

THESIS

HEMPSEED MEAL AS AN EFFECTIVE PROTEIN SUPPLEMENT FOR FINISHING

WETHERS

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ABSTRACT

HEMPSEED MEAL AS AN EFFECTIVE PROTEIN SUPPLEMENT FOR FINISHING WETHERS

To address the needs of an increasing global population and the decreased availability of arable land due to the continued expansion of monoculture farms for the use of feeding livestock, we must begin to research more sustainable methods for feeding animals and the people who consume them. This study aims to address those needs by presenting hempseed meal (HSM) as a protein alternative to more commercially available feeds such as soybean meal, which is commonly used in finishing rations on animal feeding operations (AFOs) across the United States. Forty Western White-Faced wethers were used in a completely randomized block design with five treatments to complete this task. These treatments included diets formulated to be near isonitrogenous with 0%, 5%, 10%, 15%, or 20% of diet DM as hemp seed meal, primarily as a substitute for soybean meal. Wethers were fed the diets individually for 90 d, followed by a 5 d balance trial with a total collection of urine and feces. There were no differences in DM intake ($P = 0.44$) or N digestibility ($P = 0.467$) between treatments, although there was a slight increase in P digestibility as hemp meal inclusion increased until it represented 15% of the dietary DM ($P = 0.047$). There were no differences in urinary N excretion ($P = 0.33$) or urinary urea excretion ($P = 0.34$) between treatments.

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DEDICATION

To my Grandmother Joyce Smarr who dedicated her life to education and provided encouragement through the writing of this thesis until her passing.

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CHAPTER 1: REVIEW OF LITERATURE

1.0 Domestication of Sheep and Trends in their Use as a Livestock Commodity

Sheep are one of the oldest continuously used and produced livestock species having been domesticated approximately 11,000 years ago, according to genome sequencing and retrotyping data (Chessa et al., 2009). Since we began to keep national records of sheep as livestock through the United States Department of Agriculture (USDA) in 1867 where the country kept 45 million head of sheep, that number peaking in 1884 at 51 million, and declining to just 3.1 million in 2022 (USDA-NASS, 2023). This doesn't negate the importance of sheep/lamb in our industry rather it is indicative of the positive trends in agriculture of creating more products with fewer inputs and less strain on our environment. The USDA and National Agriculture Statistic Service (NASS) states that we processed 2.1 million lambs in 2022 in the NASS Database with the national carcass weight data not having been updated since 2010, we can still suggest that based on the average processing weight of lambs between 27.22 - 36.29 kg that we are processing well over 54 million kgs of lamb meat every year. The Livestock, Dairy, and Poultry Outlook for June 2023 published by the USDA suggests that the forecast for lamb/mutton production in 2023 is 59 million kgs with a suspected decrease of 1.5% in 2024 and our import numbers landing right at around 153 million kgs with a suspected increase of 9% by 2024. The shocking number of imports into the United States demonstrates a significant need for the further streamlining and revitalization of research geared towards producing greater numbers of lamb and mutton domestically.

The USDA defines two major sectors of the industry including lamb production and stock-sheep producers with less than 20% of producers according to the 2011 Sheep Report are hair sheep producers. Those who are defined as stock-sheep producers graze their sheep on rangelands

typically in arid environments with not very much nutritional input although this has been proven to be an effective environmental control method of both invasive plants and revitalization of soils and grazing lands (Havstad, 1994). This low cost option does save the farmer money on reseeding as Colorado State University Extension suggests those cost may be between \$200-\$400 per 4046 square meters, while maintaining a flock of sheep on rangeland cost the owner fencing and if applicable a grazing fee from the Bureau of Land Management. This style of raising lambs, has however led to a longer time on pasture and had a decreased carcass weight (Gallo, 2019). Lamb producers who remained on feedlot saw greater carcass yields and a shorter time on pasture according to the same aforementioned study (Gallo, 2019). In the 2021 Sheep Industry Review, the American Sheep Industry Association (ASIA) touted record highs in the price per hundred weight (cwt) of lamb which in March of that year went up to \$271.27 a number which was greater than the average from 2015-2019 (ASIA, 2021).

These numbers indicate while small the sector for sheep production has remained steady and proves to be a continual contributor the economy of many states particularly here in Colorado where we remain amongst one of the top 10 states for lamb production. Colorado particularly has a strong industry doing more than \$111 million in gross income according to the most recent USDA-NASS data from 2010. Whether it's the use of heritage breeds for show or 1 of 10,000 lambs on a feedlot the value of sheep as a livestock commodity and member of every day American society continues to prove itself.

2.0 Feeding Sheep from Past to Future

Originating in Southwest Asia, the first species of sheep evolved from the mouflon (*Ovis orientalis*) which mainly fed on steppe grasses and herbs characteristic of their arid environment in Turkey; these include grasses mainly belonging to the Leguminosae, Gramineae, and

Umbelliferae families (Kaya et al., 1992). The diet of the mouflon is not only characteristic of what was previously eaten in the wild, but what also exist in the industry today as a recommended diet for both small-scale and commercial farmers with most of our diets including grasses and grains from the previously documented wild diets (Umberger, 2009).

Previously our neolithic ancestors used sheep for both meat and wool production, today not much has changed according to the aforementioned trends, however, due to our heavy reliance on lambs for meat our diets have had to adapt our changing needs and landscape. These changes include a heavier reliance on grains for faster growth and larger carcass yields in feedlot lambs rather than pasture raised lambs/sheep (Priolo, 2002). To further prove and demonstrate our reliance on these efficient feedlot systems and proper nutrition as a part of our further perfection of the domestication of sheep; a study was done by Priolo et al. on thirty-two whethers which were split into four groups, two on pasture and two in stalls. The purpose was to demonstrate the differences in growth, carcass quality and yield via confinement versus those lambs which had been pasture raised not based on any other factors except nutrition and access to likely a greater land area. The lambs in the stalls were fed a concentrate diet along with hay of which the concentrate diet included barley, soybean meal, and wheat amongst other ingredients. The diet is indicative of those commercially available and demonstrated a greater carcass yield (15.8 kg) in opposition to ones on pasture (14.7 kg). However, the lambs raised on pasture did indicate a lesser fat percentage ($P < 0.05$) than those raised in stalls. The study does not take into account amount of activity nor size of pen, however, it does demonstrate larger themes which speak to areas of need in the industry such as how we maintain quality as well as efficiency throughout the system of raising livestock.

