AN EVALUATION OF FEEDING HEMPSEED CAKE IN FINISHING CATTLE DIETS

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Thomas Michael Winders

In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Animal Sciences

April 2022

Fargo, North Dakota

North Dakota State University
Graduate School

Title

AN EVALUATION OF FEEDING HEMPSEED CAKE IN FINISHING CATTLE DIETS

By

Thomas Michael Winders

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Kendall Swanson
Chair

Dr. Carl Dahlen

Dr. Samat Amat

Dr. Timothy Greives

Approved:

4/11/2022
Date

Dr. Kendall Swanson
Department Chair

ABSTRACT

Understanding the potential of hempseed cake to be used as a livestock feedstuff is important for both industrial hemp and beef producers. Experiment 1 evaluated the effects of hempseed cake (HEMP) on growth performance, feeding behavior, plasma metabolite concentrations across time, and carcass characteristics when fed in finishing diets to heifers in comparison to dried corn distillers grains plus solubles (DDGS). Experiment 2 investigated the effects of HEMP on diet ruminal fermentation parameters, total tract digestibility, nutrient flow, and nitrogen balance in finishing steers in comparison to DDGS and a control (CON) diet containing no byproduct. Experiment 3 explored the effects of hempseed cake on immune parameters in response to an endotoxin (lipopolysaccharide) challenge in finishing steers in comparison to DDGS and CON. In experiment 1, the HEMP diet reduced heifer growth performance and hot carcass weight while not influencing dry matter intake, feeding behavior, or other carcass characteristics. Plasma urea nitrogen (PUN) was greater for heifers fed the HEMP diet, and glucose and total amino acid concentrations were not influence by treatment. In experiment 2, ruminal ammonia and total VFA concentrations were greatest for steers fed the HEMP diet. Furthermore, organic matter (OM) intake tended to be greater, OM total tract apparent digestibility was reduced, and N digestibility was greatest for steers fed the HEMP diet, and site of digestion was influenced by treatment. Nitrogen retention was greatest in steers fed the HEMP diet, suggesting treatment influence on N metabolism. In experiment 3, there was a treatment by hour interaction for PUN, and plasma glucose and NEFA were not influenced by treatment. Plasma IL-1α, IL-36RA, and TNF-α were lowest in steers fed the HEMP diet, and all other cytokines and total amino acid plasma concentrations were not influenced by treatment. Hempseed cake negatively influences growth performance in large part because of reduced total

tract apparent OM digestibility, while N total tract apparent digestibility and N retention are improved and immune response is influenced, so further understanding of these outcomes is needed to explore implications of feeding hempseed cake to finishing cattle.

ACKNOWLEDGMENTS

I would like to extend my gratitude and appreciation to all who helped me reach my goal of obtaining my Ph.D. First and foremost, to my advisor Dr. Kendall Swanson, words cannot fully express how grateful I am for the opportunity to learn from and work with you these past few years. Your guidance, encouragement, and expertise have helped mold me into the researcher and person I am today, and I am lucky to not only consider you a mentor, but also a friend. I would also like to thank my committee members, Dr. Carl Dahlen, Dr. Samat Amat, and Dr. Timothy Greives for the time and effort you devoted to myself and my program, for your desire to help and knowledge shared, and for simply being great, positive people to work with.

Additionally, I would like to thank the faculty members, graduate students, and staff that I had the opportunity to work with for all of your help and enjoyable experiences shared along the way. I was fortunate to have great instructors whose energy and passion for connecting with graduate students made the coursework truly enjoyable. Thank you to Sarah Underdahl, Terry Skunberg, Justin Gilbertson, Jim Kirsch, Laurie Geyer, Dr. Veselina Valkov and all other facility managers and staff who helped make my research possible. Thank you to Dr. Zac Carlson for being a great friend and mentor, to my lab mates, Kafi Mia and Macie Mosher for all the fun times together, and to Dr. Tom Peters for the years of mentorship and friendship shared with me.

I would also like to thank my family (Max, Karin, Jeremy, Jack, Andrew and Josie) for your continuous love, support, and belief in me. Times like these serve as an important reminder for how blessed I am to have such a supportive family. And finally, a special thank you to Kerri Bochantin for absolutely everything. Your unwavering love, support, encouragement, positivity, and selflessness pushed me to reach this goal and I could not have done it without you. You're simply the best and I am grateful and fortunate to have you by my side.

"Life means to me living in the fullest sense, having a healthy and fit body and an active and inquiring mind. It means feeling all the emotions like love, joy, anger, frustration, sorrow, and pain. It means laughter and crying, enjoying the company of friends, and having the tolerance to put up with jerks. Accepting authority, even when commands are silly, and being able to accept criticism from others. But most of all, life is for giving. Giving of self to others in service or love, for it is in giving of oneself that I can become part of the life of God."

– Bert Winders (1982).

DEDICATION

To my role model, hunting and fishing partner, great friend and the best Grandpa

Dr. Todd Johnson

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2-AG .....2-arachadonoyl glycerol

AA .....amino acids

AAFCO.....association of American feed control officials

ADF .....acid detergent fiber

ADG .....average daily gain

AEA .....arachidonoyl ethanolamide

AP-1 .....activator protein

BW .....body weight

CB1 .....cannabinoid receptor 1

CB2 .....cannabinoid receptor 2

CBD .....cannabidiol

CD14.....cluster of differentiation 14 protein

COX-2.....cyclooxygenase

CP .....crude protein

Cr.....chromium

DAGL .....diacylglycerol lipase

DE .....digestible energy

DEI.....digestible energy intake

DHA.....docohexaenoic

DM .....dry matter

DMI .....dry matter intake

EAA .....essential amino acids

EPA.....eicosapentanoic

FAAH.....fatty acid amide hydrolase

G:F .....gain to feed

GE .....gross energy

GEI.....gross energy intake

GRP120.....g-coupled protein receptor 120

GPR55.....g-coupled protein receptor 55

HCW .....hot carcass weight

IFNγ .....interferon gamma

IKKβ .....IkappaB kinase β

IL-2 .....interleuken-2

IL-4 .....interleuken-4

IL-6 .....interleuken-6

IL-8 .....interleuken-8

IL-10 .....interleuken-10

IL-36RA.....interleukin-36 receptor agonist

iNOS .....inducible nitric oxide synthase

IP-10.....interferon gamma-induced protein 10

JNK .....c-Jun N-terminal kinase

kcal.....kilocalorie

LBP .....LPS binding protein

LM .....longissimus muscle

LPS.....lipopolysaccharide

MAGL.....monoacylglycerol lipase

Mcal .....megacalorie

MCP-1.....monocyte chemoattractant protein-1

MD-2.....myeloid differentiation factor 2

MIP-1α.....macrophage inflammatory protein-1α

MIP-1β.....macrophage inflammatory protein-1β

MP.....metabolizable protein

MyD88.....myeloid differentiation primary response 88

N .....nitrogen

NDF .....neutral detergent fiber

n-3 .....omega-3

n-6 .....omega-6

NAPE-PLD .....N-acyl-phosphatidyl-ethanolamine-selective phospholipase D

NDF.....neutral detergent fiber

NEAA .....non-essential amino acids

NEFA .....non-esterified fatty acid

NEm .....net energy for maintenance

NEg .....net energy for gain

NF-κB .....nuclear factor kappa b

NH3.....ammonia

OM .....organic matter

PAMP.....pathogen-associated molecular pattern

PGE2.....prostaglandin E2

PGE3.....prostaglandin E3

PPARγ.....peroxisome proliferator-activated receptor gamma

PRR.....pattern-recognition receptor

PUFA .....polyunsaturated fatty acid

PUN .....plasma urea nitrogen

RDP.....rumen degradable protein

RUP.....rumen undegradable protein

SEM .....standard error of the mean

TAB1.....TAK1 binding protein

TAK1 .....transforming growth factor-b activated kinase-1

THC detla-9-tetrahydrocannabinol

TIR .....toll-interleuken-1 receptor

TIRAP .....TIR domain-containing adaptor inducing protein

TLR-4.....toll-like receptor 4

TNF-α.....tumour necrosis factor alpha

TRAM .....TRIF-related adaptor molecule

TRIF .....TIR domain-containing adaptor inducing IFN-β

TRPv1 .....transient receptor channel subfamily V member 1

VEGF-A .....vascular endothelial growth factor A

VFA.....volatile fatty acid

CHAPTER 1. INTRODUCTION AND REVIEW OF LITERATURE

1.1. Introduction

In the US, feed costs represent the largest variable cost of production, accounting for roughly two-thirds of total production costs (Anderson et al., 2005). Corn distillers grains plus solubles, the most common byproduct feed fed to finishing cattle in the US (Samuelson et al., 2016), can be volatile in terms of pricing and availability, and tends to follow the price of corn (USDA Agricultural Marketing Service, Daily Ethanol Report, 2022). Alternative feedstuffs with a similar nutrient profile to corn distillers grains plus solubles could provide producers with more flexibility, and more affordable options to meet nutrient requirements of finishing beef cattle. Hempseed cake (a byproduct of hempseed processing byproducts) is a novel feedstuff that has a similar protein concentration to corn distillers grains plus solubles. Hempseed cake is gaining interest as an alternative protein source, but cannot be fed to livestock without the Food and Drug Administration (FDA) approval through the Association of American Feed Control Officials (AAFCO; Kleinhenz et al., 2020a). The objectives of this review of literature are to explore the history of hemp production, review animal performance and digestibility results on feeding hempseed byproducts as a feedstuff for ruminants, and investigate potential immune response implications that could result from feeding hempseed byproducts. Ultimately, the goal is to gain a comprehensive understanding of what is currently known as well as uncover gaps in the literature to better understand potential implications of using hempseed cake as a feedstuff for beef cattle fed finishing diets.

1.2. Industrial Hemp Production – History and Current Trends

Cannabis sativa L., or industrial hemp, is an ancient crop thought to be indigenous to Asia (Small and Marcus, 2002), and first harvested as many as 8,500 years ago (Fike, 2016).

Historically, hemp was cultivated for its fiber properties making it useful for the production of products such as canvas and rope, but became scrutinized in the 20th century because of the psychoactive compounds found within *Cannabis Sativa* L (Hessle et al., 2008; Fike 2016). *Cannabis sativa* L. encompasses marijuana and industrial hemp, which are technically the same species. The difference has been largely driven by human selection for either the stem fiber properties of hemp, or the narcotic concentration of marijuana (Small, 2015). Because differentiation between hemp and marijuana was difficult, and fear that allowing hemp production would make marijuana more accessible (Small, 2015), all industrial hemp production was legally banned in the US beginning with the Marihuana Tax Act of 1938. This was the initial step that placed hemp on the Schedule 1 list of Controlled Substances (USDA, Agricultural Marketing Service, 2021). While it took time to completely stop industrial hemp production, this act began the process of requiring hemp growers to be federally licensed and registered in an effort to control the psychoactive varieties. Apart from a brief increase in hemp production during World War II, hemp production steadily declined in the US, until the mid-1990's when Canada and Europe reinstated legal hemp cultivation and production, restoring interest in hemp production in the US (Fike, 2016).

In recent years there has been renewed interest in industrial hemp production after its removal from the list of US Drug Enforcement Agency Schedule 1 drugs as a result of the 2018 Agricultural Improvement Act. A series of pilot studies were allowed under the 2014 Agricultural Improvement Act and lead to reinstating legal industrial hemp cultivation. By statute, industrial hemp must contain less than 0.3% delta-9-tetrahydrocannabinol [THC; dry matter (DM) basis], which is the psychoactive component of the hemp plant, and is the differentiating component between hemp and marijuana (USDA, Agricultural Marketing

Service, 2021). Cannabis containing THC concentrations exceeding 0.3% DM remains a Schedule 1 controlled substance. The interest in hemp production today has broadened from historically being a fiber product. Today, hempseed oil and byproducts have gained interest for use in human food, livestock feed, nutritional supplements, biofuel, therapeutic uses, and more, and hemp production for fiber has declined to just 0.3% of worldwide natural fiber production (Callaway, 2004; Small, 2015).

Markets for the different hemp products are not at the same stage of development, leading to some market uncertainty as well as variations in economic return between hemp products. Currently, oil products from industrial hemp are projected to generate greater profits per acre than other products that come from hemp production (Mark et al., 2020). Because the development of hemp markets in the US is still in its infancy, production and pricing uncertainty tends to lead to instability and rapid turn-over in market trends. Industrial hemp production increased steadily as a result of the pilot programs of 2014 and 2018 Agricultural Improvement Act, with 90,000 planted hemp acres reported by states in 2018, and peaking in 2019, but has since declined because of lack of economic return and limited markets for producers, with 54,000 planted hemp acres reported for 2021 (USDA NASS, National Hemp Report, 2022; Mark et al., 2020). Still, as production may increase in coming years, it is important to find a use for the byproducts produced from industrial hemp production and oil extraction (Abrahamsen et al., 2021).

1.3. Hemp Byproducts

With hempseed oil use expected to continue to increase (Mark et al., 2020) there is interest in exploring markets for the byproduct(s) created from the oil extraction process, including using it as an animal feed ingredient (Fike, 2016; Abrahamsen et al., 2021). Hempseed

oil is typically extracted using a cold-pressing mechanical process where the hempseed is pressed, removing approximately 35% of the hempseed DM as oil, and leaving approximately 65% of the hempseed as a byproduct, which is called hempseed cake (Figure 1-1A; Helstad et al., 2022). A common step taken after the production of hempseed cake is to grind it, creating a product called hempseed meal (Eriksson, 2007; Figure 1-1B).

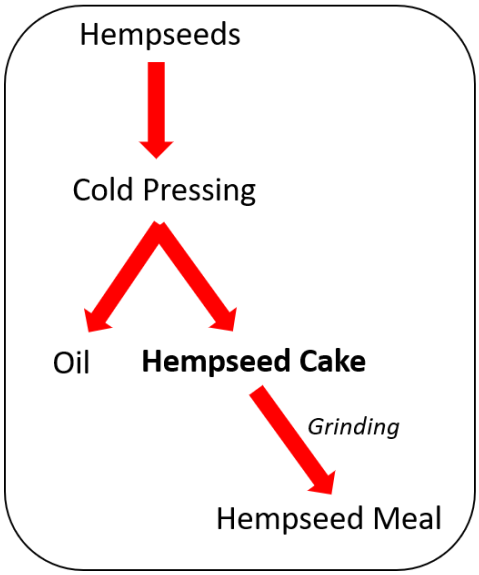

Figure 1-1. Hempseed processing into byproducts (Adapted from Eriksson, 2007 and Fike, 2016).

The literature on feeding hempseed cake or meal to animals is limited, and the terminology between hempseed cake and hempseed meal is sometimes used interchangeably (Mustafa et al., 1999; Hessle et al., 2008; Pojić et al., 2014; Abrahamsen et al., 2021; Rajasekhar et al., 2021). Hempseed byproducts have potential to be used as a feedstuff, primarily in ruminant species because of the fiber and protein concentrations (Abrahamsen et al., 2021). Hempseed byproducts are high in neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP; 51%, 39%, and 30% respectively; Chapter 2) but limited markets are available for it because hemp and hemp byproducts cannot be legally fed to livestock or pets in the US without FDA approval through the Association of American Feed Control Officials

(Kleinhenz et al., 2020b). Although feeding hemp byproducts is legal in the European Union (among other places), there are relatively few data on the nutritive value of hempseed cake as a feed ingredient for beef cattle (Hessle et al., 2008). Therefore, further understanding of the use of hempseed cake as a potential feedstuff for ruminants is needed.

