been reported that KRP extract increased lightness values, whereas PRP extract decreased the lightness values [36]. However, there was no difference between control and PSP treated patties [36]. Additionally, control and KRP patties were redder compared to other patties [36]. When evaluating the yellowness values, it was found there was no difference between the control, KRP and PSP patties however, PRP patties were significantly lower [36]. Additional studies have found that when evaluating cooked ground beef patties from cattle fed various feeding supplements, such as grape seed at a 0.01% inclusion rate, grape seed at a 0.02% inclusion rate, oleoresin rosemary extract and water-soluble oregano extract, and stored for 8 days there was no difference between the lightness or redness values [37]. However, increases in yellowness, chroma and Hue angle values for each treatment did occure [37]. Another study reported that pH of raw meat may have a lasting effect on the internal cooked color [38]. It was reported that when meat has an increased pH it decreases the percent myoglobin denatured within the meat. This in turn causes an apparent color difference within the cooked muscles [38].

3.4 Warner-Bratzler Shear Force

Warner-Bratzler Shear Force was measured on cooked goat steaks. Results indicate that HSM concentration in the diet did not alter (p = 0.122) objective measures of tenderness in goat steaks (Figure 3). Contrary to previous results when goats are fed dietary rations of 15 and 30 % pine bark, steaks can become more tender [2]. Additionally, feeding goats 0 or 15% peanut skins in the diet did not alter instrumental tenderness values of goat steaks [2]. Another study has found that when cattle are fed brassicas and in addition to postmortem aging of the *Longissimus lumborum* did not improve instrumental tenderness of strip loin steaks [39]. Additional research has stated that when connective tissue begins to denature below 60 °C, myofibrillar components will begin to denature between 65 and 80 °C [35]. When denaturation occurs, it may cause an increase in strength of the perimysium within the single muscle fibers [34].

4. Conclusions

Results from this study have suggested that inclusion of hemp seed only impacted cooking time and cook loss. It was found that HSM did not alter instrumental tenderness, while also keeping tenderness values under 4 kg, suggesting steaks remained tender regardless of the inclusion rate of HSM. More importantly, HSM did not alter cooked color regardless of inclusion level in the diet. Additional research should be focused on the evaluation of sensory characteristics of goat meat when using by-product feed ingredients such as HSM. Author Contributions: Conceptualization, V.E.Z., F.W.A, J.T.S.; methodology, V.E.Z.; validation, V.E.Z., A.L.K., K.E.C., K.A.H., N.C.K., J.K.F.; formal analysis, J.T.S.; investigation, V.E.Z. A.L.K., K.E.C., K.A.H., N.C.K., J.K.F.; data curation, V.E.Z.; writing—original draft preparation, V.E.Z.; writing—review and editing, V.E.Z., A.L.K., T.D.B., M.K.M., A.D.B., J.T.S.; supervision, J.T.S.; project administration, J.T.S.; funding acquisition, J.T.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Table 1: Diets of goats fed various levels of HSM						
Feedstuffs	Hempseed meal supplementation [^] , %					
reeasturis	0%	6 10% 20%	20%	30%		
Hempseed meal	0	10	20	30		
Cotton Seed Hulls	20	20	25	16		
Alfalfa meal	30	25	20	23.2		
Cracked Corn	31.6	30.9	27.2	27.3		
Soybean Meal	15.9	10.6	4.3	0		
Cane molasses	2.5	2.5	2.5	2.5		
Premix ^{\$}	1	1	1	1		
Total	100	100	100	100		

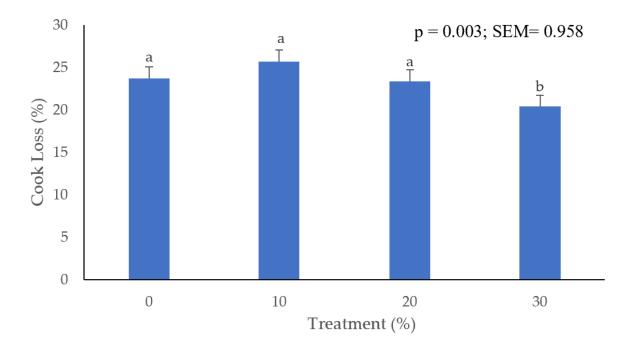


Figure 1: The influence of hempseed meal (HSM) concentration on cook loss of goat steaks. Bars lacking common letters differ (p < 0.05). Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM.