We often accomplish this efficiency piece through the use of byproducts which are often waste items and residues from other popular crop products such as corn stover, cottonseed meal, or in our case hempseed meal. Byproducts have demonstrated scientific importance in terms of lessening our environmental load particularly when raising small ruminants such as sheep which produce 6.5% of all livestock emissions as defined using the Global Livestock Environmental Assessment Model (GLEAM) (Gerber et al., 2013). While they only account for 6.5% of livestock emissions, they are producing about 430 million metric tons of CO₂-eq which essentially describes what is known as their global-warming potential (GWP) through converting the amount of other gases they emit i.e methane into carbon-dioxide equivalence (Gerber et al., 2013). Of this 430 million metric tons, about 271 are due to meat production alone, and although these numbers are based on European numbers the lamb/mutton sector demonstrates a large portion of our US Sheep market (Gerber et al., 2013). While 55% of these emissions come from enteric fermentation, another 35% comes from producing the feed (Gerber et al., 2013); which demonstrates further reasoning for feeds which require lesser inputs for greater or extended outputs allowing us to reap more from the crop for longer periods of time. Food waste and unusable biomass from agriculture production accounts for a large percentage of global agriculture emissions and luckily enough are often included in animal feeds (Salami et al., 2019). While all animals can benefit from the use of byproducts such as crop residues, ruminants specifically benefit from those byproducts derived from plants otherwise known as plant byproducts which come from the post-harvest utilization of crops into usable food products (Salami et al., 2019)(Johnson, 2009). These byproducts are able to benefit the rumen fermentative process along with utilizing the entire plant material to lessen the environmental load through assisting in the balance and disruption of the excretion of nitrogen and undegradable carbon into the environment which leads to decreased nitrous oxide and methane

emissions (Montes et al., 2013). Using plant byproducts also opens the door for a wider variety of feed ingredients to be included in the diet which also brings more questions about what should and shouldn't be fed commercially to animals entering the production cycle. The future of animal feeds is promising, with the recent passing of the 2011 Food Safety Modernization Act and the particular rulings about the Preventative Controls for Animal Food and others which impact how we feed animals, research into animal feeds has to catch up in order to expand our knowledge about utilizing these environment saving feeds to better determine efficiency and quality outputs.

3.0 Nutritional Requirements and Disorders of Domesticated Sheep

Feeding sheep while not very much different from other ruminants requires special attention to dietary differences and sensitivities which may cause illness or other nutritional deficiencies which may be latent. Their nutritional requirements can be broken down into a few major groups in which include water, protein, energy, vitamins, and minerals.

3.1 Water

Undoubtedly of these, the most important is water which should be given ad libitum to sheep and will be consumed more or less given certain environmental factors and stressors. The typical feeder lamb will consume 3.78 - 5.67 liters of water per 1.81 kgs of dry matter, given an average weight of 27.2 to 49.9 kgs that weight increases for older animals and pregnant ewes. This number is impacted by the DM percentage in the feed consumed with feed that has a lesser DM percentage requiring less water intake due to the amount acquired through consumption. During the winter, the water provided may be lower due to snowfall totals in the area where sheep are present. Wherever provided, it is of paramount importance that the water be clean and safe for consumption; this means that water should be free of fecal matter or other organic materials which may cause disease. Many diseases can be acquired from dirty water consumption and include

diseases such as listeriosis which is caused by *Listeria monocytogenes* which is a part of the normal gut flora; however, poor water conditions can cause the numbers of this bacteria to increase and cause complications such as paralysis and abortion. Dirty water brings other issues, such as pests that may carry parasites like flies and mosquitoes that prefer to breed and proliferate in and around bodies of water. Water is the most important ingredient for proper sheep nutrition, for it's deficiencies can lead to death via dehydration, therefore maintaining access to clean and safe drinking water is of extreme importance.

3.2 Protein

Protein feeds are an important part of the sheep nutrition equation as they provide the foundation for building and repairing muscle tissue within the animal. This is particularly important for greater carcass yields and overall production of the animal. The NRC recommends that at least 9% of the diet in sheep is dedicated to fulfilling the protein requirement for maintenance while growing sheep need 12% and those which are pregnant or have greater nutritional stresses need up to 15% of their diet allocated to fulfilling the protein requirement. This requirement is fulfilled through a variety of feedstuffs namely leguminous grains such as alfalfa, the diet is often bolstered using supplements such as cottonseed meal, sunflower meal etc. Studies have shown that sheep have the ability to discriminate how much protein supplementation they need when offered two different diets in varying crude protein quantity, they typically chose the feed to meet their maintenance requirement rather than one which would've likely led to overconsumption of proteins (Kyriazakis et al., 1993). The idea of overconsumption is particularly important particularly when discussing the oversupplementation of proteins which can lead to a number of diseases. Pyelonephritis, ulcerative vulvovaginitis, and posthitis are all diseases that can be acquired from excess protein consumption due to the greater urine secretion which comes from

the increased protein consumption leading to more basic urine which irritates the urethral openings and the entire urinary tract (Underwood et al., 2015). While overconsumption can lead to disease, so can the lack of dietary protein, which can make a flock more susceptible to gastrointestinal nematode parasites where research has shown that adequate supplementation and sometimes over-supplementation of quality proteins can lead to the disruption of the proliferation of those parasites (Knox, 2003). The lack of dietary protein can also manifest in a lack of energy which leads to poor gains, increased muscle wasting as the body begins to try and compensate for the lack of protein to build muscle, reduced antibody production, and thus an impaired immune system, along with poor reproductive performance as the body simply can't sustain itself without proteins to build and repair tissues.

Nonetheless, it is unavoidable to supplement protein to meet necessary yields in a commercial environment. To properly supplement protein, we determine the level of crude protein (CP) in the desired feed ingredient. We calculate this by multiplying the nitrogen concentration of the feed and multiplying that by 6.25, which is the universally acceptable conversion factor. The number acquired after completing this equation encompasses both what is known as "true protein," which is derived from plant products such as cottonseed meal, sunflower meal, etc.; as well as the non-protein nitrogen, which, unlike its true protein counterparts, comes from sources that don't contain amino acids such as urea or biuret. It isn't necessary to include these NPN sources in significant amounts, in fact, they should only be used in conjunction with a true protein source to prevent NPN poisoning (Dinning et al., 1948).

Non-protein nitrogen sources are converted into protein through a method in which the microbes present in the rumen produce ammonia which binds to the NPN sources and produces protein as the end product. The excess NPN is detoxified in the liver and excreted in the urine as

a waste product. This is an effective method of producing proteins in animals, and the products which provide NPN such as urea are often cheaper ingredients to source; in fact a Brazilian study on the economic impact of the inclusion of urea in diets given to feedlot lambs suggests that feedlots who include urea in their diets as a major source of protein have a better economic return than those who do not (Rozanski et al., 2019). Given this, it has become a cost-saving remedy for large operations and small farmers alike, increasing its prevalence on farms. However, we should be using these NPN sources with caution as we run the risk of NPN toxicity and hyperammonemia. This occurs when we have an excess production of ammonia, leading to increased work for the liver and associated detoxifying organs, which eventually begin releasing ammonia into the bloodstream. This disease leads to an acute onset of abdominal pain, respiratory distress, and eventually fatality (Thompson, 2019).

Once the CP is determined of the desired feed ingredient, the next determining factor of its effectiveness in the diet is whether it is either a rumen degradable protein (RDP) or rumen undegradable protein (RUP). Just as it is presented, RDP encompasses proteins that can be metabolized in the rumen to generate amino acids along with ammonia which assist in the aforementioned metabolism of NPN sources and stimulate further rumen fermentation. The RUP is also aptly known as “escape protein” as it bypasses the rumen and undergoes enzymatic hydrolyzation in the small intestines to extract the amino acids present and the rest is excreted as waste products. This method is effective in acquiring typically 80% of the amino acids present and is often prevalent in animal-based proteins such as fish meal (Putri et al., 2021). The RDP:RUP ratio depends on the animal and their metabolic load with those in greater metabolic distress such as pregnant animals require a greater RUP content to maintain basal metabolism while the goal for the typical production animal is to utilize as little RUP as possible to maintain animal health,

growth, and reproduction (Putri et al., 2021). Several studies agree with this notion, as it is even stated in research that an increased RDP improves overall metabolism and metabolic activity (Paula et al., 2016; Brooks et al., 2012).