1.4. Hemp Byproducts as a Feedstuff

1.4.1. Nutrient Composition of Hempseed Byproducts

Hempseed oil extraction byproducts, such as hempseed cake and hempseed meal, are used in the EU and Canada among other places as a feedstuff for livestock (Gibb et al., 2005; Hessle et al., 2008). While still illegal in the US to feed hemp products to livestock, there is growing interest in its approval (Abrahamsen et al., 2021). Of the livestock species in the US, cattle and sheep are the most logical species to feed hemp byproducts to because of the high fiber and protein concentration of this feedstuff. Currently, corn distillers grains is the most common byproduct fed to cattle (Samuelson et al., 2016), but when corn prices increase, and/or availability of distillers grains decrease, alternative protein sources could be used as a feedstuff by producers. Hempseed byproducts contain approximately 30% crude protein, and has a different amino acid profile than distillers grains (Chapter 3; Liu, 2011). This could influence animal growth performance as individual amino acids have differing rumen degradability which influences post-ruminal amino acid absorption and subsequent metabolizable supply of amino acids (Weisbjerg et al., 1996). Although hempseed cake/meal has a similar crude protein concentration as corn distillers grains, amino acid concentration and site of degradation is important to consider when formulating diets to meet rumen degradable protein (RDP) and amino acid, and metabolizable protein (MP) and amino acid requirements (Karlsson et al., 2012).

Research has suggested that hempseed meal is high in rumen undegradable protein (RUP; 61-67%), determined using *in situ* and *in vitro* approaches (Mustafa et al., 1999; Karlsson et al., 2009). More recent data in dairy cows reported that hempseed cake protein is more ruminally available than initial observations suggested. Karlsson et al. (2012) reported in situ RUP of hempseed cake to be 26%, while Karlsson and Martinsson (2011) reported in situ RUP of hempseed cake to be 29% in dairy cows and growing lambs, respectively. Differences could be because of variety of hemp, type of processing, differences in laboratory techniques, etc. Regardless, corn distillers grains plus solubles and hempseed cake have similar CP concentrations, but site of CP digestion considerations are necessary when feeding hempseed cake if formulating diets for MP supply.

Hempseed cake has a high fiber concentration relative to other protein byproducts fed to ruminants (Hessle et al., 2008), with a relatively large amount of variation between experiments that have fed hempseed cake (Abrahamsen et al., 2021). The NDF concentration has been reported to be 39-51%, and ADF concentration has ranged from 30-39% (Chapter 3; Karlsson and Martinsson, 2011; Karlsson et al., 2010; Mustafa et al., 1999). The variability may be because of differing plant varieties and/or seed processing methods used in these studies. There is typically a negative relationship between ADF concentration and digestibility (Karlsson and Martinsson, 2011; Hessle et al., 2008). Ruminants may be able to utilize hempseed byproducts more effectively than non-ruminants because of the greater fiber concentrations compared with other protein feedstuffs because mammalian enzymes cannot break down β-1,4 glycosidic bonds found within cellulose, but microbial enzymes can. Even with that in mind, increased fiber concentrations are associated with lower digestibility because lignin cannot be digested and is chemically linked to cellulose and hemicellulose, reducing their availability to microbial

enzymatic activity (Moore and Jung, 2001). Greater lignin and ADF concentrations of a feedstuff is associated with lower overall digestibility, which may lead greater DMI to compensate for lower available dietary energy (Krehbiel et al., 2006). As observed in chapter 3 of this dissertation, the ADF concentration of hempseed cake tended to increase OM intake, suggesting steers ate more to have similar energy intake.

Hempseeds contain approximately 30% oil, which may be a beneficial energetic source for ruminant animals (Gibb et al., 2005). Furthermore, fatty acid intake has been suggested to have some human health concerns, with a shift over the last 40 years towards more omega-6 fatty acids (n6) and fewer omega-3 fatty acids (n3; Ailhaud et al., 2006). The increased use of cereal grains and oilseeds for animal production may be contributing to this trend by influencing the fatty acid profile of meat (Turner et al., 2008). The ratio of n6:n3 for optimal human health is thought to be approximately 3:1 (Callaway, 2004), as omega fatty acids have been shown to decrease superoxide production, neutrophil and monocytes cell numbers, and production of pro-inflammatory cytokines (Calder, 2001).

Hempseed oil contains roughly 84% of the lipid in the form of polyunsaturated fatty acids (PUFA; Turner et al., 2008), of which 56% is linoleic (omega-6) and 22% is alpha-linolenic (omega-3; Callaway, 2004), suggesting an ideal ratio of omega fatty acids for optimal human health. Turner et al. (2008) fed hempseed cake to Swedish Red steers in comparison to soybean meal and measured the fatty acid profile of longissimus muscle samples and concluded that hempseed cake did indeed favorably shift the fatty acid profile to lower n6:n3 levels, however, the biological significance of this shift was thought to be insignificant because the magnitude of change was small. These authors attribute biohydrogenation in the rumen to be the main reason as to why a greater change in this ratio was not observed, as rumen microbes are prolific at

altering the fatty acid profile in the rumen away from saturated fatty acids towards unsaturated fatty acids, ultimately influencing the fatty acid profile incorporated into the carcass (Duckett et al., 2002).

1.4.2. Effects of Feeding Hemp Byproducts on Animal Performance

While the data are fairly limited on feeding hemp products to ruminant species, there has been an increase in recent years. The first known experiment evaluating the effects of feeding hempseed byproducts to ruminants across a series of studies by Mustafa et al. (1999). These authors first conducted an *in situ* digestibility experiment using two non-lactating Holstein cows to evaluate the ruminal DM and CP disappearance. The RUP concentration was calculated by what was left over after 12-hour ruminal incubation time. Then, the RUP portion was evaluated *in vitro*, ultimately obtaining an intestinal digestibility of CP. These authors also conducted a digestibility experiment using 20 Suffolk ram lambs fed hempseed meal at 5 different levels displacing canola meal in a barley- and brome hay-based growing diet. The five levels of hempseed meal inclusion were 0, 5, 10, and 20% of the diet DM, with the greatest inclusion level being the sole supplemental protein source in the diet. The *in situ* experiment indicated that the hempseed meal had lower DM degradability as well as a lower degradation rate than canola meal. However, the CP degradability determined from the *in situ* and *in vitro* sequence was greatest for hempseed meal, with a majority being in the form of RUP (77%). The digestibility trial showed that inclusion of hempseed meal did not influence DMI, which is supported by what others have observed (Abrahamsen et al., 2021). As hempseed meal inclusion increased, there was no influence on DM or OM digestibility, indicating that hempseed meal and canola meal have similar digestibilities. The nutrient composition of the diets containing 0% hempseed meal and 20% hempseed meal had similar NDF and ADF concentrations, likely resulting in similar

DM and OM digestibility. The results from these authors differ from what others have reported relating to site and extent of nutrient digestibility (Karlsson and Martinsson, 2011). Inconsistencies between experiments could be partially because the hempseed meal used by Mustafa et al. (1999) was heat-treated, making the CP less available in the rumen. Other differences could result from hemp variety and processing differences.

Gibb et al. (2005) evaluated the effects of feeding full-fat hempseed in barley-based finishing diets to 60 yearling steers on growth performance. Hempseed is a different feedstuff than hempseed cake or hempseed meal because it has not had oil extracted. Hempseed was included in the diet at 0, 9 or 14% (as-fed basis) after undergoing seed processing through a roller mill. Treatments were isonitrogenous, while NDF, ADF and ether extract concentration increased as inclusion of hempseed increased. Similar to Mustafa et al. (1999), hempseed inclusion did not influence DMI, but an overall DMI as a percent of BW of 1.8% is lower than what would typically be observed for finishing steers. The authors did not indicate if cattle were fed for *ad-libitum* intake, however, and noted that steers were individually housed, which has been shown to decrease feed intake (Cruz et al., 2010). Average daily gain (ADG) and feed efficiency (G:F) were not influenced by hempseed inclusion, which differs from what Hessle et al. (2008) observed when feeding hempseed cake to cattle, and final BW was not influenced by treatment.

Two feeding experiments were conducted by Hessle et al. (2008) where hempseed cake was fed in comparison to soybean meal to growing bull calves and to finishing steers. Fifty-six Swedish Red bull calves were fed hempseed cake at 1 kg/hd/d in comparison to 1 kg/hd/d of a 50:50 blend of soybean meal and rolled barley to make the diets isonitrogenous. In experiment two, 51 Swedish Red steers were fed 0.7 kg/hd/d hempseed cake compared to 0.7 kg/hd/d of the

same soybean meal/rolled barley blend previously mentioned for the first seven weeks and then switched to 1.4 kg/hd/d of each protein treatment supplement for the remainder of the finishing period. Inclusion of hempseed cake in the growing diet was approximately 20%, and 9% in the finishing ration (DM-basis). Dry matter intake was improved by 9% for bulls receiving hempseed cake during the growing experiment, and DMI was not influenced by treatment for the finishing trial. As expected, NDF intake was greater for cattle receiving the hempseed cake treatments across both experiments. Final BW were not different, resulting in similar ADG and reduced G:F for the growing experiment while ADG and G:F were not influenced by treatment for the finishing experiment. Furthermore, hot carcass weight (HCW) was not influenced by hempseed cake inclusion for finishing steers. These authors conclude that differences were not observed in the finishing experiment because greater fiber concentration and lower starch concentration resulted in improved rumen function, leading to no effects on growth performance or carcass characteristics.

Karlsson and Martinsson (2011) evaluated the effect of feeding hempseed cake on growth performance of 48 ewe lambs. These authors also conducted an *in situ* and *in vitro* study to evaluate CP degradability in the rumen and intestine, where hempseed cake was observed to have a RUP value of 29%. This agrees with what Karlsson et al. (2012) observed, and differs from Mustafa et al. (1999) observations. Interestingly, the digestibility of the RUP fraction in this experiment was only 31%, compared to 85% for Mustafa et al. (1999). Hempseed cake was fed at 22% of diet DM in a barley-based finishing diet in comparison to isonitrogenous diets that contained either peas or rapeseed cake as the protein supplement. Dry matter intake did not differ between treatments, and lambs had reduced final BW, ADG, and G:F compared to the peas and rapeseed cake treatments but was not different from control. These results agree with the

other animal growth performance experiments previously discussed. The NDF and ADF concentration of the hempseed cake diet was greater than the other diets fed, contributing to the reduction in observed growth performance results. Furthermore, the low RUP digestibility observed in situ/in vitro and subsequent reduction in absorbed amino acids in the intestine reported in this experiment also may be contributing to the reduction in growth performance.

An experiment evaluating the effects of feeding hempseed meal to 40 growing meat goats was conducted by Abrahamsen et al. (2021). These authors evaluated the influence of feeding 0, 11, 22, or 33% hempseed meal (DM-basis) on goat DMI, rumen fermentation parameters, in vitro digestibility, blood metabolites, and growth performance. Inclusion of hempseed meal displaced cracked corn and soybean meal in a timothy hay-based growing diet. As hempseed meal inclusion increased, dietary CP, NDF, and ADF increased as well, which is similar to what others have observed (Karlsson and Martinsson, 2011; Gibb et al., 2005). Although DMI and final BW were not influenced by treatment, ADG and G:F were linearly reduced as inclusion of hempseed meal increased. Furthermore, the ruminal fermentation parameter data suggest that hempseed cake reduced VFA production, and increase the acetate to propionate ratio linearly, likely resulting from greater fiber concentration, which promotes more acetate and less propionate production. Although ruminal ammonia was not measured in this experiment, plasma urea nitrogen concentration increased linearly as hempseed meal inclusion increased, which may suggest that greater amounts of ammonia were produced and absorbed across the rumen wall, similar to results reported in chapter 3 and 4 of this dissertation. The reduced ADG and G:F observed may be partially driven by the lower in vitro digestibility observed in this experiment, as hempseed cake is displacing more digestible, energetic feedstuffs resulting in reduced performance.

Karlsson et al. (2010) evaluated the effects of feeding increasing amounts of hempseed cake to dairy cows on milk production and composition. No other known studies have evaluated the effect of hempseed byproducts on milk production and composition. Hempseed cake was included at 0, 14, 23 or 32% of the diet (DM-basis), displacing a barley-based pellet. Dry matter intake was greater in the 14 and 32% hempseed cake treatments than the control and 23% hempseed cake treatments. Milk production and components increased quadratically, with the greatest response observed at 14% hempseed cake in the diet. Similar to other experiments discussed, the CP, NDF, and ADF concentrations increased as hempseed cake inclusion increased. The authors suggest that 14% hempseed cake was more beneficial than 23 and 32% hempseed cake, likely because increasing the dietary CP above the animal requirements shows no additional milk yield improvements (Broderick, 2003).

Two other studies were found that fed other industrial hemp products to ruminant animals that are likely quite different than hempseed byproducts previously discussed, but are relevant to this review. Krebs et al. (2021) fed pelleted hemp stubble to 15 male sheep over a 56-day growing period to evaluate the effects of feeding hemp stubble on DMI, nutrient digestibility, ruminal fermentation parameters, growth rate, carcass characteristics, and cannabinoid residues. While this hemp product is quite different from the hempseed meal and hempseed cake products that are the focus of this review, it warrants some consideration to better understand hemp plant usage in livestock production systems. Pelleted hemp stubble was fed at inclusion rates of 0, 28, and 56% of diet DM, displacing cereal straw as inclusion increased. Dry matter intake was not influenced by hemp stubble inclusion in the diet, while weight gain was greater for sheep fed hemp stubble. Numerical improvements in ADG and G:F were also observed for the hemp stubble treatments. Furthermore, the digestibility period portion of this experiment observed

greater DM, OM, CP, NDF, and ADF digestibility for sheep fed the 56% hemp stubble treatment compared to the control, while the 28% hemp stubble treatment was intermediate. Ruminal ammonia and VFA concentrations increased as hemp stubble inclusion increased. The CP concentration of the diet increased as hemp stubble inclusion increased, which may partially explain some of the growth performance, digestibility, and rumen parameter results. The NDF and ADF concentration of the three treatments were similar, further suggesting that the performance responses observed are likely because of the greater CP concentration in hemp stubble than other nutrient composition differences among treatments.

Kleinhenz et al. (2020a) evaluated the plasma cannabinoid concentrations in cattle following oral administration of the flower portion of industrial hemp. These authors had previously characterized cannabinoid concentrations throughout various parts of the hemp plant (Kleinhenz et al., 2020b), confirming cannabinoid concentrations were present in all parts of the plant. In the current experiment, these authors orally administered a bolus of hemp flower material in eight Holstein calves and measured plasma cannabinoid concentrations out to 96 hours after dosing. They concluded no adverse effects from hemp administration, and 5 of the 11 cannabinoids measured in the hemp were detected in the plasma, including CBD in four of the samples measured and CBD acid (CBDA) in all samples, which is the precursor to CBD. Additionally, these authors state that the concentration of the acidic form of cannabinoids was higher in plasma, indicating some differences in absorption or metabolism of cannabinoids based on their chemistry. This experiment was a one-time oral dose of hemp, so sustained feeding may lead to different plasma cannabinoid concentrations.