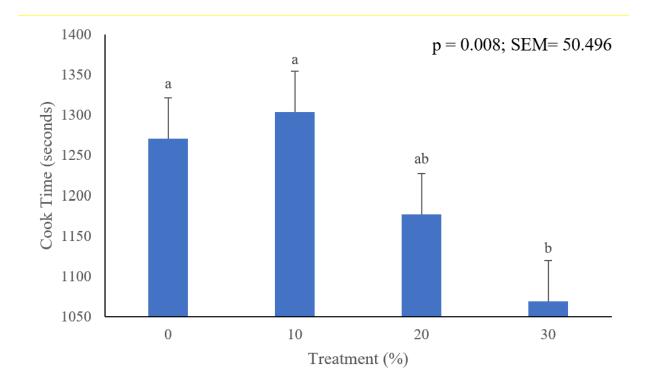


Figure 2: The influence of the various treatments on the cook time of goat steaks. Bars lacking common letters differ (p < 0.05). Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM.

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Instrumental Color ²	0	10	20	30	SEM
L*	28.01	23.72	23.44	22.34	2.554
a*	60.49	60.79	61.61	61.51	0.853
b*	17.50	16.54	17.72	17.45	0.862
Hue (°)	18.60	18.42	19.04	18.84	0.280
C*	47.25	48.62	47.47	47.57	1.147
RTB	25.63	24.85	26.07	25.74	0.753

Table 2. The effects of HSM diet supplementation on the internal cooked color of goat steaks.

¹ Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM. ²Instrumental Color values: L* values are a measure of darkness to lightness (larger values indicate a lighter color); a* values are a measurement of redness (larger values indicate a redder color); b* values are a measure of yellowness (larger value indicates a more yellow color) Hue (°) angle represents the change from the true red axis (larger numbers indicate a greater shift from red to yellow). C* (Chroma) is the measurement of total color (larger values indicate a more vivid color). RTB calculated 630 nm ÷ 580 nm. This represents the change in color from red to brown (larger values indicate a redder color). ^{a-c} Mean values within the row common superscripts differ ($p \le 0.05$). *SEM, Standard error of mean.

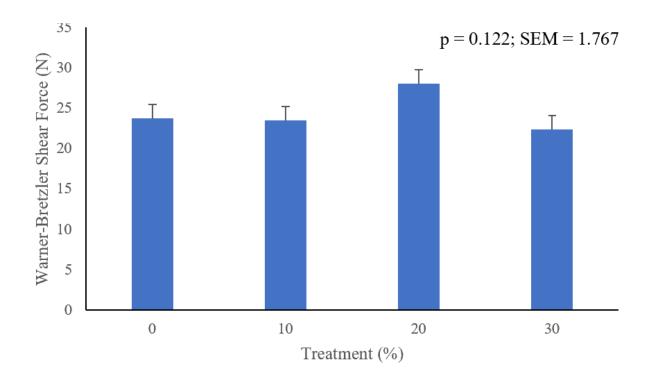


Figure 3: Average of the diet inclusion rate of hemp seed meal (HSM) supplementation on Warner-Bratzler shear force values of goat steaks. Bars lacking common letters differ (p > 0.05). Treatment defined as added dietary hempseed meal (HSM) to goat diets: 0 = no added HSM; 10 = 10% added HSM; 20 = 20% added HSM; 30 = 30% added HSM.

APPENDICIES

APPENDIX A

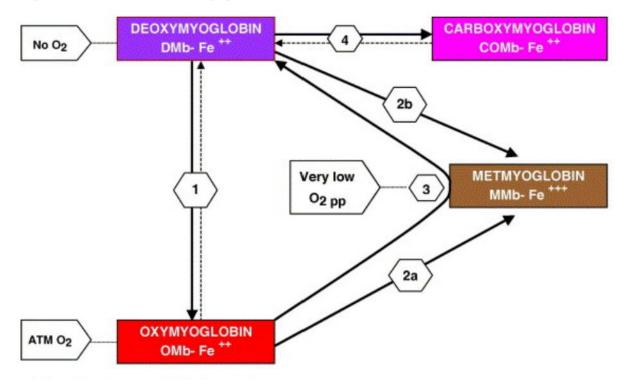


Figure 1.1 Different States of Myoglobin

(From Mancini and Hunt, 2005)