These categories allow us to classify types of proteins given to sheep, however, there are specific goals in feeding proteins to sheep which include providing necessary amino acids, hormone synthesis, muscle development, nitrogen recycling, milk/wool production, and an alternate energy source for diets lacking in necessary carbohydrates.

There are about 20 amino acids present in sheep, with 10 of them being noted as essential, and all of which can be required from NPN sources which makes ruminants different from non-ruminants which require their proteins to come only in the readily digestible form since they can't form it themselves. Of these 10 which are noted as essential, 2 are considered to be the most limiting and serve as the foundation for the other amino acids which all serve the purpose of maintaining basal metabolism, building muscle, and providing aid to various physiologic processes which include methionine and lysine.

Methionine is essential for sheep and has even gained the title of being the most limiting amino acid in growing lambs when using predominately NPN sources (Wei et al., 2017). It is also one of the major factors in wool production and has been shown to increase nitrogen retention as well through several metabolism studies which demonstrated this through the limitation of other amino acids (Fennessy et al., 1978, Reis et al., 1964, Bird et al., 1972). Methionine's role in nitrogen retention is valuable as this signals its ability to build muscle tissues and excrete less nitrogen in waste products which adds to the productivity of the animal (Hristov et al., 2019). It also serves as a methyl group donor working particularly with cysteine to increase intakes and yields, making it essential for biosynthesis for many other AAs, DNA synthesis, and the production

of neurotransmitters (Löest et al., 1999). Without these basic physiologic processes such as DNA synthesis, sheep could be more susceptible to cancer and various other mutations destroying their production value and rendering them ill. The presence of methionine in the diet although necessary proves to demonstrate both benefits and detriments when deficient as its presence is linked to oxidative stress metabolism (Liu et al. 2020, Maddineni et al., 2013). A study completed by the Chinese Academy of Agricultural Sciences on lambs given a methionine-restrictive diet followed by subsequent refeeding demonstrated that while the body adapts to a deficiency in methionine evidenced by an increase in glutathione and superoxide dismutase, which in turn leads to the extermination of free radicals, the body has a difficult time adjusting when that deficiency is met and while the redox state is met through accommodations made within the animal; the resulting impact is a deficiency in the hepatic metabolism which can expose the liver to a variety of toxicities (Liu et al., 2020). While this proves to be a detriment the positive outcome of the redox status is similar in another study that demonstrated the ability to increase the lifespan and decrease the prevalence of age-related illness in rats given a methionine-restricted diet which again relied on the prevalence of greater glutathione levels in the blood to explain redox status in the rats (Maddineni et al., 2013).

Lysine is the second limiting amino acid in sheep and is important in collagen production which in turn leads to muscle growth or, given a deficiency, lack thereof. A study published in the *Journal of Animal Science* using rumen-protected lysine and methionine proved an increase of about 33% in N retention in lambs compared to those not given lysine or methionine can then positively impact nitrogen balance to maintain muscle growth and yields (Oke et al., 1986). While there is a paucity of new information regarding the impacts of lysine on sheep physiology and growth, there are several studies aside from the aforementioned which demonstrated little to no

impact on wethers given a high-energy diet with lysine supplementation which gained marginally over the period of 15 days (Harbers et al., 1961). However, this does not negate its importance and merits further study.

3.3 Energy

Energy feeds offer sheep valuable resources in calories, allowing them to complete everyday tasks from walking to giving birth. These feeds are typically comprised of cereal grains such as corn and barley and are valuable portions of the nutritional equation and can provide a challenge for those livestock that are on pasture, as forages may not always be able to meet the necessary energy requirements. Sheep on pasture often experience greater energy expenditure due to increased walking distances and grazing range, often increasing their energy requirements compared to those in more concentrated settings such as a feedlot (Osuji, 1974). This decreased energy expenditure was referred to by early researchers as the cost of muscular activity, which includes standing, drinking, and scratching; and when measured in a respiration chamber the energy loss is negligible this number however dramatically increases given a wider range to graze (Blaxter, 1967).

Energy is often measured in megajoules (MJ) in the realm of ruminant nutrition but reflects the amount of calories in human equivalent terms given to the animal, which is measured in a few different categories based on how the energy is utilized. The overall amount of energy consumed is known as gross energy (GE) and describes the total amount of MJ/kg DM ingested by the animal. GE tells us what we need to give the animal to meet the basic energy requirements prior to being digested, once digested we can then calculate digestible energy (DE) which is the amount of energy actually absorbed by the animal. The rest of what isn't absorbed is passed into the feces and the lower this number is the better, since it allows the producer to be more efficient in the way an

animal as fed since the less waste produced, the more profitable we can be in terms of gains to livestock. However, feces doesn't represent the entirety of waste as we can lose energy in urine and the gaseous form of methane, what is then left over is known as metabolisable energy (ME) which is the amount available for growth; it's not necessarily the amount utilized for growth as some will be lost as heat during the growth process. The energy not lost as heat is known as the net energy (NE) which is the energy used for overall growth and production, this is the number researchers and producers value as it demonstrates the energy necessary for improved animal yields.

We lose energy in a number of different ways, which don't always have to be sequelae due to previous disease yet common methods include pregnancy and livestock used for milk production which have high metabolic loads and can be more susceptible to poor growth due to increased stresses on the body. When feeding ruminants particularly sheep whom may be pregnant there is a significant increase in the heat produced by the dam of an average of 85 kcal/kg during pregnancy with the NE requirements during the last 100 days decreasing from an average of 16.1 kcal/kg to 12.1 kcal/kg to 85 days prior to conception (Rattray et al., 1974). Feeding sheep who are producing milk while typically not for production purposes show no difference in energy loss based on various diets; although there is still energy lost that should be accounted for based on the increased strain on the body to produce milk (Gallo et al., 2019).

While cereal grains is a significant portion of how we provide energy to livestock, there are several other feed types in which we are able to find a similar result in gain and productivity. One of those methods is by feeding oils which are typically plant-based and proves to be a great alternative for meeting the energy requirements of sheep. A study done on sheep used for meat production utilizing various plant-based oils proves that specifically canola oil and rice bran oil

provided a lesser cost to producers while not compromising average daily gain (ADG) and meeting the energy requirements of sheep (Le et al., 2018). Understanding this may assist in us using a greater number of feed byproducts for production purposes which accomplish a similar mission, another study demonstrated this by utilizing residual cooking oils. While the residual frying oils did decrease intake, they didn't impact the digestibility of feedstuffs when used as an additive (Peixoto et al., 2016).

Other alternative feedstuffs to fulfill the energy requirement of sheep may not be as common due to the extra processes required in order to make them viable feedstuffs. Various methods of processing feedstuffs include what are known as wet and dry methods and are meant to enhance the nutritional profile or even palatability of the feedstuff utilized to maximize production. Although most of these methods which include processes such as dry rolling, grinding, pelletization etc. aren't used to enhance the energy/fat quality of the feed, they can increase digestibility which can lead to positive feed effects.

There aren't many known nutritional diseases that are sequelae to energy deficiency although the major energy-related cause of illness seems to be in pregnant animals which is pregnancy toxemia. This disease occurs in animals that are typically overweight or are expecting twins and typically occurs in late gestation (Ji et al., 2023). A negative energy balance is the major cause for this disease as a pregnant ewe that is improperly fed may become overweight and due to a lack of proper nutrients begin metabolizing fat storage which disrupts the metabolic process and builds ketone bodies leading to toxemia (Ji et al., 2023). This toxemia can eventually lead to death for both the dam and fetus; which can only be corrected by proper diet and exercise to meet the needs of a pregnant animal.