Taken together, these experiments evaluating the usage of hemp products as ruminant feedstuffs show some consistent results. These data suggest that DMI is not negatively

influenced by dietary inclusion of hemp products, and has been shown to increase in DMI (Karlsson et al., 2010). While hempseed products do not seem to influence DMI to a large extent, they seem to have a consistently negative influence on animal growth performance measures. All previously discussed experiments apart from Krebs et al. (2021) and Karlsson et al. (2010) observed a decrease in one or several performance metrics, including final BW, ADG, G:F, and HCW. Krebs et al. (2021) observations on growth performance is likely an outlier in comparison to the other experiments because pelleted hemp stubble has a different nutrient composition than hempseed meal or hempseed cake, and because the feedstuff it displaced in the diet (cereal straw) was of lower quality than the hemp stubble itself, as illustrated by the lower CP and similar NDF and ADF concentration. The Karlsson et al. (2010) results should also be discussed separately because it is the only experiment to date that has evaluated the effects of hempseed cake on milk production in dairy cows. In general, dairy cows are fed more CP and dietary fiber than beef cattle, so the greater milk yield observed in that experiment may not be applicable to finishing beef cattle growth performance because dietary protein concentrations are lower and concentrate inclusions are typically greater than that of dairy cow diets.

Overall, the reduction in performance in ruminants fed hempseed byproducts is likely because of greater ADF concentration in these byproducts compared to most protein feedstuffs. The lower digestibility of ADF compared with other components within feedstuffs indicate that as ADF concentration increases, OM digestibility will decrease, and this was consistently observed throughout these experiments. Another factor potentially negatively influencing the growth performance and efficiency of ruminants fed hemp byproducts is the high CP concentration. If MP provided in the diet exceeds the animal's MP requirement, a larger amount of amino acids are deaminated, with the amine group undergoing conversion to urea through the

urea cycle. Urea production has an energetic cost and could be decreasing energy utilization efficiency, ultimately negatively influencing growth performance (Jennings et al., 2018). While these initial experiments are helpful in establishing trends for feeding hemp byproducts, more work is needed to evaluate the effect of feeding hempseed cake in finishing diets in comparison to typically used feedstuffs. Furthermore, better understanding of the effects of hempseed cake on ruminal fermentation parameters, nutrient digestibility, nutrient flow, and nitrogen retention are needed to determine implications of feeding hempseed cake in finishing diets. Lastly, there is a lack of data in regards to animal health when hemp byproducts are fed. Kleinhenz et al (2020b) did not observe any adverse effects (reactions or behavioral influences) when dosing industrial hemp to cattle, while all other ruminant studies feeding hemp products did not investigate health influences, so an evaluation of the immune response is warranted.

1.5. The Effects of Hemp Products on the Immune System

While interest in hemp production has been renewed recently, hemp has a long history of being cultivated for not only fiber production, but food and medicinal purposes, dating back at least 3,000 years in China (Callaway, 2004). Hempseed oil contains cannabinoids, specifically cannabidiol (CBD), which may have health and immune function benefits associated with its anti-inflammatory properties (Leizer et al., 2000). Furthermore, hempseeds contain 27-38% oil, and the oil contains a 3:1 ratio of n6:n3, which is suggested to be optimal for human health and is one reason as to why hemp products are being evaluated for their therapeutic potential (Pojić et al., 2014).

1.5.1. Cannabinoids

Cannabis sativa L. is composed of terpenes, carbohydrates, fatty acids, amides, amines, phytosterols, phenolic compounds, and compounds specific to this plant, called cannabinoids.

Cannabinoids are mono- to tetracyclic meroterpenoid compounds found within *Cannabis sativa* L, with over 100 compounds currently that have been isolated (Bruni et al., 2018; Pellati et al., 2018). The hemp plant has varying concentrations of cannabinoids based on location in the plant, and in general cannabinoids have become a recent area of interest for various types of research. Kleinhenz et al. (2020b) evaluated cannabinoid concentrations in hemp and observed that the greatest cannabinoid concentration are in the leaves and flower, and the lowest in the stalks, but actual cannabinoid concentrations within hempseed cake were not evaluated by these authors. Physiological concentrations of cannabinoids needed to elicit a physiological response in cattle are not yet known.

The primary psychoactive cannabinoid is THC, and is found in greater concentrations in marijuana strains of hemp, and is the most extensively studied cannabinoid (Burstein, 2015). Delta-9-tetrahydrocannabinol was isolated in 1964, which ultimately allowed for industrial fiber-type hemp to be chemically differentiated from drug-type hemp because THC concentration in fiber-type hemp is very low (Pellati et al., 2018; McPartland et al., 2015), ultimately leading to the renewal of interest in hemp production observed today. Cannabidiol is the most abundant cannabinoid in hemp and has received an increase in attention because of its pharmacological actions without exerting the psychotropic actions associated with THC (Di Marzo and Piscitelli, 2015; Bruni et al., 2018). Cannabidiol and THC are considered to be 'sister' molecules that are synthesized by nearly identical enzymes found in *Cannabis* L (de Meijer et al., 2003). While they are structurally very similar, their physiological functions vary, largely driven by the way they interact with the endocannabinoid system.

1.5.2. Endocannabinoid System

The endocannabinoid system is a lipid-signaling system present in all living animals and is comprised of two endocannabinoids, five enzymes, and 2 cannabinoid receptors. The two endocannabinoids are arachidonyl ethanolamide (AEA), and 2-arachadonoyl glycerol (2-AG; Di Marzo and Piscitelli, 2015). The cannabinoid metabolizing enzymes are N-acyl-phosphatidyl-ethanolamine-selective phospholipase D (NAPE-PLD), diacylglycerol lipases (DAGL) α and β, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). The two receptors are cannabinoid receptor 1 (CB1) and 2 (CB2; Rohbeck et al., 2021). However, the definition and subsequent functions of the endocannabinoid system are still being elucidated, so more molecules are likely to be included as more investigations take place. While over 100 cannabinoids have been isolated, only THC and its acid form, THCA, are capable of binding with high affinity to CB1 and CB2 because they mimic the endocannabinoids (Di Marzo and Piscitelli, 2015). CBD on the other hand has very low affinity for CB1 and CB2 and is considered an antagonist to THC (McPartland et al., 2015).

CB1 is largely found in the brain, mainly in the frontal cortex, basal ganglia and cerebellum, which is why THC exhibits psychotropic effects because of the affinity it has for CB1 (Bruni et al., 2018). CB1 is also found in adipose tissue, the gastrointestinal tract, spinal cord, adrenal and thyroid glands, liver, reproductive organs and immune cells of mammals. CB2 on the other hand is primarily expressed in immune cells, but can also be found in chondrocytes, osteocytes, and fibroblasts, and is thus considered to be the peripheral cannabinoid (Bruni et al., 2018). While CBD has low affinity for CB1 and CB2, it is of interest for therapeutic uses primarily because it has high antioxidant and anti-inflammatory activity, along with anxiolytic, anti-arthritic, and anticonvulsant properties (Pellati et al. 2018; McPartland et al., 2015). For

this reason (among others), CBD is of interest for therapeutic uses to further understand the influence it has on the endocannabinoid system, inflammation, and the immune system in general.

1.5.3. CBD and Inflammation

The effect of CBD on inflammation has not been evaluated extensively in cattle, but there is increasing focus on the effects of CBD on inflammation in other species (Ribeiro et al., 2015; Pagano et al., 2016). While the influence of CBD on the immune system and endocannabinoid system is not completely understood (Pellati et al., 2018), there are several proposed mechanisms that could influence cytokine production and subsequent inflammation. While there are only two confirmed cannabinoid receptors within the endocannabinoid system, exogenous cannabinoids can interact with several other molecular targets that can influence cellular signaling, such as g-protein coupled receptor 55 (GRP55), transient receptor channel subfamily V member 1 (TRPV1). Exogenous CBD is thought to interact with the endocannabinoid system by downregulating CB2 and GPR55, and upregulating TRPV1. This leads to a decrease in cyclooxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), and cytokine production, ultimately reducing the inflammatory response to infection (Pellati et al., 2018; Bruni et al., 2018). Furthermore, CBD has been suggested to restore gut permeability and serve as an antioxidant potentially through peroxisome proliferator-activated receptor gamma modulation (PPARγ), reducing inflammation and improving immune function (Pellati et al., 2018; McPartland et al., 2015). The downregulation of CB2 and GPR55 and upregulation of TRPV1 along with the upregulation of PPARγ is a proposed mode of action for how CBD can influence the immune system inflammatory response (Figure 1-2).

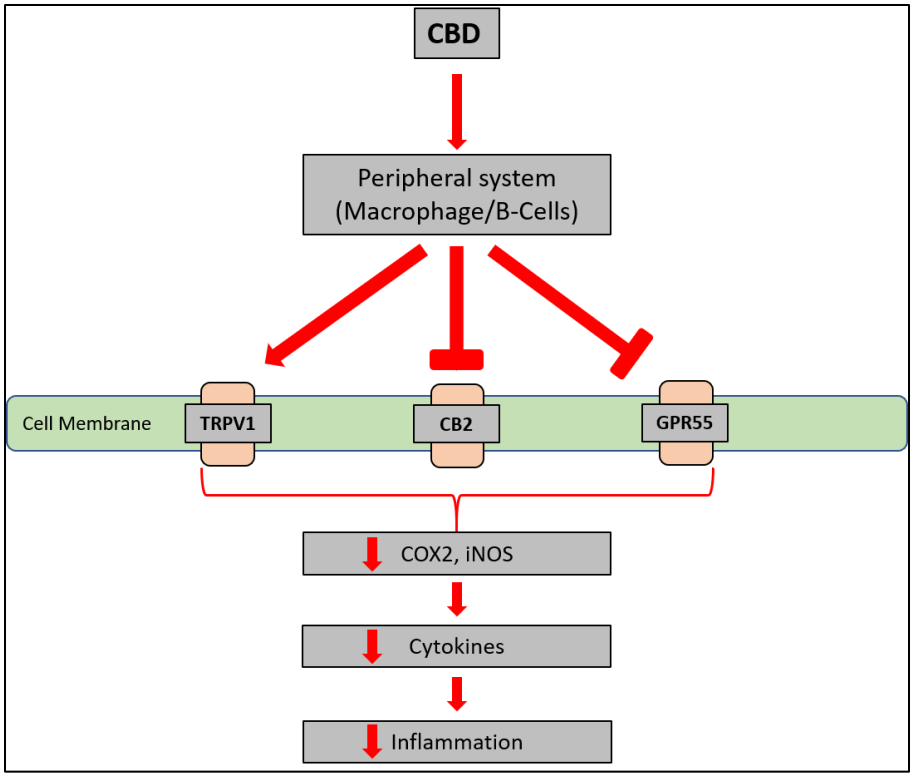

Figure 1-2. General representation of the signaling pathways involved in CBD anti-inflammatory effects. Cannabinoids reduce peripheral inflammation by acting at TRPV1, CB2, and GPR55 receptors; these interactions lead to downregulation of enzymes involved in the production of prostaglandins, reactive oxygen species, and cytokines (Adapted from Pellati et al., 2018).

Another potential mode of action that has been proposed for CBD's anti-inflammatory effects has focused on the adenosine A2A receptor (Burstein, 2015). Adenosine A2A receptors can downregulate over-reactive immune cells, which can protect tissues from excess inflammation that would otherwise result. Providing an adenosine A2A receptor agonist to mice resulted in an increase in intracellular cAMP, which exhibits immunosuppressive properties (Ohta et al. 2001). Ohta et al. (2001) found that TNF-α and IFNγ, two pro-inflammatory cytokines, were reduced in serum, and tissue damage was reduced as well. Cannabidiol has been shown to upregulate A2A signaling by inhibiting equilibrative nucleoside transporter (Ribeiro et al. 2012), which could decrease inflammation (Carrier et al., 2006). Overall, CBD has been shown to decrease pro-inflammatory markers, such as IL-1β, IL-2, IL-6, IL-8, TNF-α, and IFNγ

(McPartland et al., 2015) as well as COX-2 and iNOS (Burstein, 2015). Furthermore, Kleinhenz et al (2020a) confirmed that cannabinoids are indeed absorbed across the rumen wall and into plasma in cattle administered with hemp flower orally, indicating that feeding hemp products does lead to cannabinoids in circulation. While these authors did not evaluate the physiological influence of cannabinoids in circulation on the immune system, they established the potential for future work looking into these effects.

1.5.4. Fatty Acids and Inflammation

Hempseed oil contains a high concentration of polyunsaturated fatty acids (84-90%), of which 56% is linoleic (omega-6) and 22% is alpha-linolenic (omega-3) fatty acids, making it a great source of essential fatty acids (Callaway, 2004). Omega-3 fatty acids, have been shown to exert anti-inflammatory effects (Oh et al., 2010). There are several mechanisms as to how omega-3 fatty acids may influence inflammation: biochemical competition between omega-3 and omega-6 fatty acids for enzymes required to synthesize prostaglandin (PGE_2) and COX-2; activation of PPARγ and its anti-inflammatory properties; inhibition of TLR's including TLR4; and a new target, identified as GPR120 (Im, 2016). Alpha-linolenic acid can be a precursor for eicosapentanoic (EPA) and docosahexaenoic (DHA) fatty acids, the major fatty acids in fish oil, that are known to have preventative as well as therapeutic roles in inflammation (Belluzzi, 2002; Oh et al., 2010). Omega-3 fatty acids have been shown to be beneficial for inflammatory responses (Solinas et al., 2007) potentially though their interaction with GPR120. GPR120 is highly expressed in adipose tissue as well as pro-inflammatory cytokines, and omega-3 fatty acids, such as EPA and DHA, exert anti-inflammatory properties through their association with GPR120. The proposed mechanism suggests that only in the presence of DHA or EPA can GPR120 bind with β-arrestin, which leads to the complex internalizing, where β-arrestin then

also binds with TAK1 binding protein (TAB1). This interaction of β-arrestin and TAB1 blocks TAB1 from binding with transforming growth factor -β activated kinase 1 (TAK1), blocking the ability of TAK1 to upregulate the IKKβ and NF-κB inflammatory pathway as well as the JNK and AP1 inflammatory pathway, ultimately downregulating TLR4 and TNF-α signaling pathways (Oh et al., 2010).

Additionally, linolenic acid, a precursor for EPA and DHA, promotes the synthesis of anti-inflammatory eicosanoids, such as PGE_3, as well as anti-inflammatory cytokines such as IL-4 and IL-10. Linoleic acid on the other hand is a precursor for arachidonic acid and has been shown to upregulate pro-inflammatory eicosanoids like PGE_2, while also promoting the release of pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6 and subsequent acute phase protein synthesis (Schmitz and Ecker, 2008; Araujo et al., 2010). Farran et al. (2002) observed improved health and immune response of cattle fed diets containing high levels of linolenic acid, potentially resulting from similar mechanisms previously described.

Both the cannabinoid and fatty acid mechanisms discussed primarily focus on the oil component of hempseeds. During hempseed cake production much of the oil is extracted, but not all of it is removed. Hempseed cake oil concentration has been reported to be between 9.5-12.7% (DM-basis; Turner et al., 2008; Karlsson et al., 2012), which may be greater than the oil concentration than dried corn distillers grains plus solubles currently fed. This oil concentration and fatty acid profile difference between these feedstuffs could have potential effects on performance and immune function. Furthermore, while cannabinoids are not explicitly produced by the hempseed, they are present in both the oil and the non-oil components of the seed (Leizer et al., 2000). So, while most of the oil is removed during mechanical cold-pressing, there is still an appreciable amount left in the hempseed cake. Because of the amount of CBD and omega-3

fatty acids necessary to elicit physiological responses has not been established, there is reason to evaluate the potential ability for low concentrations to elicit a physiological response on the immune system.