APPENDIX B

Thiobarbituric Acid Reactive Substances (TBARS)

Chemicals:

Water – HPLC grade or distilled deionized water Potassium phosphate (monobasic) KH2PO4 Potassium phosphate (dibasic) K2HPO4 Ethylenediaminetetraacetic acid (EDTA) n-Propyl gallate (PG) Trichloroacetic acid (TCA) 2-Thiobarbuturic acid (TBA) 1, 1, 3, 3, Tetraethoxypropane (TEP)

Reagents:

50mM phosphate buffer – pH 7.0, shelf-life = 2 weeks

Prepare 50mM monobasic potassium phosphate solution – weight out 3.40g KH2PO4, place in a 500 ml volumetric flask, dissolve and bring to volume with distilled-deionizedwater (pH will be approximately 4.5).

Prepare 50mM dibasic potassium phosphate solution – weight out 8.71g K2HPO4, place in a 1 L volumetric flask, dissolve and bring to volume with distilled deionized water (pHwill be approximately 8.5). Prepare at least 4 L of the dibasic solution each time.

Using a 2 L beaker, combine approximately 500 ml of dibasic and 100 ml of monobasic solutions. Mix and monitor the pH of the combined solution as you continue to add moreof each solution until the volume is in excess of 1 L. The pH of this solution will be slightly greater than 7.0.

Add 1.0g of EDTA and 1.0g of PG. Allow the solution to mix for one hour, as PG isextremely slow to dissolve.

30% TCA

Use extreme care when making, as TCA is corrosive (clean up any spills immediately). Weigh 300g of TCA into a 2 L beaker, add 1000 ml of distilled deionizedwater. If less is needed, weigh out 30g and add 100 ml of distilled deionized water.

0.02M TBA

Make fresh daily (250 ml is enough for 125 samples). Weigh out 0.7208g TBA, and placeinto a 250 ml volumetric flask. Add 250 ml of distilled deionized water. The

use of low heat while mixing will accelerate the dissolving process, but use extreme caution as too much heat will destroy the solution

Store all reagents under refrigerated conditions, but do not store solutions in the coldest regions of the refrigerator as some of these solutions will freeze at low temperatures.

Analysis:

General notes: Prepare and turn on water bath-set temperature at 100 °C. It takes approximately 1h for the water bath to reach the desired temperature. If a sipper unit is being used, it is necessary to prepare at least 3 blanks and then run at least one working standard with each run.

For raw meat samples:

- 1. Weigh out 2.0g (1.95 to 2.05g) of minced meat into a labeled 50 ml disposable centrifuge tube. Record the exact weight of the sample.
- 2. Add 8 ml of prepared phosphate buffer to the tube.
- 3. Add 2 ml of TCA to the tube and homogenize for 20 to 30 secs.
- 4. Filter homogenate through a Whatman (No. 4) filter paper, collecting the clear filtrate into labeled tubes. (It is OK to stop at this point, but the tubes containing the filtrate must be sealed and stored in a refrigerator).
- 5. Remove 2 ml of the sample filtrate and place it into a labeled glass test tube. Prepare duplicate tubes for each sample at this point (i.e., tube "A" and tube "B").
- 6. Prepare three "Blank" tubes, using 2 ml of distilled-deionized water.
- 7. Prepare one "Standard" tube, using 2 ml of phosphate buffer. (Note: after this point, time is extremely critical. Make sure that the water bath is at the correct temperature and level prior to continuing).
- 8. Add 2 ml of TBA to each tube including the blanks and standard.
- 9. Cover tubes with aluminum foil and place them into the hot water bath for 20 min.
- 10. Remove tubes from hot water bath and place into the ice water bath for 15 min.
- 11. Read absorbance at 533 nm.
- 12. Multiply absorbance by 12.21.
- 13. Report TBARS as mg/kg of malonaldehyde.

Standards:

1, 1, 3, 3 tetraethoxypropane (TEP)Stock standard solution 0.02M solution-0.44g (0.5 ml) to 100 ml of distilled water ($2 \times 10-5$ moles/ml)

Working standard solution

Dilute 0.5 ml of TEP stock standard to 500 ml ($2 \times 10-8$ moles/ml).

Standards for standard curve

Dilute each of the following amounts of TEP working solution in 50 ml volumetric flasks withdistilled water.