3.4 Vitamins and Minerals

The inclusion or lack thereof of vitamins and minerals can lead to a significant number of deficiencies and diseases within the ruminant animal, particularly the sheep. Vitamins are typically more easily attainable in the diet as they are found naturally in plant material consumed by the animal and are often required daily. A significant vitamin required by sheep is vitamin E which is found in many forages and is required for muscle growth and function, a deficiency of which has been proven to cause muscular dystrophy in developing lambs (Culik et al., 1951). The same study completed in 1951, demonstrated the lambs susceptibility to pneumonia and paralysis given a diet absent of vitamin E, reversible only long term through the incorporation of vitamin E back into the diet (Culik, 1951). Vitamin A is additionally an important factor in a sheeps diet as it bolsters the immune system of animals universally and should also be included daily in the diet. A study done on commercial wethers given high protein diets with reduced mineral levels demonstrated an increased prevalence of polyuria in sheep which consumed diets which were absent of vitamin A (Webb et al., 1968). This increase in urinary waste leads to a decrease in ME along with an increased risk of dehydration in the animal further placing that animal in jeopardy. Vitamin D which is provided by the sun's ultraviolet rays are also valuable in maintaining sheep metabolism, while that may not be something we supplement. Studies done at higher altitudes demonstrate that we may not even have a need for vitamin D supplements as the elevation doesn't impact levels of vitamin D in the milk or blood (Kohler et al., 2013).

While the presence of the aforementioned vitamins is generally universal, the presence of minerals isn't as many areas of the country may suffer from a variety of mineral deficiencies which can lead to disease in livestock. One of the more clear cases of this is selenium which is variable here on the front range of Colorado, but can have detrimental impacts if not supplemented

properly. Selenium works in tandem with vitamin E to reduce free radicals in the body and assist in redox reactions; it's even been cited that they can work to decrease heat stress on merino sheep exposed to high heat in which they measured excreted Se and Vitamin E to determine their impacts on the organs of the animal (Underwood, 1977, Alhidary et al., 2015).

4.0 The Role of Hempseed Meal in the Global Agriculture Landscape

Hemp production began in China and was brought to Europe in the early 16th century where it was used for both fiber and traditional chinese medicines (Dempsey, 1975). Since making it's mark in Europe it was brought to the Americas in 1945 by the Puritans who used it for fiber purposes as well; from there, in 1775 it became a predominant crop across several states in what would become the United States and up until the 1860s the US Navy demanded a greater production of hemp due to it's value as a fiber source (USDA, n.d). Eventually, likely due to it's boom in production the US enacted the Marijuana Tax Act of 1937 which made hemp illegal to grow without a license which eventually contributed to the demise of it's commercial boom.

Today all sectors of the hemp industry account for more than \$824 million of our nation's agricultural landscape according to the USDA with the sector for industrial hemp being the largest (USDA, 2021). However, in the livestock industry our main sector for consumable product is the hempseed sector which is valued at over \$41.5 million with a smaller number of producers yet a steady yield. The median price for seed totals \$219 valued at \$11.20 per pound with the nation producing and consuming more than 1.68 million pounds of hempseed each year.

With all of the new developments in hemp and the agriculture industry, the US government extended broader opportunities to research hemp in the 2014 Farm Bill where states and universities were allowed to complete research projects based on improving our knowledge of hemp and how we can utilize it to progress the industry. Since then, countless pieces of information

has emerged about its use medicinally, however, not as much has been learned about how we can sustainably use it as a livestock feed source.

5.0 Producing Hempseed as a Consumable

Hempseed meal is produced through a process in which the seed is dehulled and the oil is extracted as aforementioned through a process known as cold pressing (Shurlock, n.d). Dehulling is the process in which a hull is removed from the seed to decrease the fiber content of a seed and also increases both the protein content, palatability, and digestion of the seed (Singh, 1995). The dehulling process is necessary for hemp since it is thought to be a trypsin inhibitor which requires heat treatment noted to be the most effective way of improving protein digestibility due to thermal processing having the ability to inactivate the thermolabile proteins particularly those which are trypsin inhibitors (Aviles et al., 2018).

The heat treatment of legumins particularly soybeans isn't new to the animal feed industry and is required to be boiled at 100 °C for a period of 9 min, cooking for a 7 to 30 min period, a microwaving period of 3 min and autoclaving session for 15 min at 121 °C (Aviles et al., 2018). While there is a paucity of knowledge as to the particular temperature required to mitigate some of the trypsin inhibition properties of hemp we can postulate that just as soybeans as well as other legumins, we may be losing some nutrient value past a certain thermal gradient. Previous studies demonstrate that similar to soybeans heat treatment past 80 °C significantly reduced solubility which will likely affect nutrient composition (Raikos et al., 2015). In the same study determining heat treatment of hempseed meal, we can determine that not only was solubility impacted, there were large insoluble protein aggregates formed after 80 °C which impacts digestibility likely making the feed less economic (Raikos et al., 2015).

6.0 Feeding Hempseed to Ruminants

When feeding hempseed meal as a protein source, we focus on two particular sources of proteins that represents the majority of it's protein output which include edestin and albumin. Edestin is the first and most common protein, making up between 60 and 80 percent of the total protein content. Albumin makes up the rest. While the two work together to produce essential protein from hempseed, there are some notable differences between them, and each has its own advantages and disadvantages. For instance, the globulin edestin dissolves and absorbs more slowly than its albumin cousin due to its lesser solubility. Blood contains the protein albumin, which among other things aids in transport and the control of osmotic pressure. Edestin and other globulins aid in the prevention of infections and the preservation of liver health. Greater or lesser levels indicate infection or disease, which can be lethal to animals, although both are beneficial in the diet when present in typical amounts. Edestin is a seed storage protein that is also referred to as a legumin protein, implying that it is present in legumes as a major storage protein (Malomo et al., 2015). Edestin is found in the muscle and cells of sheep, is an essential component of the digestion of food. In addition to its use in promoting weight gain in sheep, edestin is also used to increase milk yield and muscle mass in cattle (Maingi et al. 700). Research has found that greater levels of edestin in the meat of sheep results in an increased nutrient absorption in the animals that consume the meat (Kang et al. 145). Although the molecular structure and function of edestin is still being studied, it is known that edestin plays an important role in the breakdown of carbohydrates and proteins, helping to make them easier to digest (Palomino et al. 233). It's more easily digestible since it is a globular protein rather than a fibrous one. While globular proteins are coiled and densely packed, making them more easily digestible, fibrous proteins are frequently long polypeptide chains that are insoluble. Several patents for employing edestin as a blood plasma

substitute have recently been developed, citing its ability to biosynthesize hormones and antibodies in particular. Edestin is remarkably similar to blood plasma (Edestin, 2005). According to Stewart et al. supplementing diets with edestin can increase gains in weaned lambs by up to 25% compared to a non-treated group (Stewart et al. 257). It helps to improve wool quality as well, by helping to increase the number of staple-length fibers produced (Stewart et al. 259). In addition, edestin also helps influence the immune system of the animal consuming it (Wei et al. 121). Aspartic and glutamic acids, two essential amino acids, are also abundant in edestin (Wang et al., 2019). As our understanding of edestin and its purposes increases, the health benefits to animals consuming its presence in the form of sheep meat will allow consumers to make better informed nutritional decisions.