1.6. Lipopolysaccharide and Inflammation

1.6.1. Overview of Lipopolysaccharide

Lipopolysaccharide (LPS) is an innate immune system activator and can induce systemic inflammation and potentially sepsis if the amount of LPS exceeds certain limits (Lu et al., 2008; Freudenberg et al., 2008). LPS is considered an endotoxin and is a major structural component found in the outer membrane of gram-negative bacteria exclusively. LPS is comprised of three domains: lipid A, a core oligosaccharide, and an O side chain (Lu et al., 2008). Furthermore, LPS has two forms, smooth (S) and rough (R) with the S form consisting of all three parts, while the R form consists only of lipid A and the core oligosaccharide (Figure 1-3). Both S and R forms are considered endotoxins because lipid A contains the biological activity of the entire molecule (Freudenberg et al., 2008).

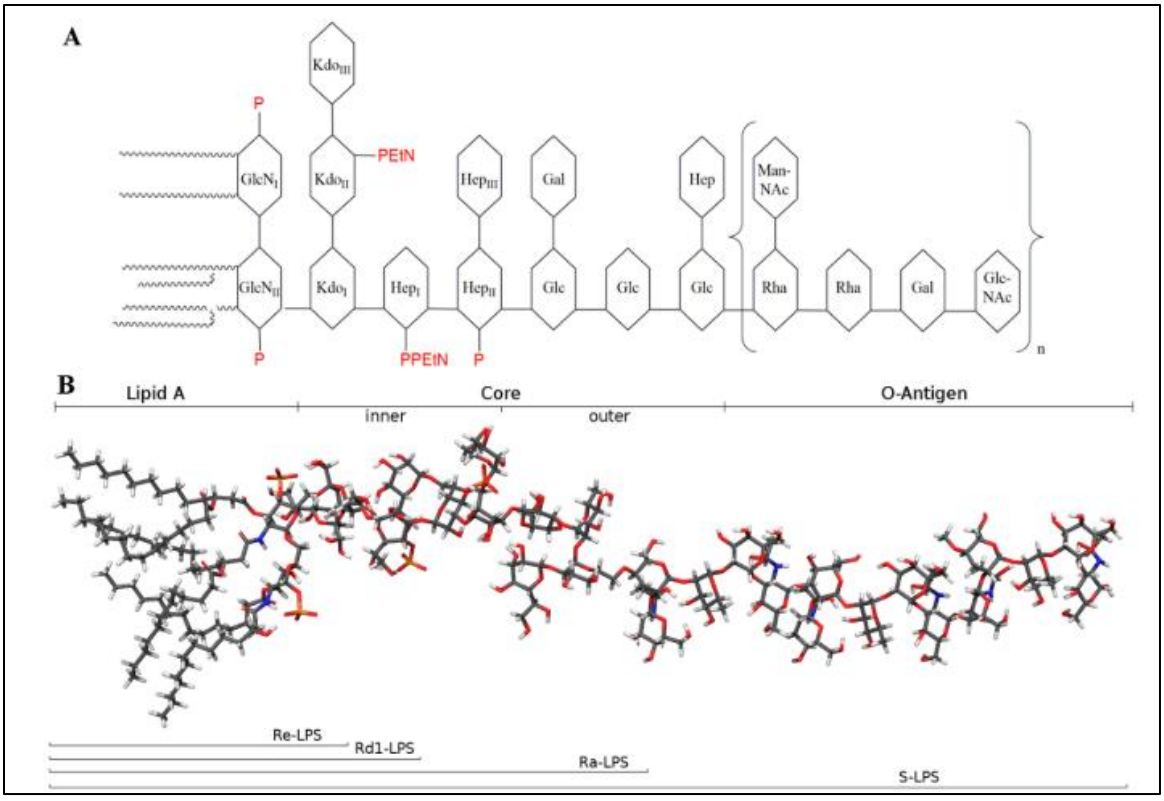

Figure 1-3. *E. coli* LPS: smooth-lipopolysaccharide (S-LPS) is composed by lipid A, core and O-antigens; truncated rough-LPS (R-LPS) are named Ra, Rd1, and Re depending on the number of sugar units of the core. (A) Schematic representation (B) Chemical structure (Cochet and Peri, 2017).

1.6.2. LPS Mode of Action

Pathogens, like LPS, contain pathogen-associated molecular patterns (PAMPs) that are detected by innate immune system cells pattern-recognition receptors (PRRs), such as toll-like receptor 4 (TLR4). When PRR detect PAMP it signals the detection and presence of infectious material, which ultimately will stimulate an inflammatory response (Kieser and Kagan, 2017). The inflammatory process is initiated after PAMP detection by LPS-binding protein (LPB), which extracts the LPS monomer from the whole LPS molecule, and transfers it to the cluster of differentiation 14 protein (CD14; Cochet and Peri, 2017) located at the plasma membrane of the innate immune cells (Kieser and Kagan, 2017). CD14 then transfers the LPS monomer to myeloid differentiation factor 2 (MD-2), which forms a hexamer complex with TLR4, MD-2,

and LPS. LPS is transferred from LBP to this complex because it has greater affinity for LPS (Gioannini et al., 2004). While TLR4 cannot bind directly to LPS, it enhances the binding of LPS to MD-2 (Lu et al., 2008).

Once this hexamer has been formed, MD-2 initiates Myeloid differentiation primary response 88 (MyD88)-dependent signaling on the intracellular side of the innate immune cell (Cochet and Peri, 2017). Taking a step back, TLR4 recruits multiple downstream adaptors through interactions with toll-interleukin-1 receptor (TIR) domains, including MyD88, TIR domain-containing adapter protein (TIRAP), TIR domain-containing adaptor inducing IFN-β (TRIF), and TRIF-related adaptor molecule (TRAM). TLR4 signaling occurs through MyD88-dependent and TRIF-dependent pathways to continue the intracellular signaling to continue the inflammation cascade (Figure 1-4; Lu et al. 2008).

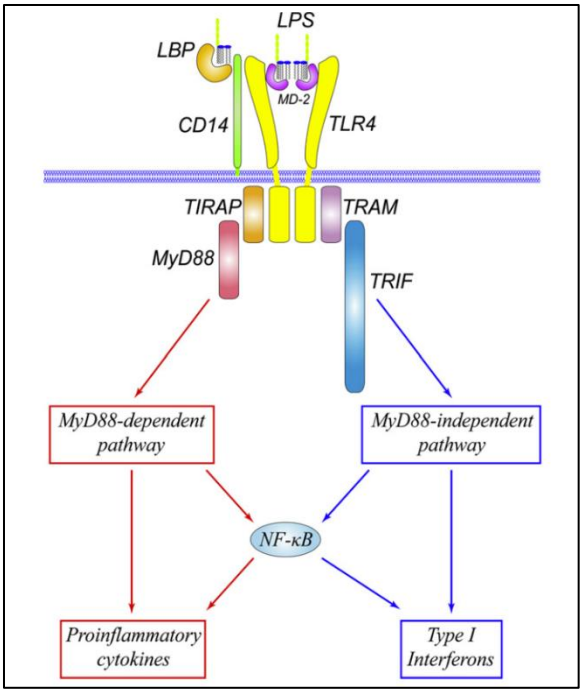

Figure 1-4. Overview of LPS/TLR4 signaling LPS recognition is facilitated by LBP and CD14, and is mediated by TLR4/MD-2 receptor complex. LPS/ TLR4 signaling can be separated into MyD88-dependent and MyD88- independent pathways, which mediate the activation of proinflammatory cytokine and Type I interferon genes (Lu et al., 2008).

The MyD88-dependent pathway leads to the formation of the myddosome, which stimulates nuclear factor-κB (NF-κB) and activator protein-1 (AP1), ultimately upregulating the production of cytokines within the innate immune cell (macrophages and lymphocytes, primarily; Figure 1-5). This is the cell membrane pathway initiates the inflammatory response. The TRIF-dependent pathway is the intracellular pathway to initiate an inflammatory response, and proceeds by triffosome formation and subsequent endocytosis of the TIR domains, stimulating AP1 and NF-κB, which leads to upregulation of cytokine production (Kieser and Kagan, 2017). While there are still unknown components within these pathways, it has been suggested that the endocytosis pathway can proceed independent of TLR4-TRIF signaling through the functions of CD14, and can even occur before TLR4 signaling has initiated

inflammatory pathways (Zanoni et al., 2011). The myddosome and triffosome pathways complement each other and help upregulate AP1 and NF-κB (Kieser and Kagan, 2017).

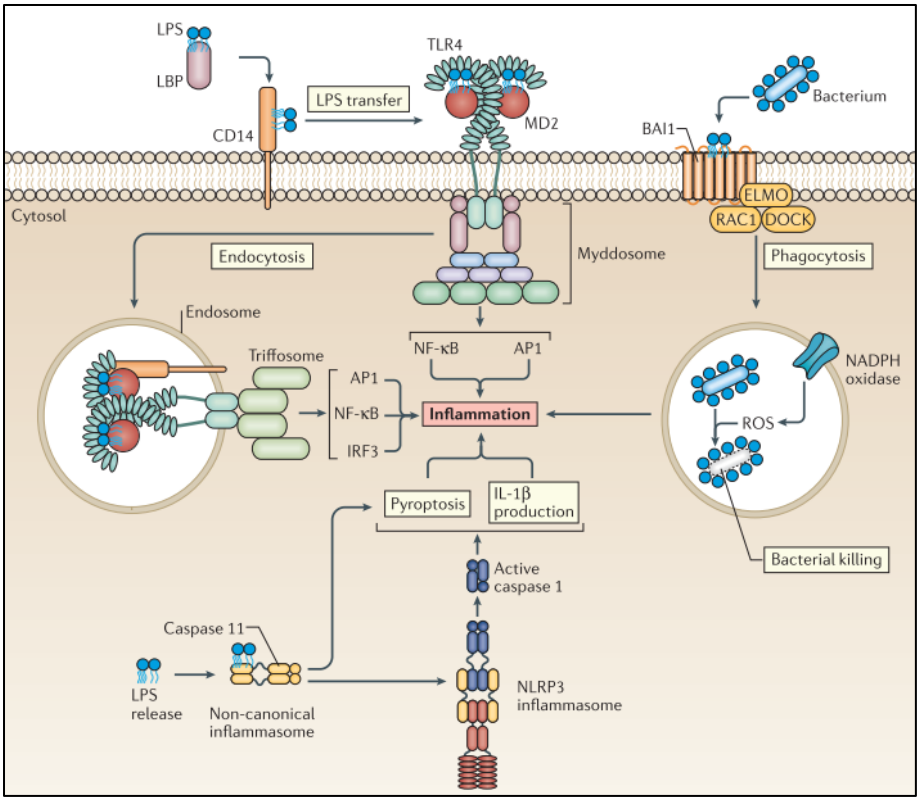

Figure 1-5. Pathways for LPS to promote inflammation (Kieser and Kagan, 2017).

1.6.3. LPS as a Tool to Evaluate Immune Response

LPS from *Escherichia Coli* (*E. coli*) is often used as a research tool because it stimulates inflammation more so than other LPS-containing Gram-negative bacteria (Rosadini and Kagan, 2017) and initiates moderate morbidity without causing mortality (Burdick Sanchez et al., 2020). Administration of LPS to evaluate an immune response has been conducted across a variety of species, including mice, sheep, cattle, and humans amongst others. Typically, the effects of LPS on the immune system is characterized by a rapid increase in pyrogenic cytokines and subsequent body temperature, leukocytes, acute phase protein production, hypoglycemia, and insulin production, often resulting in reduced DMI and animal performance (Jacobsen et al.,

2005; Kvidera et al., 2016; Oh et al., 2017; Littlejohn et al., 2019). Furthermore, glycogenolysis and gluconeogenesis will increase (McGuinness, 1994) as well as an increase in plasma non-esterified fatty acids (NEFA) concentrations (Kvidera et al., 2017) in attempt to compensate for the increased energetic of the immune response (Kvidera et al., 2016). However, the NEFA response has been observed to occur later in the immune response cascade than glucose depletion or insulin production (Kvidera et al., 2017). Dose of LPS provided is important, can vary depending on species, and has been suggested that cattle may be several thousand times more sensitive to LPS than laboratory animals (Jacobsen et al., 2005). The literature shows a range of 2 ng/kg to 2 μg/kg for experiments in cattle (Burdick Sanchez et al., 2020).

The immune response to LPS administration is typically quite quick, with pyrogenic cytokines being upregulated within 1-2 hours and body temperature increasing within 1-3 hours post-administration (Littejohn et al., 2019), whereas some acute phase proteins may continue to rise across a 24 hour period (Waggoner et al., 2009; Oh et al., 2017). Intravenous injections are the most common method of administering LPS in livestock species, while intraperitoneal injections are often used in laboratory animals (Ribeiro et al., 2015).

Cytokines are chemical messengers between immune cells (You et al., 2011) and are commonly studied in animal models subjected to an LPS challenge because they respond to LPS administration and are thought to mediate the metabolic response to bacterial infection (Waldron et al., 2003). There are many different cytokines, often described as pro-inflammatory or anti-inflammatory and are primarily produced and secreted by macrophages and monocytes during times of stress (Calder, 2001; You et al., 2011; Littlejohn et al., 2019). Of the pro-inflammatory cytokines, TNF-α, IL-1α, IL-1β, and IL-6 are considered pyrogenic and initiate the febrile response typically observed post-LPS administration (Burdick Sanchez et al., 2020). Of these,

TNF-α is typically the first cytokine to be increased in circulation, and has the ability to stimulate the release of other pro-inflammatory cytokines, including IL-6 and IFNγ (Burdick Sanchez et al. 2020). Furthermore, TNF-α, IL-1 and IL-6 activate neutrophils, monocytes, and macrophages to initiate phagocytosis of foreign bacteria, stimulate T and B lymphocyte production, and upregulate production of other cytokines (Calder, 2001). Pro-inflammatory cytokines increase in response to bacterial infection, which triggers anti-inflammatory cytokines, such as IL-4 and IL-10 to be upregulated to mediate the inflammatory response (You et al., 2011).

In general, cytokine production should increase in response to LPS administration. This response is necessary and beneficial for the host to mount an immune respo nse, however, prolonged and/or over-production of cytokines can have negative consequences that occur under inflammatory conditions (Calder, 2001). The upregulation of inflammation through cytokine production can have detrimental implications on animal performance and health, as anorexia and catabolic processes often accompany and activated immune system (Figure 1-6).

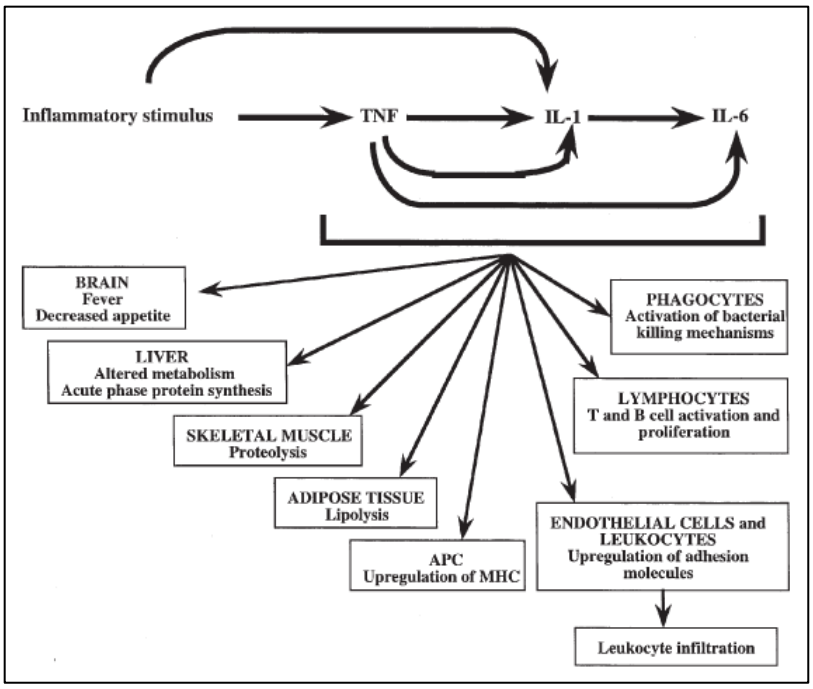

Figure 1-6. Key roles of proinflammatory cytokines in mediating the host innate immune response and in integrating the innate and acquired immune responses (Calder, 2001).