<u>TEP</u>	Concentration of "Standard"	Absorbance
1 ml (4.4 µg)	0.088 µg/ml	0.03
2 ml (8.8 µg)	0.176 µg/ml	0.06
4 ml (17.6 µg)	0.352 µg/ml	0.123
5 ml (22.0 µg)*	0.44 µg/ml	0.150
10 ml (44.0 µg)	0.88 µg/ml	0.30
20 ml (88.0 µg)	1.76 µg/ml	0.60
40 ml (176.0 µg)	3.52 µg/ml	1.20

*This standard should have an Absorbance in the proximity of 0.150. Range may be 0.130 to 0.170, depending upon the accuracy of solutions and dilutions.

References:

Kuntapanit, C. 1978. Beef muscle and adipose lipid deterioration as affected by nutritional regime, vacuum aging, display, and carcass conditioning. Ph.D. dissertation. Kansas State University. pg. 117.

Witte, V. C., Krause, G. F., & Bailey, M. E. 1970. A new extraction method for determining 2- thiobarbituric acid values for pork and beef during storage. Journal of Food Science, 35,582-585

APPENDIX C

Aerobic Plate Count Method

Materials & Equipment:

5g sample of Raw Product 3M Sample Bag W/ Filter Sterile 50mL 3M Butterfield's Buffer Stomacher Lab Blender 2 Glass test tubes with 9mL of 3M Butterfield's Buffer (autoclaved) 1mL Pipetting tips and Pipette Vortex Mixer Petrifilm® aerobic plate count (APC) plates 3M Petrifilm Spreader Incubation chamber (36.0 °C)

Procedure:

- 1. Extract 5-gram sample from respective packaging material.
- 2. Place sample in 3M Sample Bag W/ Filter Sterile.
- 3. Add 50mL 3M Butterfield's Buffer to sample bag.
- 4. Place sample bag and contents in stomacher lab blender for 60 seconds.
- 5. With pipette extract 1mL from sample bag and plate sample on APC plate.
- 6. Use 3M Petrifilm Spreader to spread sample evenly.
- 7. With pipette extract 1mL from sample bag and place in dilution two tube.
- 8. Vortex dilution two tube.
- 9. With pipette extract 1mL and place in dilution three tube.
- 10. With pipette extract 1mL from dilution two tube and plate sample on APC plate.
- 11. Use 3M Petrifilm Spreader to spread sample evenly.
- 12. Vortex dilution three tube.
- 13. With pipette extract 1mL from dilution three tube and plate sample on APC plate.
- 14. Use 3M Petrifilm Spreader to spread sample evenly.
- 15. Vortex dilution four tube.
- 16. With pipette extract 1 mL from dilution four tube and plate sample on APC plate.
- 17. Use 3M Petrifilm Spreader to spread sample evenly.

- 18. Vortex dilution five tube.
- 19. With pipette extract 1 mL from dilution five tube and plate sample on APC plate.
- 20. Use 3M Petrifilm Spreader to spread sample evenly.
- 21. Vortex dilution six tube.
- 22. With pipette extract 1 mL from dilution six tube and plate sample on APC plate.
- 23. Use 3M Petrifilm Spreader to spread sample evenly.
- 24. Vortex dilution seven tube.
- 25. With pipette extract 1 mL from dilution seven tube and plate sample on APC plate.
- 26. Use 3M Petrifilm Spreader to spread sample evenly.
- 27. Vortex dilution eight tube.
- 28. With pipette extract 1 mL from dilution eight tube and plate sample on APC plate.
- 29. Use 3M Petrifilm Spreader to spread sample evenly.
- 30. Vortex dilution nine tube.
- 31. With pipette extract 1 mL from dilution nine tube and plate sample on APC plate.
- 32. Use 3M Petrifilm Spreader to spread sample evenly.
- 33. Vortex dilution ten tube.
- 34. With pipette extract 1 mL from dilution ten tube and plate sample on APC plate.
- 35. Use 3M Petrifilm Spreader to spread sample evenly.
- 36. Incubate APC plates at 36 °C \pm 1 °C for 48h.
- 37. Interpret plates by counting colonies.

References:

3M, P. (n.d.). 3M Petrifilm Aerobic Count Plates https://multimedia.3m.com/mws/media/1804005O/3m-petrifilm-standard-rapidplatecomparisonac-rac.pdf.