Albumin is a water-soluble protein that is used as a transport protein. It's easily digested and aids in hormone and medication transfer as well as osmotic pressure regulation and has the ability to attach to hydrophobic molecules, allowing it to transport nutrients from a lipid surface to a target cell for absorption. Albumin contains high levels of essential amino acids such as methionine and cysteine which contributes to it being considered a high quality protein source especially in protein-deficient diets. While not apart of this study, the inclusion of albumin likely makes hempseed a viable product for sheep with difficulty digesting some proteins in certain forages. It's included at significant amounts in other commercially available products such as blood meal, fish meal, and dried distillers grains. Among other crops that are strong in protein, such as soya protein, hemp possesses one of the highest seed sources albumins, which boosts the overall digestion of hempseed (Wang et al., 2019).

Hempseed contains all of the necessary essential amino acids, and its amino acid composition is comparable to that of other high-quality proteins, making it very valuable for our

meat-producing animals. Hempseed meal contains a high percentage of aspartic acid, glutamic acid, and arginine, with aspartic acid and glutamic acid accounting for the majority of the whole plant protein (Wang et al., 2019). Although only arginine is a necessary amino acid, the other amino acids help the animal maintain its general health by preventing major weight loss, dietary changes, and overall health. When compared to other similar feedstuffs, we can discover arginine in large amounts, with roughly 12 percent compared to the average of 7% in wheat, soy, and rapeseed (Wang et al., 2019). However, the amino acid profile of hempseed varies depending on how it is processed; some of the highest levels of amino acids can be found in our hemp protein meal or whole hempseed (Wang et al., 2019).

Glutamic acid is one of the more abundant free amino acids in ruminants, particularly in sheep and goats, it is found in milk products produced by these small ruminants as well as cows. While it can be found in the body and doesn't need to be supplemented significantly, it is a significant producer of nitrogen in its amide form of glutamine which acts as a nitrogen transporter to maintain nitrogen balance within our livestock species. It is also a free amino acid which means it is more readily available in terms of metabolism and feedstuffs with greater numbers of FAAs are more bioavailable in protein synthesis. FAAs also are particularly important when it comes to fetal development and the ability to produce growing young due to their ability to oxidize and be converted into glucose through gluconeogenesis without compromising the viability to protein synthesis of other feed ingredients. While the rates of free amino acids in the rumen are low quite often, it doesn't necessarily mean they are non-existent, however, glutamic acid is found in our bloodstream as glutamate is a significant neurotransmitter and just as its counterpart GABA both are affected by a glutamic acid precursor. Glutamate is essential and its deficiency in both animals and humans can lead to anemia. Its main function in sheep however is to facilitate the transfer of

ammonia between tissue for nitrogen exchange which is essential in protein metabolism. This will then have an effect on the urea cycle which relies heavily on the inclusion of glutaminase for the elimination of ammonia from the body which is the sole purpose of the urea cycle (Chaudry, 2002).

Aspartic acid is the second most abundant in hempseed meal and also extremely valuable for the reproduction and birth of a lamb. Aspartic acid is one of the 20 amino acids that make up the proteins in sheep and other animals. It's a non-essential amino acid, meaning that it can be produced by the body from other amino acids and does not have to be obtained directly from the diet. It is also a negatively charged amino acid that plays a role in pH buffering in the blood through assisting in maintaining the acid-base balance. In our research, the more important role is in the metabolic processes which include the Krebs cycle as a main pathway for producing energy throughout the body. In the urea cycle which as aforementioned is valuable for nitrogen metabolism it assists in the removal of excess nitrogen from the body. While it is found in many other proteins fed to small ruminants particularly sheep, in hemp it plays an important role as a catalytic residue which are specific amino acids that play a vital role in catalyzing chemical reactions. They're found on the active sites of enzymes and form interactions with the substrate molecule to begin the reaction with a reduced activation energy of the reaction which increases it's likelihood of being successful. While there are particular types of catalytic residues their particular functions aren't imperative to this study as we did not measure the microbiological components of hempseed being fed to the sheep in this particular study. However, catalytic residues play an important role in the function and performance of other nutritional components such as zinc, iron, copper, and other metals. The requirements for aspartic acid in sheep vary, but is greater in lambs who rely a little more on aspartic acid for normal growth and development. The inclusion of aspartic acid is a vital amino acid for protein metabolism in sheep and it's inclusion

at a significant rate in hempseed is vital to the overall function of hempseed as a valuable protein feed alternative.

Phytic acid is the common name for inositol hexaphosphate (IP6) and is extremely pertinent to this study and as we continue to look for feed alternatives particularly in seeds as it is present in a wide variety of seeds as a mineral binding agent. Often seen as a prominent anti-nutritional factor in feeds containing seeds, recent studies have discussed the mitigation of negative effects through various processing techniques to reap positive environmental impacts. . In the realm of ruminant nutrition, we have to focus on phytic acid as an additive as it's a phosphorus-containing compound which means it can also bind to minerals and impact their availability to be absorbed within the digestive tract. Our greatest concern in the addition of phytic acid is in the metabolization of total P, Ca, Fe, Zn, and Na content which contributes to it's categorization as an anti-nutritional factor (Bala et al. 2023). To combat this we have begun to use phytase in animal feeds to mitigate the impacts of phytic acid and enhance digestion, particularly valuable in monogastric animals and poultry who are much less likely to digest phytic acid-containing compounds as easily as our ruminant species (Vohra et al. 1965). Studies completed in poultry species saw this introduction of phytase and inorganic phosphorus sources into the diets of animals being fed diets high in phytic acid as a necessity for growth and the maintenance of essential nutrient factors particularly phosphorus (Selle et al., 2000).

It is not clear why these mechanisms of disruption to the absorption and digestion of other nutrients occurs, however, a recent study in pigs and poultry offers up a few theories as to why the pathways are disrupted. The study demonstrates when we have protein-phytase complexes used to measure the interaction between the two molecules that are then measured at above isoelectric and below isoelectric points, these are the points at which the pH has no charge essentially operating

as low and high pH's to mimic a more acidic and basic environment (Selle et al., 2000). When below isoelectric the phytate binds directly to proteins and prevents enzyme breakdown such as that of digestive enzymes like pepsin. When above isoelectric we still get the same protein binding but with more complex structures still preventing breakdown by those digestive enzymes. Another theory discussed is that due to phytic acid increasing mucin production within the body, we begin to see our amino acids be carried away undigested as mucin isn't processed in the gut (Selle et al., 2012).

Regardless of the mechanism the impacts of phytate greatly impact not only gut health, but the environmental and economic impacts felt by over-supplementation of phytic acid containing compounds. When we use phytase in animal feeds we release bound phosphorus through hydrolyzing phytase which reduces the phosphorus excretion in the manure (Selle et al., 2000). This nutritionally functional and sustainable practice of including phytase in animal feeds reduces the need for inorganic phosphorus which also decreases our feed costs making this practice economically viable for farmers as well (Cowieson et al., 2011). While the industry continues to acknowledge the perspective of phytate and phytic acid as an ANF, there are new perspectives on the inclusion of phytic acid as a nutritional benefit with antioxidant properties. A recent study suggests that phytic acid defends against oxidative stress and lipid peroxidation which maintains and modulates gut health for growth (Gao et al., 2007). However, studies such as these are limited and there remains a paucity of evidence on beneficial roles of phytate supplementation in animal feeds.