1.7. Experiments Evaluating CBD and the Immune System

While the influence of CBD on immune response parameters after LPS administration has not been evaluated in cattle, Kleinhenz et al (2020a) has indicated the absorptive potential of cannabinoids when orally administering hemp material to cattle and confirmed that cannabinoids are indeed present in plasma. Furthermore, there has been some promising research conducted in various species showing reduced inflammation with CBD supplementation, likely from the proposed mechanisms of action described earlier. Costa et al. (2004) provided CBD at 0, 5, 7.5, 10, 20, and 40 mg/kg BW to mice that were subjected to a carrageenan injection, which is used to initiate an inflammatory response similar to that of LPS. The authors were interested in COX-2 and subsequent PGE_2 concentrations, as these are known to increase during immune responses as a result of pyrogenic cytokine upregulation (Broom, 2007). Costa et al. (2004) observed that as CBD concentration increased, COX-2 measured in the paw tissue and plasma PGE_2

decreased. These authors suggest that the antioxidant properties of CBD may be the reason for this reduction in inflammatory indicators. Perhaps, as previously discussed, supplemental CBD could be resulting in decreased pyrogenic cytokines by downregulation of GPR55 and CB2, while upregulating TRPV1, resulting in reduced COX-2 activity and PGE_2 synthesis.

Ribeiro et al. (2015) conducted an experiment evaluating the effects of supplemental CBD on lung function and inflammation in mice when challenged with LPS. CBD was injected intraperitoneally (IP) at 0, 20, or 80 mg/kg BW. Decreases in TNF-α, IL-6, and two chemokines (MCP-1, and MIP-2), which are a subcategory of cytokines, were all reduced when 80 mg/kg BW of CBD was administered. All cytokines besides IL-6 were decreased in mice administered with 20 mg/kg BW compared to the control group treated with LPS. Furthermore, total leukocytes as well as myeloperoxidase, which is an indicator of neutrophil infiltration, were decreased for both CBD treatments compared to the control. These authors suggest that while the mode of action for anti-inflammation effects of CBD is not completely understood, CBD is decreases NF-κB activation and subsequent cytokine production. Once again, this points towards the possible mode of action described earlier, implicating an upregulation of TRPV1 and/or downregulation of GPR55 and CB2 and reducing downstream cytokine production. These same authors conducted a different experiment (Ribeiro et al., 2012) exploring the effects of supplementation of CBD on inflammation and concluded that the decreased inflammation that was observed when providing 20 mg/kg BW of CBD to mice challenged with LPS was a result of increased adenosine signaling. This suggests that the upregulation of adenosine receptor A2A is likely an important mechanism mediating the anti-inflammatory effects of CBD. However, Riberio et al. (2012) concluded that other factors could be involved as well, and more research is needed.

Pagano et al. (2016) evaluated the effect of pure CBD and CBD-BDS, which is obtained from a high-CBD containing strain of *Cannabis* L compared to a control treatment on inflammatory markers in response to dinitrobenzenesulfonic acid (DNBS) injection in the colon. DNBS is used in a similar fashion as LPS to initiate an immune response. These authors also evaluated oral and IP administration of both CBD and CBD-BDS. Both CBD treatments were delivered on an equal CBD-basis at 0, 5, 10, and 30 mg/kg BW via IP injection, and 0, 10, 30, and 60 mg/kg BW via oral administration. Liver and colon samples were collected to evaluate CBD concentration from oral administration to identify if each CBD form was metabolized similarly in these tissues. These authors used the ratio of colon weight to colon length to determine amount of inflammation occurring in the colon, with a greater the ratio indicating greater inflammation. Interestingly, CBD-BDS decreased the colon weight to colon length ratio both orally and IP, whereas pure CBD did not influence the colon weight to length ratio, suggesting differences between CBD and CBD-BDS influence on inflammation. Myeloperoxidase was decreased in the colon tissue as well for the CBD-BDS treatment, implying inflammation was decreased (these authors did not report myeloperoxidase concentration for the pure CBD treatment, however). CBD was found in greater concentration in colon tissue for the pure CBD treatment compared to CBD-BDS, while CBD-BDS treatment had greater CBD concentration in the liver. These results imply that even though oral administration of pure CBD led to more CBD in the colon, it did not influence inflammation parameters. These authors suggest that CBD-BDS has other cannabinoids present in the product as well, and this could be the reason that CBD-BDS had a greater effect on reducing inflammation. This is interesting when considering that hempseed cake fed to cattle in the experiments conducted for

this dissertation contains CBD as well as other cannabinoids, which could influence results observed.

Overall, there is strong evidence throughout the literature to suggest that CBD exhibits anti-inflammatory effects in mice (Costa et al., 2004; Kozela et al., 2010; Ribeiro et al., 2012; Li et al., 2013; Ribeiro et al., 2015), and humans (Oláh et al., 2014; White, 2019). While the mechanism of action of how CBD influences on inflammatory pathways is still not completely understood (Kozela et al., 2010; White, 2019), CBD may have beneficial effects on immune function. Directly applying these results to ruminants is inexpedient, but there are some intriguing results observed that could potentially influence ruminant physiology in a similar manner, warranting further investigation.

1.8. Experiments Evaluating Fatty Acids and the Immune System

In general, saturated fatty acids are pro-inflammatory, unsaturated fatty acids are neutral to somewhat pro-inflammatory, and omega-3 PUFAs are anti-inflammatory (Im, 2016). Fish oil is the most common dietary addition to provide omega-3 fatty acids to ruminants, and fish oil contains high levels of omega-3 fatty acids largely from consuming algae that contain high concentrations of omega-3. Carvalho et al. (2018) provided a microalgae product to finishing steers to determine the ability of omega-3 fatty acids in this form to bypass ruminal biohydrogenation. While omega fatty acid biohydrogenation can be quite high (Duckett et al. 2002), PUFA's have been shown to inhibit some bacterial species that are responsible for biohydrogenation (Jenkins et al., 2008), potentially sparing some omega-3 fatty acids from complete biohydrogenation.

Carvalho et al. (2018) observed an increase in plasma omega-3 and a decrease in plasma omega-6 fatty acids, shifting the ratio of n6:n3 in a potentially favorable direction. A similar

increase in plasma omega-3 fatty acids was observed when flaxseed, which has a fatty acid profile that contains 54% linolenic acid, was fed to growing steers (Farran et al., 2008). While Carvalho et al. (2018) did not address immune function, the ability to have omega-3 bypass ruminal biohydrogenation could potentially be observed when feeding hempseed cake, ultimately influencing immune response and inflammation.

The inclusion of PUFA in animal diets has been shown to influence the immune response, but the mechanisms involved are still not completely understood (Araujo et al., 2010). Farran et al. (2002) fed diets containing tallow (no omega-3 PUFAs), flaxseed (contains omega-3 PUFAs), and microalgae (contains omega-3 PUFAs) to finishing steers challenged with LPS injections to determine potential immune response influences of dietary lipid supplementation. These authors observed that steers that received flaxseed and microalgae had reduced rectal temperatures, while TNF-α, and acute phase proteins were not influenced by treatment. They conclude that dietary lipid source may have a significant influence on immune response, but further understanding of mechanisms controlling this is needed.

Farran et al. (2008) conducted another series of experiments evaluating the effects of flaxseed, tallow and full-fat soybeans (contains high concentration of omega-6 PUFAs) on immune response parameters when challenged with LPS. Once again, rectal temperature was decreased, while cytokines and acute phase proteins were not influenced by flaxseed treatment. These authors indicate that the temperature reduction may be related to decreased PGE_2 synthesis of immune cells, as omega-3 fatty acids are known to compete with arachidonic acids for synthesizing enzymes, ultimately reducing PGE_2. An experiment by Araujo et al. (2010) evaluated the effect of a rumen-protected PUFA supplementation and a rumen-protected saturated fatty acid compared to no lipid supplementation on inflammation parameters in beef

calves that were under stress as a result of transportation to the feedyard. The supplemented PUFA contained 3% linolenic and 28% linoleic omega fatty acids. These authors observed a reduction in the acute phase protein, haptoglobin, across the first 8 days at the feedyard after transportation. Other experiments have shown a reduction in cytokines when supplementing linolenic acid in various forms to dairy cows (Rezamand et al., 2009).

Although the results of experiments feeding omega-3 fatty acids to cattle have not consistently decreased cytokine or acute phase protein production, many studies in lab species (Yaqoob and Calder, 1995; Oh et al., 2010; Li et al., 2013) and humans (Calder, 1996; Wigmore et al., 1997) have shown the potential of linolenic acid and its derivatives (EPA, DHA) to have a positive influence immune function. For more information on this topic, refer to a review by Im (2016). Amount of omega-3 fatty acid in the plasma is likely important, and because ruminant microbes can convert PUFA to saturated fatty acids quite efficiently, the amount of omega-3 PUFA available in plasma may be lower than the concentrations needed to influence cytokine and acute phase protein production.

1.9. Literature Summary

Industrial hemp production and subsequent hempseed oil extraction offers intriguing opportunities to utilize byproducts from this industry as feedstuffs for ruminants. Hempseed cake, the most commonly studied hempseed oil byproduct, likely has greater potential use in ruminant diets because of the high fiber and protein concentration, while still containing appreciable lipid concentrations. Ruminants, particularly cattle, are best-equipped of the domesticated livestock species in the US to utilize hempseed cake because of their ability to convert fiber that is indigestible to mammalian enzymes, into energy to support efficient animal growth. The current data on hemp byproducts as a feedstuff for ruminants suggest that intake is

not influenced, while ADG and G:F are consistently decreased, likely resulting in lower final BW. Furthermore, the fatty acid profile and cannabinoids within hempseed cake have been shown to have positive influences on immune response in other species, suggesting that there could be a positive immune response when fed to cattle as well. However, there are no known experiments in cattle that have evaluated this. Overall, there is a lack of research evaluating the performance, digestibility, and health implications of feeding hempseed cake to beef cattle. The objectives of these experiments in this dissertation were to 1) evaluate the effects of hempseed cake on growth performance, feeding behavior, plasma metabolite concentrations across time, and carcass characteristics when fed in finishing diets to heifers in comparison to dried corn distillers grains plus solubles (Chapter 2), 2) investigate the effects of dietary inclusion of hempseed cake on ruminal fermentation parameters, nutrient total tract apparent digestibility, nutrient flow, and nitrogen balance in finishing steers in comparison to dried corn distillers grains plus solubles and a control diet containing no byproduct (Chapter 3), and 3) explore the effects of dietary inclusion of hempseed cake on immune parameters in response to an endotoxin challenge in finishing steers in comparison to dried corn distillers grains plus solubles and a control diet containing no byproduct (Chapter 4).

1.10. Literature Cited

Abrahamsen, F. W., N. K. Gurung, W. Abebe, G. P. Reddy, K. Mullenix, and S. Adhikari. 2021. Effects of feeding varying levels of hempseed meal on dry matter intake, rumen fermentation, in vitro digestibility, blood metabolites, and growth performance of growing meat goats. Appl. Anim. Sci. 37:681–688. doi:10.15232/aas.2021-02153.

Ailhaud, G., F. Massiera, P. Weill, P. Legrand, J. Alessandri, and P. Guesnet. 2006. Temporal changes in dietary fats: Role of n−6 polyunsaturated fatty acids in excessive adipose

tissue development and relationship to obesity. Prog. Lipid Res. 45:203–236. doi:10.1016/j.plipres.2006.01.003.

Anderson, R. V., R. J. Rasby, T. J. Klopfenstein, and R. T. Clark. 2005. An evaluation of production and economic efficiency of two beef systems from calving to slaughter. J. Anim. Sci. 83:694–704. doi:10.2527/2005.833694x.

Araujo, D. B., R. F. Cooke, G. R. Hansen, C. R. Staples, and J. D. Arthington. 2010. Effects of rumen-protected polyunsaturated fatty acid supplementation on performance and physiological responses of growing cattle after transportation and feedlot entry. J. Anim. Sci. 88:4120–4132. doi:10.2527/jas.2009-2684.

Belluzzi, A. 2002. n -3 Fatty acids for the treatment of inflammatory bowel diseases . Proc. Nutr. Soc. 61:391–395. doi:10.1079/pns2002171.

Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370–1381. doi:10.3168/jds.S0022-0302(03)73721-7.

Broom, M. 2007. Physiology of fever. Paediatr. Nurs. 19:40–44. doi:10.7748/paed.19.6.40.s32.

Bruni, N., C. Della Pepa, S. Oliaro-Bosso, E. Pessione, D. Gastaldi, and F. Dosio. 2018. Cannabinoid delivery systems for pain and inflammation treatment. Molecules. 23. doi:10.3390/molecules23102478.

Burdick Sanchez, N. C., J. A. Carroll, P. Rand Broadway, T. S. Edrington, I. Yoon, and C. R. Belknap. 2020. Some aspects of the acute phase immune response to a lipopolysaccharide (LPS) challenge are mitigated by supplementation with a Saccharomyces cerevisiae fermentation product in weaned beef calves. Transl. Anim. Sci. 4:1–13. doi:10.1093/tas/txaa156.

Burstein, S. 2015. Cannabidiol (CBD) and its analogs: A review of their effects on inflammation. Bioorganic Med. Chem. 23:1377–1385. doi:10.1016/j.bmc.2015.01.059.

Calder, P. 2001. Polyunsaturated fatty acids and immunity. Lipids. 36:1007–1024. doi:10.1007/s11745-001-0812-7.

Calder, P. C. 1996. Immunomodulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. Proc. Nutr. Soc. 55:737–774. doi:10.1079/PNS19960069.

Callaway, J. C. 2004. Hempseed as a nutritional resource: An overview. Euphytica. 140:65–72. doi:10.1007/s10681-004-4811-6.

Carrier, E. J., J. A. Auchampach, and C. J. Hillard. 2006. Inhibition of an equilibrative nucleoside transporter by cannabidiol: A mechanism of cannabinoid immunosuppression. Proc. Natl. Acad. Sci. U. S. A. 103:7895–7900. doi:10.1073/pnas.0511232103.

Carvalho, J. R. R., K. M. Brennan, M. M. Ladeira, and J. P. Schoonmaker. 2018. Performance, insulin sensitivity, carcass characteristics, and fatty acid profile of beef from steers fed microalgae. J. Anim. Sci. 96:3433–3445. doi:10.1093/jas/sky210.

Cochet, F., and F. Peri. 2017. The role of carbohydrates in the lipopolysaccharide (LPS)/toll-like receptor 4 (TLR4) Signalling. Int. J. Mol. Sci. 18. doi:10.3390/ijms18112318.

Costa, B., M. Colleoni, S. Conti, D. Parolaro, C. Franke, A. E. Trovato, and G. Giagnoni. 2004. Oral anti-inflammatory activity of cannabidiol, a non-psychoactive constituent of cannabis, in acute carrageenan-induced inflammation in the rat paw. Naunyn. Schmiedebergs. Arch. Pharmacol. 369:294–299. doi:10.1007/s00210-004-0871-3.

Cruz, G. D., J. A. Rodríguez-Sánchez, J. W. Oltjen, and R. D. Sainz. 2010. Performance, residual feed intake, digestibility, carcass traits, and profitability of angus-hereford steers housed in individual or group pens. J. Anim. Sci. 88:324–329. doi:10.2527/jas.2009-1932.