Phytic acid remains a prominent ANF in animal feeds, which presents a challenge to the use of HSM and other feeds containing seeds. The approach of this author is to view it as an ANF and find ways to combat the mineral degradation effects.

Pepsin inhibition is another factor we are concerned with as leguminous feeds including HSM tend to contain protease inhibitors. These protease inhibitors are beneficial biopesticides which are plants that function to decrease pests through their production of elements such as cysteine and serine which play pivotal roles in protease inhibition in response to phytopathogen attack (Rodriquez-Sifuentes et al., 2020). While this role in combatting pest and even potentially potent agrochemicals is valuable, it serves as a challenging factor to feeding ruminants. Similar to some of the proposed aforementioned theories surrounding phytic acid, pepsin inhibitors simply bind to the site of action and prevent pepsin from completing its mechanism of action (Ryan et al., 1990).

An anti-nutritional factor (ANF) is simply stated are properties which are naturally occurring in plants and make it difficult for the animal to unlock the full potential of other minerals given to supplement the diet. Typically these ANFs use legumes to complete their binding process to minerals such as Calcium, Zinc, Iron etc., typically those which are tri or divalent which suggests that they are more complicated to break down metabolically which leads to deficiencies and impacts overall gains and lead to inadequate intakes (Ravindran et al., 2007)(Cheeke, 1989). Arguably even more detrimental than their ability to bind and lock minerals resulting in inadequate intakes is their ability to occasionally contain enzyme inhibitors which disrupt protein and carbohydrate breakdown, which we understand metabolically can lead to overall poor health and inadequate gain (Liener, 1994). The aforementioned concept is present in soybeans which we have worked to improve the delivery through further feed processing, as discussed in that portion of the paper. To mitigate the impacts of ANFs impacting feed quality and intake, we can use some of the same feed processing methods as aforementioned, particularly chemical processing via heating, fermentation, and the addition of acids/alkalis to reduce the concentration of the negative impacts

of ANFs in feedstuffs which improves quality (Fenwick et al., 1991). Another additive proven to positively impact the quality of feedstuffs through mitigating ANFs is the addition of enzymes which assist in breaking down ANFs to increase their availability of nutrients already previously bound. The best example of this is the addition of phytase to the diet of feeds with significant phytic acid overload to improve the availability of Ca, Na, and P (Ravindran et al., 2007). Another step taken by plant geneticists is to even modify the plant varieties to contain lesser levels of ANFs to reduce the presence further in feed processing which can prove to be a long-term strategy (Jones et al., 1999).

6.1 Can Hempseed Meal Combat Common Metabolic Disease Patterns?

The short answer is that prior to further research we are unable to definitely determine if hempseed meal can alter the course of disease or reduce negative impacts on animals with an increased metabolic load such as given a parasitic infection.

CHAPTER 2: HEMPSEED MEAL AS AN EFFECTIVE PROTEIN SUPPLEMENT FOR FINISHING WETHERS

1.0 Introduction

As our global agriculture landscape continues to change, it has become more important than ever to find sustainable alternatives to feed livestock. Hempseed and its derivatives may provide alternative sources of nutrients for inclusion in livestock diets, however, due to the paucity of research on hemp and its byproducts, there is no authorization of the inclusion of these products in food animal diets. We hypothesized that the digestibility and use of hempseed meal would be similar to other livestock protein sources. As a crucial factor in determining the value of HSM as a feedstuff we looked at the overall digestibility of HSM by examining the DMI along with the ADF and NDF as factors for healthy rumen function and fill. We examined the role of nitrogen and phosphorus as key factors in growth, reproduction, and metabolism along with their potential environmental implications when excreted. While previous studies have aimed to demonstrate the use of HSM as protein supplement for growth, there remains a paucity of research regarding the metabolism and digestibility of nutrients included in the diet. This study aims to close the gap by utilizing feedlot wethers to examine the effects of the inclusion of various levels of HSM and their impact on DMI and fiber, nitrogen, and phosphorus utilization and retention.

2.0 Materials and Methods

This study was conducted between June 2020 and July 2021 at the Colorado State University Agricultural Research Development and Education Center (ARDEC) in Fort Collins, Colorado. All methods were approved through the Institutional Animal Care and Use Committee (IACUC) IACUC# - 1485.

The experiment was divided into 2 balance trials separated by year and by treatment using 40 white-faced wethers with an average starting weight of 43.47 kg in year 1 and an average starting weight of 91.01 kg in year 2. The sheep were housed in individual stalls in an open-air covered facility at ARDEC prior to their inclusion in the trial. Each stall was outfitted with a watering device and feeding bucket, which would house individual diets for each sheep based on their treatment color. They did have the ability to view and interact with their neighbor through metal sheep fencing and their flooring was comprised of wood shavings.

Sheep were gathered once a week to collect weights during the feeding period and blood was also collected during these days to collect blood chemistry results on each of the participants. Sheep were then returned to their individual pens until their designated feeding period for the balance trial. Each sheep was fed the diet for 90 days at an inclusion of 0,5,10,15, or 20% of the diet.

2.1 Balance Trial

Balance studies were completed on all of the participants starting on Sunday of each week where sheep were placed into individual metabolism crates. These crates were raised and outfitted with a feeding trough and watering device which the sheep would have to push with their nose to activate. These watering devices, however, were checked twice a day to ensure no sheep succumbed to dehydration during the study and had access to water *ad-libitum*. The crates were outfitted with slatted floors in which we placed a rubber mat on top to increase welfare while allowing room for urine to escape through the floors for collection.

We collected ORTS, feces, and urine from each sheep each day to be weighed in the morning to allow for exact feeding times each day in 5-minute increments to keep our collection data as close to 24 hours as possible. ORTS which are the amount of feed leftover each day or by

some definitions, what the animal has refused to eat each day was collected to determine dry matter intake and digestibility. The feces were collected to determine the digestibility of various vitamins, minerals, and hemp inclusion in the diet based on treatment. Urine was collected to do the same, however, we measured urine quality to determine urinary urea nitrogen and overall urea nitrogen levels to further determine hempseed meal's viability as a high source of protein. Post-collection all of the samples were frozen up until the date of analysis to protect the individual biological data. The samples were then sent to Dairy One for individual feed analysis. These results were then converted to grams per day to determine the actual intake on a per-day basis of the individual.

Each trial included 4-5 individuals representative of our 5 treatment groups of various inclusion of hemp in the diet. This study was considered blind and treatments were separated by color rather than hemp seed meal inclusion rate even after statistical analysis. The trials began on Wednesday with an adjustment period of 2 days beginning on Monday where sheep were placed into metabolism crates with the diet specific to their treatment and adjusted to the new indoor setting in the CSU Metabolism Barn at ARDEC. They were the only participants in this area and contrary to the environment prior to beginning the feeding trial were not allowed interaction with their neighbor due to crate placement. They did, however, have exposure to light and were allowed to maintain typical circadian cycles. The trials concluded on Sunday. On Monday, the second set of sheep were then placed in the metabolism crates and the first set were placed back into their individual stalls.

2.2 Statistical Analysis

Feces and ORTS samples were analyzed by Dairy One forage services. They also analyzed the urine samples which yielded urea nitrogen and urinary urea nitrogen values for each individual involved in the trial.