Duckett, S. K., J. G. Andrae, and F. N. Owens. 2002. Effect of high-oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. J. Anim. Sci. 80:3353–3360. doi:10.2527/2002.80123353x.

Eriksson, M. 2007. Hemp seed cake as a protein feed for growing cattle. MSc thesis, student report 128. Swedish University of Agricultural Sciences. Department of Animal Environment and health. Skara. Retrieved from: https://stud.epsilon.slu.se/10740/.

Farran, T. B., C. D. Reinhardt, D. A. Blasi, J. E. Minton, T. H. Elsasser, J. J. Higgins, and J. S. Drouillard. 2008. Source of dietary lipid may modify the immune response in stressed feeder cattle. J. Anim. Sci. 86:1382–1394. doi:10.2527/jas.2007-0116.

Farran, T. B., M. F. Spire, J. J. Sindt, S. P. Montgomery, C. M. Coetzer, H. J. LaBrune, J. E. Minton, J. J. Higgins, T. H. Elsasser, and J. S. Drouillard. 2002. Immune response in feeder cattle fed different sources of dietary lipid. Kansas Agric. Exp. Stn. Res. Reports. 5–9. doi:10.4148/2378-5977.1677.

Fike, J. 2016. Industrial Hemp: Renewed Opportunities for an Ancient Crop. CRC. Crit. Rev. Plant Sci. 35:406–424. doi:10.1080/07352689.2016.1257842.

Freudenberg, M. A., S. Tchaptchet, S. Keck, G. Fejer, M. Huber, N. Schütze, B. Beutler, and C. Galanos. 2008. Lipopolysaccharide sensing an important factor in the innate immune response to Gram-negative bacterial infections: Benefits and hazards of LPS hypersensitivity. Immunobiology. 213:193–203. doi:10.1016/j.imbio.2007.11.008.

Gibb, D. J., M. A. Shah, P. S. Mir, and T. A. McAllister. 2005. Effect of full-fat hemp seed on performance and tissue fatty acids of feedlot cattle. Can. J. Anim. Sci. 85:223–230. doi:10.4141/A04-078.

Gioannini, T. L., A. Teghanemt, D. Zhang, N. P. Coussens, W. Dockstader, S. Ramaswamy, and J. P. Weiss. 2004. Isolation of an endotoxin-MD-2 complex that produces Toll-like receptor 4-dependent cell activation at picomolar concentrations. Proc. Natl. Acad. Sci. 101:4186–4191. doi:10.1073/pnas.0306906101.

Helstad, A., E. Forsén, C. Ahlström, I. C. Mayer Labba, A. S. Sandberg, M. Rayner, and J. K. Purhagen. 2022. Protein extraction from cold-pressed hempseed press cake: From laboratory to pilot scale. J. Food Sci. 87:312–325. doi:10.1111/1750-3841.16005.

Hessle, A., M. Eriksson, E. Nadeau, T. Turner, and B. Johansson. 2008. Cold-pressed hempseed cake as a protein feed for growing cattle. Acta Agric. Scand. A Anim. Sci. 58:136–145. doi:10.1080/09064700802452192.

Im, D. S. 2016. Functions of omega-3 fatty acids and FFA4 (GPR120) in macrophages. Eur. J. Pharmacol. 785:36–43. doi:10.1016/j.ejphar.2015.03.094.

Jacobsen, S., T. Toelboell, and P. H. Anderson. 2005. Dose dependency and individual variability in selected clinical, haematological and blood biochemical responses after systemic lipopolysaccharide challenge in cattle. Vet. Res. 36:167–178. doi:10.1051/vetres.

Jenkins, T. C., R. J. Wallace, P. J. Moate, and E. E. Mosley. 2008. Board-Invited Review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. J. Anim. Sci. 86:397–412. doi:10.2527/jas.2007-0588.

Jennings, J. S., B. E. Meyer, P. J. Guiroy, and N. Andy Cole. 2018. Energy costs of feeding excess protein from corn-based by-products to finishing cattle. J. Anim. Sci. 96:653–669. doi:10.1093/jas/sky021.

Karlsson, L., M. Finell, and K. Martinsson. 2010. Effects of increasing amounts of hempseed cake in the diet of dairy cows on the production and composition of milk. Animal. 4:1854–1860. doi:10.1017/S1751731110001254.

Karlsson, L., M. Hetta, P. Udén, and K. Martinsson. 2009. New methodology for estimating rumen protein degradation using the in vitro gas production technique. Anim. Feed Sci. Technol. 153:193–202. doi:10.1016/j.anifeedsci.2009.06.010.

Karlsson, L., and K. Martinsson. 2011. Growth performance of lambs fed different protein supplements in barley-based diets. Livest. Sci. 138:125–131. doi:10.1016/j.livsci.2010.12.010.

Karlsson, L., M. Ruiz-Moreno, M. D. Stern, and K. Martinsson. 2012. Effects of temperature during moist heat treatment on ruminal degradability and intestinal digestibility of protein and amino acids in hempseed cake. Asian-Australasian J. Anim. Sci. 25:1559–1567. doi:10.5713/ajas.2012.12213.

Kieser, K. J., and J. C. Kagan. 2017. Multi-receptor detection of individual bacterial products by the innate immune system. Nat. Rev. Immunol. 17:376–390. doi:10.1038/nri.2017.25.

Kleinhenz, M. D., G. Magnin, S. M. Ensley, J. J. Griffin, J. Goeser, E. Lynch, and J. F. Coetzee. 2020a. Nutrient concentrations, digestibility, and cannabinoid concentrations of industrial hemp plant components. Appl. Anim. Sci. 36:489–494. doi:10.15232/aas.2020-02018.

Kleinhenz, M. D., G. Magnin, Z. Lin, J. Griffin, K. E. Kleinhenz, S. Montgomery, A. Curtis, M. Martin, and J. F. Coetzee. 2020b. Plasma concentrations of eleven cannabinoids in cattle following oral administration of industrial hemp (Cannabis sativa). Sci. Rep. 10:1–7. doi:10.1038/s41598-020-69768-4.

Kozela, E., M. Pietr, A. Juknat, N. Rimmerman, R. Levy, and Z. Vogel. 2010. Cannabinoids Δ9-Tetrahydrocannabinol and Cannabidiol Differentially Inhibit the Lipopolysaccharide-activated NF-κB and Interferon-β/STAT Proinflammatory Pathways in BV-2 Microglial Cells. J. Biol. Chem. 285:1616–1626. doi:10.1074/jbc.M109.069294.

Krebs, G. L., D. W. De Rosa, D. M. White, B. L. Blake, K. C. Dods, C. D. May, Z. X. Tai, E. H. Clayton, and E. E. Lynch. 2021. Intake, nutrient digestibility, rumen parameters, growth rate, carcase characteristics and cannabinoid residues of sheep fed pelleted rations containing hemp (*Cannabis sativa* L.) stubble. Transl. Anim. Sci. 5:1-13. doi: 10.1093/tas/txab213.

Krehbiel, C. R., J. J. Cranston, and M. P. McCurdy. 2006. An upper limit for caloric density of finishing diets. J. Anim. Sci. 84:E34-E49. doi: 10.2527/2006.8413_supplE34x

Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. S. Fernandez, and L. H. Baumgard. 2017. Glucose requirements of an activated immune system in lactating Holstein cows. J. Dairy Sci. 100:2360–2374. doi:10.3168/jds.2016-12001.

Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L. H. Baumgard. 2016. Technical note: A procedure to estimate glucose requirements of an activated immune system in steers. J. Anim. Sci. 94:4591–4599. doi:10.2527/jas.2016-0765.

Leizer, C., D. M. Ribnicky, A. Poulev, D. Dushenkov, and I. Raskin. 2000. The composition of hemp seed oil and its potential as an important source of nutrition. J. Nutraceuticals, Funct. Med. Foods. 2:35–53. doi:10.1300/J133v02n04_04.

Li, X., Y. Yu, and C. D. Funk. 2013. Cyclooxygenase-2 induction in macrophages is modulated by docosahexaenoic acid via interactions with free fatty acid receptor 4 (FFA4). FASEB J. 27:4987–4997. doi:10.1096/fj.13-235333.

Littlejohn, B. P., N. C. B. Sanchez, J. A. Carroll, D. M. Price, R. C. Vann, T. H. Welsh, and R. D. Randel. 2019. Influence of prenatal transportation stress on innate immune response to an endotoxin challenge in weaned Brahman bull calves. Stress. 22:236–247. doi:10.1080/10253890.2018.1523895.

Liu, K. 2011. Chemical composition of distillers grains, a review. J. Agric. Food Chem. 59:1508–1526. doi:10.1021/jf103512z.

Lu, Y. C., W. C. Yeh, and P. S. Ohashi. 2008. LPS/TLR4 signal transduction pathway. Cytokine. 42:145–151. doi:10.1016/j.cyto.2008.01.006.

Mark, T., J. Shepherd, D. Olson, W. Snell, S. Proper, and S. Thornsbury. 2020. Economic Viability of Industrial Hemp in the United States: A Review of State Pilot Programs. United States Dep. Agric. 11.

Di Marzo, V., and F. Piscitelli. 2015. The Endocannabinoid System and its Modulation by Phytocannabinoids. Neurotherapeutics. 12:692–698. doi:10.1007/s13311-015-0374-6.

McGuinness, O. P. 1994. The impact of infection on gluconeogenesis in the concious dog. Shock. 2:336–343. doi:10.1097/00024382-199411000-00007.

McPartland, J. M., M. Duncan, V. Di Marzo, and R. G. Pertwee. 2015. Are cannabidiol and Δ9-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br. J. Pharmacol. 172:737–753. doi:10.1111/bph.12944.

de Meijer, E. P. M., M. Bagatta, A. Carboni, P. Crucitti, V. M. C. Moliterni, P. Ranalli, and G. Mandolino. 2003. The Inheritance of Chemical Phenotype in Cannabis sativa L. Genetics. 163:335–346. doi:10.1093/genetics/163.1.335.

Moore, K. J., and H. J. G. Jung. 2001. Lignin and fiber digestion. J. Range Manag. 54:420–430. doi:10.2307/4003113.

Mustafa, A. F., J. J. McKinnon, and D. A. Christensen. 1999. The nutritive value of hemp meal for ruminants. Can. J. Anim. Sci. 79:91–95. doi:10.4141/A98-031.

Oh, D. Y., S. Talukdar, E. J. Bae, T. Imamura, H. Morinaga, W. Q. Fan, P. Li, W. J. Lu, S. M. Watkins, and J. M. Olefsky. 2010. GPR120 Is an Omega-3 Fatty Acid Receptor Mediating Potent Anti-inflammatory and Insulin-Sensitizing Effects. Cell. 142:687–698. doi:10.1016/j.cell.2010.07.041.

Oh, J., M. Harper, F. Giallongo, D. M. Bravo, E. H. Wall, and A. N. Hristov. 2017. Effects of rumen-protected Capsicum oleoresin on immune responses in dairy cows intravenously challenged with lipopolysaccharide. J. Dairy Sci. 100:1902–1913. doi:10.3168/jds.2016-11666.

Oláh, A., B. I. Tóth, I. Borbíró, K. Sugawara, A. G. Szöllõsi, G. Czifra, B. Pál, L. Ambrus, J. Kloepper, E. Camera, M. Ludovici, M. Picardo, T. Voets, C. C. Zouboulis, R. Paus, and T. Bíró. 2014. Cannabidiol exerts sebostatic and antiinflammatory effects on human sebocytes. J. Clin. Invest. 124:3713–3724. doi:10.1172/JCI64628.

Pagano, E., R. Capasso, F. Piscitelli, B. Romano, O. A. Parisi, S. Finizio, A. Lauritano, V. Di Marzo, A. A. Izzo, and F. Borrelli. 2016. An orally active Cannabis extract with high content in cannabidiol attenuates chemically-induced intestinal inflammation and hypermotility in the mouse. Front. Pharmacol. 7:1–12. doi:10.3389/fphar.2016.00341.

Pellati, F., V. Borgonetti, V. Brighenti, M. Biagi, S. Benvenuti, and L. Corsi. 2018. Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer. Biomed Res. Int. 2018. doi:10.1155/2018/1691428.

Pojić, M., A. Mišan, M. Sakač, T. D. Hadnadev D Signev, B. Šarić, I. Milovanović, and M. Hadnadev. 2014. Characterization of byproducts originating from hemp oil processing. J. Agric. Food Chem. 62:12346–12442. doi:10.1021/jf5044426.

Rajasekhar, K., S. Fausto, S. Byron, C. Frank, B. Chris, C. Rodney, and W. Eric. 2021. Characterization of the Nutritional and Safety Properties of Hemp Seed Cake as Animal Feed Ingredient. Int. J. Livest. Prod. 12:53–63. doi:10.5897/ijlp2020.0750.

Rezamand, P., K. M. Hunt, J. E. Williams, S. Shields, and M. A. McGuire. 2009. Effects of trans fatty acids on markers of in- flammation. FASEB J. 23:910.16. doi: 10.1096/fasebj.23.1_supplement.910.16.

Ribeiro, A., V. I. Almeida, C. Costola-De-Souza, V. Ferraz-De-Paula, M. L. Pinheiro, L. B. Vitoretti, J. A. Gimenes-Junior, A. T. Akamine, J. A. Crippa, W. Tavares-De-Lima, and J. Palermo-Neto. 2015. Cannabidiol improves lung function and inflammation in mice submitted to LPS-induced acute lung injury. Immunopharmacol. Immunotoxicol. 37:35–41. doi:10.3109/08923973.2014.976794.

Ribeiro, A., V. Ferraz-De-Paula, M. L. Pinheiro, L. B. Vitoretti, D. P. Mariano-Souza, W. M. Quinteiro-Filho, A. T. Akamine, V. I. Almeida, J. Quevedo, F. Dal-Pizzol, J. E. Hallak, A. W. Zuardi, J. A. Crippa, and J. Palermo-Neto. 2012. Cannabidiol, a non-psychotropic plant-derived cannabinoid, decreases inflammation in a murine model of acute lung

injury: Role for the adenosine A 2A receptor. Eur. J. Pharmacol. 678:78–85. doi:10.1016/j.ejphar.2011.12.043.

Rohbeck, E., J. Eckel, and T. Romacho. 2021. Cannabinoid receptors in metabolic regulation and diabetes. Physiology. 36:102–113. doi:10.1152/physiol.00029.2020.

Rosadini, C. V., and J. C. Kagan. 2017. Early innate immune responses to bacterial LPS. Curr. Opin. Immunol. 44:14–19. doi:10.1016/j.coi.2016.10.005.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico state and Texas tech university survey. J. Anim. Sci. 94:2648–2663. doi:10.2527/jas.2016-0282.

Schmitz, G., and J. Ecker. 2008. The opposing effects of n−3 and n−6 fatty acids. Prog. Lipid Res. 47:147–155. doi:10.1016/j.plipres.2007.12.004.

Small, E. 2015. Evolution and Classification of Cannabis sativa (Marijuana, Hemp) in Relation to Human Utilization. Bot. Rev. 81:189–294. doi:10.1007/s12229-015-9157-3.

Small, E., and D. Marcus. 2002. Hemp: A New Crop with New Uses for North America Hemp: A New Crop with New Uses for North America. 1–39.

Solinas, G., C. Vilcu, J. G. Neels, G. K. Bandyopadhyay, J.-L. Luo, W. Naugler, S. Grivennikov, A. Wynshaw-Boris, M. Scadeng, J. M. Olefsky, and M. Karin. 2007. JNK1 in Hematopoietically Derived Cells Contributes to Diet-Induced Inflammation and Insulin Resistance without Affecting Obesity. Cell Metab. 6:386–397. doi:10.1016/j.cmet.2007.09.011.