Sample data were analyzed using R-Studio and an ANOVA was run on the data. In this study, we ran a Levene's Test to yield an F-value as well as a one-way ANOVA. During the ANOVA, we adjusted using the Tukey method for comparing a value with 5 estimates and a significance level of .05 was used to determine significance while anything below .1 is considered a trend towards significance. The analysis was done by year and then by treatment to determine the impact of various hemp inclusion levels in the diet.

3.0 Results

The results are reflective of a 5-day total collection of feces and urine with the exception of 4 occurrences which are reflective of a 3-day feeding period due to illness resulting in early termination of the subjects time in the study. These occurrences were accounted for in the statistical analysis by retaining and accounting for the data up until the termination of the feeding period while in the metabolism crates used during the balance trial thus ending their participation in the project. The wethers which make up these occurrences were not all from a particular group and include those being feed with 0%,5%,10%,15% or 20% inclusion of HSM in the ration.

The diets utilized in this study were designed to be near isonitrogenous and near isocaloric, yet yielded some differences upon feed analysis post diet formulation (Table 1). HSM was included to represent a linear increase of dietary hemp with the alternate protein source being soybean meal. Soybean meal inclusion represented a linear decrease to ensure that the diets were isonitrogenous with a relatively equal exchange of HSM and soybean meal while cottonseed meal remained the same across all diets. Varying ground corn inclusion was used to make the diets near isocaloric as an energy source. Dry matter intake (DMI), DM digestibility, neutral detergent fiber (NDF), acid detergent fiber (ADF), along with nitrogen (N) and phosphorus (P) intake and excretion are summarized in Table 2.

Throughout the 2 year study period, the wethers across treatments gained and consumed feeds based on the diet provided. On 5 instances subjects were removed from the study due to declining health mostly due to lack of feed consumption to meet maintenance requirements in the post-transitional period into metabolism crates for the collection period. In one instance, one of the wethers in the 15% HSM ration developed urinary calculi early on in their lifecycle on day 32 of the feeding trial as discovered on necropsy at the Colorado State University- Veterinary Teaching Hospital after being humanely euthanized using a captive bolt. Animal removals were evenly spread across all treatments with 1 animal per treatment being removed from the study due to the aforementioned declining health statuses prior to the termination of the balance trial.

Intake across treatments increased with HSM inclusion up to 10% and 15%, the lowest intake which was demonstrated at 0% HSM inclusion (1130.7 g/d). The highest intake being seen at 10% inclusion (1596.2 g/d). There was no statistical significance amongst these groups ($P = 0.445$). Increased hemp inclusion showed a trend of decreasing digestibility, from the highest digestibility being 81.1% for the control, and 74.9% at a 20% HSM inclusion rate ($P = 0.061$).

NDF and ADF values varied between treatments with the highest NDF for the 5% HSM (50.7%) and the highest ADF for the 10% inclusion rate (50.4%). There were no trends seen in the NDF data, however, the ADF demonstrated a trend although this data and the differences were not statistically significant (NDF: $P = 0.229$, ADF: $P = 0.070$).

N intake increased with HSM supplementation at a relatively steady rate up until 15% (44.5 g/d) where we see the intake peak and drop off at a 20% inclusion rate. While the output of both urine and fecal N followed a similar trend, neither of these parameters demonstrated significance ($P > 0.1$). N digestibility presented a non-statistically significant increase at 5% and 10% HSM

(57.6% and 58.5%, respectively) when compared to the control (34.3%), these changes while demonstrating these trends did not prove to be statistically significant ($P = 0.264$).

Phosphorus utilization varied across the treatments particularly with intake and excretion. We see the highest intake and digestibility at 15% inclusion. While the intake was not significant between the treatments ($P = 0.296$), the digestibility demonstrated significance ($P = 0.047$). Retained P was $P = .050$ and only became positive at a 10% and 15% inclusion, although negative these results did not yield statistical significance after ad-hoc analysis was run.

3.1 Weight Gain and Performance

The beginning weight between groups varied minimally from an average of 55.47 kg in the 20% treatment group to 60.91 kg in the 15% treatment group; although these values varied they demonstrated no statistical significance ($P = 0.91$). The end weights prior to processing for these groups also varied from 75.52 kg in the 20% treatment group to 84.11 kg in the 5% treatment group, as with the beginning weights, the end weights demonstrated no statistical significance ($P = 0.86$). The intercept which represents the initial weights was similar across treatments with the highest seen at 61.07 kg in the 15% treatment group and the lowest at 54.86 kg in the 20% treatment group, representing the lack of statistical significance that we see in the aforementioned data regarding initial weights ($P = 0.89$). The slope, representing the daily weight gain, ranged from 0.18 kg/day on average in the 15% treatment group to 0.2859 kg/day in the 5% treatment group, with no statistically significant difference represented between groups ($P = 0.63$).

3.2 Blood Chemistry

Blood chemistry was monitored to assess overall organ health when introducing HSM. Across the different treatment groups there were no statistically significant differences observed in any of the blood chemistry parameters. The blood pH remained narrow and consistent with a

range of 7.36 to 7.42, which is a normal range and not indicative of a state of metabolic acidosis/alkalosis. There was no difference between treatments ($P = 0.83$). No difference was observed in respiratory gas exchange vis-à-vis the $p\text{CO}_2$ and $p\text{O}_2$ values ($P = 0.56$ and $P = 0.55$ respectively).

The electrolyte levels which include Na, K, and ionized Ca were consistent between treatment groups ($P = 0.91$, 0.91 , and 0.61 , respectively). Na ranged from 145.8 mM to 146.7 mM and represented no statistical significance ($P = 0.91$). The K ranged from 4.22 mM to 4.42 mM and ionized Ca ranged from 145.8 mM to 146.7 mM neither represented statistical significance representing ($P = 0.91$) and ($P = 0.61$) respectively. Glucose levels remained consistent throughout the study ranging from 68.4 to 72.3 mg/dL and did not differ between treatments ($P = 0.81$). Mean hematocrit ($P = 0.76$) and body temperature ($P = 0.92$) also did not differ between treatments.

4.0 Discussion

The purpose of this study was to evaluate the impact of hempseed meal inclusion on intake, animal performance, and dietary characteristics of HSM inclusion in feedlot wether diets by examining various inclusions of HSM at increments of 5 from 0%-20%.

4.1 Dry Matter Intake and Digestibility

While consumption varied across the treatments the group with a 10% HSM inclusion rate demonstrated the highest intake at 7981.1 g/day with the lowest intake being the control at 5653.7 g/day. The data demonstrates that there was greater intake in groups fed 5%-15% compared to the control and 20% groups, none of these findings were statistically significant ($P=0.445$), suggesting that the inclusion of HSM up to the measured 20% doesn't adversely impact the DMI of sheep fed this diet. The variance in the intake between the control and HSM groups warrants further study into the underlying mechanisms such as palatability and diet efficiency. In terms of the

digestibility, similarly, there was no significance between the treatments ($P=0.061$) with the results demonstrating a steady downward regression in digestibility from a 0% inclusion to a 20% inclusion rate suggesting that the inclusion of HSM played an insignificant yet noticeable factor in the overall DM digestibility. With the results demonstrating no significance, we can assert that the inclusion of HSM in the tested population doesn't compromise the overall feed intake or digestibility.