Turner, T., A. Hessle, K. Lundström, and J. Pickova. 2008. Influence of hempseed cake and soybean meal on lipid fractions in bovine M. longissimus dorsi. Acta Agric. Scand. A Anim. Sci. 58:152–160. doi:10.1080/09064700802492354.

USDA, Agricultural Marketing Service. 2021. Establishment of a domestic hemp production program, final rule. 7CFR Part 990. Fed. Resist. 86:5596–5691

USDA, National Agriculture Statistics Service. 2022. National hemp report. Retreived from https://downloads.usda.library.cornell.edu/usdaesmis/files/gf06h2430/xd07hw825/v692v917t/hempan22.pdf

USDA, Agricultural Marketing Service. 2022. Daily ethanol report. Retreived from https://www.ams.usda.gov/mnreports/lsdethanol.pdf

Waggoner, J. W., C. A. Löest, J. L. Turner, C. P. Mathis, and D. M. Hallford. 2009. Effects of dietary protein and bacterial lipopolysaccharide infusion on nitrogen metabolism and hormonal responses of growing beef steers1. J. Anim. Sci. 87:3656–3668. doi:10.2527/jas.2009-2011.

Waldron, M. R., T. Nishida, B. J. Nonnecke, and T. R. Overton. 2003. Effect of lipopolysaccharide on indices of peripheral and hepatic metabolism in lactating cows. J. Dairy Sci. 86:3447–3459. doi:10.3168/jds.S0022-0302(03)73949-6.

Weisbjerg, M. R., T. Hvelplund, S. Hellberg, S. Olsson, and S. Sanne. 1996. Effective rumen degradability and intestinal digestibility of individual amino acids in different concentrates determined in situ. Anim. Feed Sci. Technol. 62:179–188. doi:10.1016/S0377-8401(96)00970-4.

White, C. M. 2019. A Review of Human Studies Assessing Cannabidiol's (CBD) Therapeutic Actions and Potential. J. Clin. Pharmacol. 59:923–934. doi:10.1002/jcph.1387.

Wigmore, S. J., K. C. H. Fearon, J. P. Maingay, and J. A. Ross. 1997. Down-Regulation of the Acute-Phase Response in Patients with Pancreatic Cancer Cachexia Receiving Oral

Eicosapentaenoic Acid is Mediated via Suppression of Interleukin-6. Clin. Sci. 92:215–221. doi:10.1042/cs0920215.

Yaqoob, P., and P. Calder. 1995. Effects of Dietary Lipid Manipulation upon Inflammatory Mediator Production by Murine Macrophages. Cell. Immunol. 163:120–128. doi:10.1006/cimm.1995.1106.

You, Z., C. Luo, W. Zhang, Y. Chen, J. He, Q. Zhao, R. Zuo, and Y. Wu. 2011. Pro- and anti-inflammatory cytokines expression in rat's brain and spleen exposed to chronic mild stress: Involvement in depression. Behav. Brain Res. 225:135–141. doi:10.1016/j.bbr.2011.07.006.

Zanoni, I., R. Ostuni, L. R. Marek, S. Barresi, R. Barbalat, G. M. Barton, F. Granucci, and J. C. Kagan. 2011. CD14 Controls the LPS-Induced Endocytosis of Toll-like Receptor 4. Cell. 147:868–880. doi:10.1016/j.cell.2011.09.051.

CHAPTER 2. INFLUENCE OF HEMPSEED CAKE INCLUSION ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, FEEDING BEHAVIOR AND BLOOD PARAMETERS IN FINISHING HEIFERS

2.1. Abstract

As the hemp industry continues to develop in the US, there is interest in feeding byproducts of industrial hemp production to livestock. A completely randomized design experiment using crossbred finishing heifers [initial body weight (**BW**) ± SE = 494 ± 10 kg] was conducted to determine the effects of feeding hempseed cake in a corn-based finishing diet (10% forage) formulated to meet or exceed ruminally degradable and metabolizable protein requirements on growth performance, carcass characteristics, feeding behavior, and plasma parameters. Dietary treatments were inclusion of 20% [dry matter (DM) basis] of: dried corn distillers grains plus solubles (DDGS, n = 16), or hempseed cake (HEMP, n = 15). Cattle were housed in two pens, had *ad-libitum* access to feed and water, and individual intakes and feeding behavior were monitored using the Insentec feeding system. Cattle were fed treatment diets for 111 days, and every 14 days BW were measured, and blood samples collected. Blood plasma was analyzed for glucose, urea nitrogen, and individual amino acids and results analyzed using repeated measures analysis in SAS. Final BW, average daily gain, gain:feed, and hot carcass weight decreased by 2.3%, 7.7%, 7.7%, and 2.6% respectively ($P \leq 0.05$) in heifers fed the HEMP diet than in heifers fed the DDGS diet. Net energy for maintenance and gain (Mcal/kg of feed, DM-basis), estimated based on heifer intake and performance, were greater ($P = 0.02$) for the DDGS diet than the HEMP diet. All other performance and carcass characteristics were not different ($P \geq 0.20$) between treatments. Heifers fed the HEMP diet had greater ($P < 0.05$) plasma urea nitrogen concentration in samples from each collection day compared to heifers fed

the DDGS diet, although there was a treatment by day interaction ($P < 0.01$) because of variability in the magnitude of treatment differences over time. Plasma glucose concentration was not influenced ($P = 0.17$) by dietary treatment. Plasma concentrations of total amino acids, non-essential amino acids, and essential amino acids were not different between treatments ($P \geq 0.09$), although there were several interactions between treatment and day ($P \leq 0.04$) for individual amino acids. These data suggest that hempseed cake has a lower NE_m and NE_g relative to dried corn distillers grains plus solubles when adequate metabolizable protein is supplied, while still providing adequate nutrition to support acceptable performance of finishing cattle.

2.2. Introduction

Industrial hemp has been produced for thousands of years, largely for its fiber, but production started to decrease as cheaper alternatives became favored (Fike, 2016). The Marihuana Tax Act of 1938 stopped industrial hemp production in the United States and placed hemp on the Schedule 1 list of the Controlled Substances Act (USDA, Agriculture Marketing Service, 2021). In recent years there has been renewed interest in industrial hemp production after its removal from the list of US Drug Enforcement Agency Schedule 1 drugs as a result of the 2018 Agricultural Improvement Act. A series of pilot studies were allowed under the 2014 Agricultural Improvement Act and lead to reinstating industrial hemp production cultivation. By statute, industrial hemp must contain less than 0.3% delta-9-tetrahydrocannabinol (THC), which is the psychoactive component of the hemp plant, and is the differentiating component between hemp and marijuana (USDA, Agriculture Marketing Service, 2021). Cannabis with a THC level exceeding 0.3% (DM-basis) remains a Schedule 1 controlled substance. Industrial hemp is produced for its fibers that are used in papers, textiles and many other products that value the

fiber strength, and more recently for the oil found in the hempseed that is used for medicines, paints, detergents, cooking and other uses (Fike, 2016). Processing of the hempseed for oil extraction has increased with the rise in demand for hemp oil for human use (Mark et al., 2020). Hempseed oil extraction creates a byproduct that is high in neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP; 51%, 39%, and 30% respectively) but limited markets are available for it because hemp and hemp byproducts cannot be fed to livestock or pets without FDA approval through the Association of American Feed Control Officials (Kleinhenz et al., 2020).

Because of the nutrient profile of hempseed cake with relatively high CP and ADF concentration, it could be a useful feed ingredient for ruminants because of the potential as protein source and because of the ability of ruminal microbes to convert fiber to usable energy. Furthermore, hemp and hemp byproducts could have therapeutic benefits when fed to livestock because of the 3:1 ratio of linoleic to linolenic omega polyunsaturated fats (Kleinhenz et al., 2020), which is thought to be optimal for human health (Leizer et al., 2000). While most of the seed oil is removed during pressing, hempseed cake contains roughly 7% oil, which could be beneficial if used as a feedstuff. Although feeding hemp byproducts is legal in the European Union (among other places), there are relatively few data on the nutritive value of hempseed cake as a feed ingredient for beef cattle (Hessle et al., 2008). Currently, corn distillers grains is the most common byproduct fed in finishing diets in the US and has similarities in nutrient composition to hempseed cake (CP, NDF, ether extract; Samuelson et al., 2016; Table 2-1). Therefore, the objectives of this study were to determine the effect of including hempseed cake, as compared to dried corn distillers grains plus solubles, in finishing diets on growth performance, carcass characteristics, feeding behavior, and plasma metabolites in heifers.

2.3. Materials and Methods

All animal care and management practices were approved by the North Dakota State University Institutional Animal Care and Use Committee prior to the initiation of the study.

2.3.1. Animals, Experimental Design, and Dietary Treatments

A 111-day finishing study was conducted at the North Dakota State University Beef Cattle Research Complex in Fargo, ND. Thirty-one angus-crossbred finishing heifers [initial body weight (BW) ± SE = 494 ± 10 kg; average age = 19 months] were used. Heifers were weighed two consecutive days at the initiation of the experiment and were assigned randomly to one of two dietary treatments with 16 heifers per pen and one pen per treatment. The dried distillers grains plus solubles treatment (DDGS) contained 55% dry-rolled corn, 20% corn silage, 20% dried corn distillers grains plus solubles, and 5% supplement (DM-basis). The hempseed cake treatment (HEMP) contained the same ingredients except hempseed cake replaced DDGS (DM-basis; Tables 2-1). The 20% inclusion level for dried corn distillers grains plus solubles and hempseed cake was selected as this is a common inclusion level for similar byproducts feeds used in practice (Samuelson et al., 2016).

Table 2-1. Diet ingredients and nutrient composition of diets containing dried corn distillers grains plus solubles (DDGS) or hempseed cake (HEMP).

	Treatments[1]		Byproducts[2]	
Ingredient, % of diet DM	DDGS	Hemp	DDGS	Hemp
Dry-rolled corn	55	55	-	-
Dried distillers grains plus solubles	20	0	-	-
Hempseed cake	0	20	-	-
Corn silage	20	20	-	-
Supplement	5	5	-	-
Fine ground corn	1.82	1.82	-	-
Limestone	2	2	-	-
Salt	0.1	0.1	-	-
Urea	1	1	-	-
Vitamin premix[3]	0.01	0.01	-	-
Trace mineral premix[4]	0.05	0.05	-	-
Rumensin-90[5]	0.02	0.02	-	-
Nutrient analyses, %[6]				
Dry matter	66.0	65.1	88.8	90.9
Ash	5.79	6.39	5.49	8.52
Starch	43.7	43.2	6.93	1.14
Crude protein	14.8	15.8	29.6	33.9
Ether extract	3.47	3.38	5.70	7.39
NDF	29.1	30.4	49.6	50.4
ADF	11.4	16.3	15.6	36.3
Calcium	0.69	0.78	0.02	0.19
Phosphorus	0.44	0.53	0.92	1.68
Calcium:phosphorus	1.56	1.48	0.02	0.11

[1]Treatment nutrient analyses for the complete diet.
[2]Byproduct nutrient analyses for the individual byproducts (DDGS and Hemp).
[3]Contained 48,510 kIU/kg vitamin A and 4,630 kIU/kg vitamin D.
[4]Contained 3.62% calcium, 2.56% copper, 16% zinc, 6.5% iron, 4% manganese, 1,050 mg/kg iodine, and 250 mg/kg cobalt.
[5]Formulated to supply monensin (Rumensin-90, Elanco Animal Health, Greenfield, IN) at 40 mg/kg.
[6]Average of samples taken weekly.

Cattle were adapted to the finishing diet over a 20-day step-up period. This was accomplished with byproduct (hempseed cake or DDGS) held constant at 20% with corn silage at 60% for step 1 and displaced by dry-rolled corn until corn silage constituted 20% of the diet. Treatments were formulated to provide 10% forage (assuming corn silage contains 50% forage) and to meet or exceed ruminally degradable protein intake and metabolizable protein

requirements. The supplement was formulated to provide 40 mg/kg of monensin (Rumensin, Elanco Animal Health, Greenfield, IN). Urea was added to both diets at 1% of diet DM to ensure ruminally degradable protein requirements were met (NASEM, 2016).

On d 1, heifers were implanted with 140 mg trenbolone acetate and 14 mg estradiol (Revalor H, Merck Animal Health, Kenilworth, NJ). Body weights were collected on day 0, 1, 2, 3, 7, 14 and then every 14 d until day 98, and a final BW at slaughter (d 112-120). Carcass data were collected after cattle were slaughtered at the North Dakota State University Meat Laboratory via captive bolt stunning and exsanguination. Cattle were slaughtered on 5 days across a 9-day period to examine the effects of withdrawal period (0, 1, 4, 7, and 8 day) from feeding hempseed cake on cannabinoid residues in plasma and tissues (Chakrabarty et al., 2021). All cattle were offered the DDGS diet for all days on feed after day 111. Cattle were assigned randomly to slaughter date with equal treatment representation each day. Final BW was collected at slaughter, and regression analysis was used to calculate final BW before the withdrawal periods began (day 111). Day 111 was used as the final BW for all growth performance measures. The carcass was chilled for a minimum of 24 hours and fat thickness, longissimus muscle (LM) area, and USDA marbling scores were recorded, and yield grade was calculated. Because hemp is not currently approved to be fed to cattle entering the human food chain, all cattle fed hempseed cake were harvested and disposed of at the completion of the experiment.

2.3.2. Feeding Behavior Measurement

Feeding behavior data were collected daily over the course of the 111-day feeding period (November to February) using the Insentec BV Feeding System (Hokafarm Group, Marknesse, The Netherlands). Each heifer received a radio frequency identification tag in the right ear

before the initiation of the experiment to allow for use of the Insentec automated feeding system (Hokofarm B.V.). This system allows for monitoring feeding behavior characteristics, quantified as number of visits to the bunk, meals (defined as visits separated by ≤ 7 minutes combined into one), time eating (per visit, meal and day), and eating rate (per visit, meal, and minute; Montanholi et al., 2010). Feeding behavior data were summarized as the average of each heifer over the entire feeding period, including the adaptation period. Each visit to the feed bunk was captured and used to quantify feeding behavior.

2.3.3. Feed Sample Analyses

Feed total mixed ration samples were collected weekly for DM analysis and dried at 60 ºC in a forced-air oven for 48 h and then ground to pass a 1-mm screen. Ground aliquots were analyzed for DM, organic matter (OM), nitrogen (N), and ether extract (AOAC, 1990). Neutral detergent fiber and ADF were quantified as described by Van Soest et al. (1991). Crude protein concentration was calculated as 6.25 x N. Samples were also analyzed for starch concentration (Herrera-Saldana and Huber, 1989).

2.3.4. Blood Sample Collection and Analyses

Blood samples were collected from all heifers before feed delivery in the morning via jugular venipuncture into tubes containing sodium heparin (Becton Dickinson, Rutherford, NJ). Blood sample collection was performed on days 0, 2, 3, 7, 14, 28, 42. 56, 70, 84 and 98. Immediately upon collection, plasma was isolated by centrifugation (3,000 x g at 4 ºC) and stored at -20 ºC until later analysis. Plasma samples were analyzed for amino acid concentrations on samples from days 0, 7, 56 and 98. Plasma samples were analyzed for glucose and urea N (PUN) concentrations on all days of plasma collection.