4.2 Fiber Utilization

The digestible structural components relating to fiber measured using NDF demonstrated no significance between groups ($P = 0.229$) The digestibility of the structural components relating to fiber represented what is expected of a leguminous plant such as an early blooming alfalfa which averages about 40%, a trend seen in the data of HSM inclusion. This indicates an increased energy availability and decreased fill, allowing for a greater DMI only limited by rumen fill. We saw the most highly energy available diet with an inclusion rate of 20% (mean = 40.7%) outside of the control group (mean=38.5%). NDF trends were similar to ADF, demonstrating no significance ($P = 0.070$) of the undigestible portions of our feedstuff. Our least digestible according to the sample, had a HSM inclusion rate of 10% (mean=50.7%) representing values seen in more undigestible feedstuffs such as straw. Given the variability of the data and significance values, we can still assert that HSM can be included without negatively impacting the overall digestibility of plant material/fiber included.

4.3 Nitrogen Utilization

The measured nitrogen utilization included a variety of factors which impacted the overall digestibility. N intake showed no significance amongst the treatments ($P=0.171$) and the fecal output of N remained consistent between the treatments ($P=0.507$). While the fecal excretion was

consistent, the urine output of N varied a bit more, yet provided no significance ($P=0.273$) and the utilization efficiency of N measured by the UUN followed the same trend of no significance ($P=0.273$). In terms of digestibility for N, it's greatest efficacy was seen at 10% while there was a steady increase of digestibility from 0%-10%; none of these findings were statistically significant suggesting again that the inclusion of HSM doesn't adversely impact N absorption in vivo ($P=0.467$). When measuring the nitrogen retention, we discovered a negative balance in the control on average (-15.4 g/day), while the treatments with HSM inclusion had a positive retention rate seen most significantly at the 15% inclusion (7.1 g/day). This can be due to a variety of factors although these differences weren't significant ($P=.264$) including weight loss and stress, while inadequate protein could also be a factor, this again is not represented across the data measured as significant. This indicates a measured benefit based of the data for the inclusion of HSM in improving N retention, protein utilization, and growth in sheep populations.

4.4 Phosphorus Utilization

Phosphorus represents an important mineral in the bodies of humans and the animals we consume for growth. In the measured population, we see no difference in the intake ($P=0.296$) or fecal output ($P=0.750$) of phosphorus; however, there was statistical significance seen in the digestibility of phosphorus ($P=0.047$) with the greatest difference being between the group with 15% HSM inclusion (mean= 58.9%) and the control (mean=33.9%). While this could be due to the absorption and utilization of other minerals in the body, it may suggest that HSM at even a moderate inclusion rate can positively impact the overall digestibility of phosphorus and improve overall mineral balance while reducing waste and environmental impact of excretion. Similarly to N retention, P retention demonstrated a negative value in the control and the 5% inclusion rate, but offered positive values in the 10% and 15% inclusion groups indicating that moderate inclusion

rates can improve phosphorus retention as a consequence of increased digestibility. While these are assumptions, the role of phosphorus in diets with HSM inclusion deserve further investigation.

5.0 Conclusion

The data demonstrates a clear need for further investigation as it pertains to studies regarding the feeding of HSM such as it's utilization on farm and palatability; yet this research lays the foundation for the inclusion of HSM at rates up to 20% and proves that even moderate amounts of HSM inclusion are non-detrimental to the overall health and nutrient utilization in our whether population. Our study demonstrated that there was no limiting statistical significance in the digestibility or metabolism which would hinder it's inclusion in the diet of the tested values and should provoke further thought into the inclusion of HSM in commercial diets.

Table 1. Diet composition based on hempseed meal inclusion.

Treatment	Percentage of diet DM				
	0	5	10	15	20
Cottonseed hulls	15	15	15	15	15
Ground corn	61.39	59.99	58.67	57.29	55.99
Hemp meal	0	5	10	15	20
Soybean meal	14.45	10.85	7.24	3.62	0
Molasses, wet	7	7	7	7	7
Limestone	1.65	1.75	1.83	1.93	2
Dicalcium phosphate	0.5	0.4	0.25	0.15	0
Vitamin/mineral premix	0.01	0.01	0.01	0.01	0.01
Nutrient content, DM basis					
DM%	89.36	89.50	89.63	89.77	89.90
CP%	13.94	13.95	13.95	13.94	13.94
NEm, Mcal/kg	1.89	1.85	1.81	1.77	1.72
NEg, Mcal/kg	1.26	1.22	1.17	1.13	1.09
Ca, g/kg	8.18	8.27	8.18	8.28	8.16
P, g/kg	4.31	4.36	4.31	4.36	4.32

Table 2: Digestibility of diets based on treatment.

Item	Treatment					SEM	P-Value
	0% HSM	5% HSM	10% HSM	15% HSM	20% HSM		
DMI, g/d	1130.7	1521.4	1596.2	1594.8	1299.8	456	.445
DM digestibility, %	81.1	79.7	79.3	75.9	74.9	0.89	.061
NDF	38.5	50.7	43.8	45.1	40.7	1.89	.229
ADF	32.9	45.2	50.4	46.3	38.6	2.17	.070
N							
N intake, g/d	29.0	34.6	37.7	44.5	26.4	2.59	.171
Feces N, g/d	7.1	8.7	8.7	9.3	6.8	0.52	.507
Urine N, g/d	233	99.4	76.1	132	136	23.0	.273
Urine urea-N, g/d	16.6	7.1	9.7	9.5	5.4	1.64	.273
N digestibility, %	34.3	57.6	58.5	53	52	4.48	.467
N retained, g/d	-15.4	2.9	5.9	7.1	5.5		.264
P							
P intake, g/d	6.5	7.7	8.3	9.1	5.8	0.53	.296
Feces P, g/d	4.1	4.6	4.1	3.5	3.4	0.30	.750
P digestibility %	33.9	38.6	51	58.9	38.6	2.99	.047
P retained, g/d	-1.8	-1.4	0.04	2.03	-0.94	0.16	.05

Table 3. Growth performance of wethers fed diets with 0, 5, 10, 15, or 20% of dietary DM as hemp meal (n = 8/treatment).

Item	Treatment					<i>P</i> – value ¹
	0	5	10	15	20	
Beginning weight, kg	58.84	58.38	57.74	60.91	55.47	0.91
End weight, kg	80.68	84.11	82.62	77.57	75.52	0.86
Intercept, kg ¹	58.43	58.38	57.72	61.07	54.86	0.89
Slope, kg/d	0.2443	0.2859	0.2693	0.1853	0.2230	0.63

Table 4. Blood chemistry of wethers fed diets with 0, 5, 10, 15, or 20% of the dietary DM as hemp meal (n = 8/treatment).

Item	Treatment					<i>P</i> – value ¹
	0	5	10	15	20	
pH	7.36	7.42	7.38	7.39	7.41	0.83
pCO ₂ , mmHg	45.22	43.05	44.55	39.49	39.99	0.56
pO ₂ , mmHg	45.00	47.67	48.67	50.86	52.43	0.55
HCO ₃ , mM	25.00	27.27	26.00	23.47	24.84	0.53
Na, mM	145.8	146.3	145.8	146.7	146.0	0.91
K, mM	4.42	4.22	4.35	4.23	4.24	0.91
Ionized Ca, mM	1.37	1.35	1.28	1.33	1.27	0.62
Glucose, mg/dL	68.4	72.3	69.67	68.86	71.57	0.81
Hematocrit, %	32.0	30.5	30.5	30.14	30.86	0.76
Temperature	39.76	39.88	39.41	39.81	39.71	0.92

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