Plasma amino acid concentrations were analyzed by reversed phase ultra-performance liquid chromatography after pre-column derivatization of amino acids with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Salazar et al., 2012; Lemley et al., 2013) and using an ethylene bridged hybrid C_{18} column (2.1 x 150 mm; 1.7 μm; Waters Corp., Milford, MA, USA). Total amino acids, total essential amino acids and total non-essential amino acids were calculated by summing the amino acid concentrations within each category for each heifer. Essential amino acids consisted of histidine, arginine, threonine, lysine, methionine, valine, isoleucine, leucine, phenylalanine, and tryptophan. Non-essential amino acids consisted of asparagine, glutamic acid, glutamine, glycine, aspartic acid, serine, alanine, proline, and tyrosine. Total amino acids consisted of all essential and non-essential amino acids listed previously.

Plasma glucose concentration was analyzed using the hexokinase/glucose-6-phosphate dehydrogenase method (Farrance, 1987) using the Infinity glucose hexokinase kit (Thermo Trace, Louisville, KY, USA). Plasma urea N was determined using the urease/Berthelot procedure (Chaney and Marbach 1962; Fawcett and Scott 1960) using the QuantiChrom urea assay kit (BioAssay Systems, Hayward, CA, USA).

2.3.5. Dietary Energy Calculations

Dietary net energy for maintenance (NE_m) and NE for gain (NE_g) were calculated using the Galyean (2009) net energy calculator, which is based on NRC (1996) net energy equations. The calculator inputs are initial BW, final BW, target endpoint with choice quality grade assumed, dry matter intake (DMI), and average daily gain (ADG). The NE_m and NE_g of hempseed cake were calculated assuming NE_m and NE_g values of DDGS (2.21 and 1.52 Mcal/kg respectively; NASEM, 2016) and based on the inclusion rate of dried corn distillers grains plus solubles and hempseed cake at 20% of diet DM. To calculate the NE_m of hempseed cake, the

following formula was used to establish the NE_m (represented by x in this equation) of the base diet (byproduct removed): NE_m Mcal/kg of the total diet = (0.2 × 2.21 Mcal/kg) + (0.8 × x). The HEMP diet NE_m was set equal to 0.8 × 1.825 (base diet NE_m, Mcal/kg calculated in previous step) + (0.2 × x), where x is equal to the NE_m of hempseed cake. Calculations for dietary NE_m and NE_g were done for individual heifers.

2.3.6. Statistical Analysis

Data were analyzed using the MIXED procedure in SAS 9.4 (SAS Inst Inc., Cary, NC) as a completely randomized design. One heifer was removed from the analyses from the HEMP treatment group dataset because the heifer was pregnant. Initial BW was used as a covariate for performance and carcass data. Amino acid, glucose and PUN data were analyzed as repeated measures using the MIXED procedure of SAS, with day as the repeated variable. Five covariance structures (autoregressive 1, compound symmetry, Toeplitz, unstructured, and ante-dependence 1) were compared for each analysis using repeated measures, with the lowest fit statistic type selected (compound symmetry and ante-dependence 1 were found to be lowest fit statistics for all measures). Heifer was the experimental unit (n = 16 for DDGS; n = 15 for HEMP). Treatment and day (for plasma metabolites and amino acids) were included in the model as fixed effects and treatment by day interactions were tested. When treatment by day interactions were present, interactive means were compared using the least significant different method. Treatment differences were considered significant when $P \leq 0.05$.

2.4. Results and Discussion

2.4.1. Growth Performance

Heifers fed the DDGS diet had greater ($P \leq 0.05$) final BW, ADG, and gain:feed (G:F) than heifers fed the HEMP diet (Table 2-2), while DMI was not influenced ($P = 0.94$) by

treatment. The observed lack of effect on DMI is similar to what Mustafa et al, (1999) also reported that DMI was not influenced in sheep fed hempseed meal at 20% of the diet, while others have reported increased DMI when hempseed cake is included at 20% of the diet DM in comparison to a mixture of soybean meal and rolled barley fed to growing calves (Hessle, 2008). Furthermore, when hempseed cake was included in dairy rations up to 31% of diet DM, DMI increased (Karlsson et al., 2010). Total concentrations of 10 cannabinoids (including CBD and THC) were 13 mg/kg of hempseed cake (DM-basis). Heifers fed the HEMP diet had a DMI of 14.13 kg, 20% of which was hempseed cake, resulting in an average daily total cannabinoid intake of 36.7 mg/hd/d.

The observed decrease in ADG and G:F in the present experiment is similar to what Hessle (2008) observed when hempseed cake was included in diets for growing cattle, and differs from what Gibb (2005) observed where final BW, DMI, and G:F were not different between steers fed barley-based diets with or without inclusion of dry-rolled hempseed. Whole hempseeds contain more oil than hempseed cake (28.4% vs 7.4% oil, respectively) and could explain the lack of difference in performance reported by Gibb (2005). Similarly, when hempseed cake was included at 22% of a barley-based diet (DM-basis) as a protein source to lambs, no differences in final BW, ADG or G:F were observed when compared to a barley-based diet without an additional protein source (Karlsson and Martinsson, 2011). These authors indicate that high insoluble fiber concentration, and low RUP digestibility could have played a role in lack of performance response observed by feeding hempseed cake. Discrepancies between experiments evaluating hempseed byproducts could be because of potential associative effects when fed with differing combinations of nutrients, and also could be because of the feedstuff being displaced by the hemp byproduct. The decrease in ADG and G:F observed in the

current experiment could be a consequence of the increase in ADF concentration in the HEMP diet as it had approximately 5 percentage units greater ADF than the DDGS diet (Table 2-1), which may have resulted in lower digestibility and reduced available energy (Karlsson and Martinsson, 2011).

The average NE_m and NE_g for hempseed cake were calculated to be 1.73 and 1.10 Mcal/kg, respectively, which is comparable to canola meal, which was calculated to have NE_m and NE_g values of 1.81 and 1.18 Mcal/kg, respectively when included at 20% of diet DM (Nair et al., 2015). Dietary NE_m and NE_g (Mcal/kg of feed, DM-basis) was greater ($P = 0.02$) for DDGS compared to HEMP diets (Table 2-2). Predicted dietary energy was lower for the HEMP diet than the DDGS diet, and NE_g values for both HEMP and DDGS diets were lower than industry averages of 1.50 Mcal/kg NE_g, likely because of the greater initial BW of the heifers used in this experiment compared to typical initial BW which may have negatively influenced growth potential over the feeding period. Hempseed cake was calculated to have 64% the feeding value of DDGS. Carlson (2017) calculated the feeding value of DDGS to be 112% compared to corn, giving hempseed cake a feeding value of roughly 76% relative to corn. Collectively, the reduced predicted net energy and feeding value of hempseed cake relative to DDGS indicate that finishing cattle performance should be reduced compared to cattle receiving typical finishing rations containing DDGS at the current inclusion rate of 20% (DM-basis). Diets were formulated to meet or exceed ruminally degradable protein and metabolizable protein requirements, so the estimated net energy and feeding value of hempseed cake is likely more dependent on the quality of dietary energy more so than dietary protein.

Table 2-2. Performance and carcass characteristics of heifers fed diets containing dried corn distillers grains plus solubles (DDGS) or hempseed cake (HEMP).

	Treatments[1]			
Performance[2]	DDGS	Hemp	SEM	*P*-Value
Initial BW, kg	493	497	17	0.80
Final BW, kg	699	683	7.4	0.05
DMI, kg	14.16	14.13	0.36	0.95
ADG, kg	1.83	1.69	0.07	0.05
G:F	0.130	0.120	0.004	0.02
NE_m, Mcal/kg feed	1.92	1.82	0.04	0.02
NE_g, Mcal/kg feed	1.28	1.19	0.04	0.02
Carcass characteristics				
HCW, kg	422	411	4.9	0.03
Dressing %	60.44	60.51	0.5	0.90
LM area, cm^2	96.6	94.0	2.8	0.37
Fat thickness, cm	1.74	1.66	0.16	0.61
Marbling score[3]	512	498	21	0.48
Calculated YG[4]	3.41	3.35	0.24	0.81

[1]Treatments consisted of 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Performance measures analyzed over the 111-day feeding period.
[3]Marbling score: 400 = $Slight^{00}$, 450 = $Slight^{50}$, 500 = Small, etc.
[4]Yield Grade (YG) = 2.50 + (0.9843 x rib fat thickness, cm) + (0.2 x 2.5% kidney, pelvic, and heart fat), + (0.0084 x hot carcass weight) – (0.496 x LM area, cm^2; USDA, 2016).

2.4.2. Feeding Behavior

No differences were observed ($P \geq 0.32$) in feeding behaviors between treatments (Table 2-3). While the effect of hempseed cake on cattle feeding behavior has not been reported elsewhere, the lack of effect is not surprising because of the observed lack of response in DMI. However, data from other species have suggested that cannabinoids from hemp can influence feed intake and feeding behavior (Engali, 2012). Additionally, some have reported that differences in dietary fiber concentration (Swanson et al., 2017) and fatty acid profile (Benson et al., 2001) can influence feed intake and feeding behavior. The cannabinoid concentration and differences in dietary fiber or fatty acid profile likely were not great enough in the current experiment to elicit changes in feed intake or feeding behavior.

Table 2-3. Feeding behavior of heifers fed diets containing dried corn distillers grains plus solubles (DDGS) or hempseed cake (HEMP).

	Treatments[1]			
Item	DDGS	Hemp	SEM	*P*-Value
Events, per d				
Visits[2]	56.5	51.2	5.5	0.35
Meals[3]	10.3	10.2	0.6	0.87
Time eating, min				
Per visit	2.64	2.82	0.31	0.56
Per meal	13.6	13.4	0.9	0.75
Per day	138	135	8	0.67
Eating rate, kg				
Per visit	0.27	0.30	0.03	0.32
Per meal	1.40	1.44	0.08	0.68
Per min	0.11	0.11	0.01	0.79

[1]Treatments consisted of 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Visit defined as each time the Insentec system detected a heifer at the bunk.
[3]Meal defined as eating periods combined if break between was not longer than 7 minutes.

2.4.3. Carcass Characteristics

Hot carcass weight (HCW) was greater ($P = 0.03$) in heifers fed the DDGS diet than the HEMP diet while all other carcass characteristics were not different ($P \geq 0.37$; Table 2-2). The decreased HCW measured in HEMP heifers is likely because of the decrease in ADG and its subsequent influence on decreased final BW. The observation that other carcass characteristics were not influenced by treatment is consistent with previous research comparing diets with and without inclusion of hemp products (Hessle et al., 2008; Gibb et al., 2005).

2.4.4. Plasma Amino Acids, Glucose, and PUN

Amino acid concentrations in plasma are influenced by many factors including feed digestibility, absorption, amino acid metabolism, protein deposition, and tissue protein turnover (Hammond, 1983; LaPierre et al., 2006; Bergen, 2008). Essential amino acids, non-essential amino acids, and total plasma amino acid concentrations were not different between treatments ($P = 0.53$; Table 2-4). Lack of differences between treatments may indicate that feeding

hempseed cake did not result in large changes in amino acid availability or utilization compared to dried corn distillers grains plus solubles, although plasma concentrations of many individual amino acids were influenced by treatment or treatment by day interaction.

Plasma tryptophan concentration was lower ($P = 0.04$) and plasma alanine concentration was greater ($P = 0.02$) in heifers fed the HEMP diet compared to heifers fed the DDGS diet. There were treatment by day interactions ($P \leq 0.04$) for plasma concentrations of the essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, and valine because treatment did not influence concentration on day 0 but did on other days and because of variability in concentrations over time. Plasma arginine concentration was greater ($P = 0.04$) in heifers fed the HEMP diet than in heifers fed the DDGS diet on day 98. Plasma histidine and valine concentrations were lower ($P \leq 0.02$) on day 56 for heifers fed the HEMP diet compared to heifers fed the DDGS diet. Plasma isoleucine and methionine concentrations were lower ($P < 0.01$) on day 7 for heifers fed the HEMP diet compared to heifers fed the DDGS diet. Plasma leucine concentration was lower ($P < 0.01$) on days 56 and 98 for heifers fed the HEMP diet compared to heifers fed the DDGS diet. Plasma lysine concentration was greater ($P = 0.01$) on days 7 and 98 for heifers fed the HEMP diet compared to heifers fed the DDGS diet. Hempseed cake has a greater lysine concentration than DDGS (1.07% vs 0.85%; Liu, 2011) and likely explains the observed increase in plasma lysine concentration. Lysine is typically the first limiting amino acid in corn-based diets (NASEM, 2016), so further research to better define the metabolizable lysine concentration of hempseed cake is warranted. Plasma phenylalanine concentration was lower ($P = 0.02$) on day 7, 56 and 98 for heifers fed the HEMP diet compared to heifers fed the DDGS diet. There were treatment by day interactions ($P \leq 0.04$) for plasma concentrations of the non-essential amino acids: aspartic acid, glutamine, proline, and tyrosine

because of variability in concentrations after day 0. Heifers receiving the HEMP diet had greater ($P < 0.01$) day 7, and lower ($P < 0.01$) day 56 plasma aspartic acid concentration compared to heifers fed the DDGS diet. Plasma glutamine concentration was greater ($P < 0.01$) on day 98 for heifers fed the HEMP diet compared to the DDGS diet. Lastly, plasma proline and tyrosine concentrations were lower ($P = 0.01$) on days 56 and 98 for heifers fed the HEMP diet compared to heifers fed the DDGS diet. Many of the interactions likely resulted from differing amino acid profiles and digestibility between hempseed cake and dried corn distillers grains plus solubles. Because of the many interactions of hempseed cake supplementation over time, further research is needed to better examine the biological relevance of changes in plasma amino acid concentrations resulting from feeding hempseed cake.

Table 2-4. Plasma amino acid concentrations of heifers fed diets containing dried corn distillers grains plus solubles (DDGS) or hempseed cake (HEMP).

	Treatments[1]											
	DDGS[2]				Hemp[2]					*P*-Value[3]		
	Day				Day				SEM	Trt	Day	TxD
Item, µM	0	7	56	98	0	7	56	98				
EAA												
Arginine	86.3[a]	76.5[a]	109.3[bc]	98.3[abc]	92.5[ab]	99.5[abc]	111.0[c]	129.0[d]	7.9	< 0.01	L	0.04
Histidine	66.2	58.1	90.2	73.8	67.3	56.3	73.0	83.0	5.6	0.49	L	0.06
Isoleucine	113[a]	172[b]	145[b]	152[b]	118[a]	102[a]	119[a]	143[b]	14	< 0.01	L	< 0.01
Leucine	219[b]	123[a]	308[c]	323[c]	226[b]	134[a]	188[b]	225[b]	20	< 0.01	L	< 0.01
Lysine	84.6[b]	55.3[a]	120.4[d]	113.5[d]	90.6[b]	92.8[bc]	114.3[cd]	141.9[e]	9.8	< 0.01	L	0.01
Methionine	25.5[a]	43.2[e]	38.3[cde]	34.9[cd]	27.8[ab]	25.4[ab]	32.6[bc]	40.0[de]	3.8	0.07	L	< 0.01
Phenylalanine	71.9[bc]	69.7[b]	94.0[d]	94.9[d]	68.5[b]	50.5[a]	68.1[b]	80.2[c]	5.3	< 0.01	L	0.01
Threonine	66.4	57.3	86.3	75.0	72.4	66.2	81.5	84.3	7.1	0.25	L	0.53
Tryptophan	47.4	54.1	72.4	69.9	46.4	42.3	59.8	67.4	4.3	0.03	Q	0.09
Valine	282	209	379	418	290	245	308	372	27	0.24	L	0.06
Total EAA	1,063	918	1,442	1,453	1,099	913	1,155	1,366	93	0.09	L	0.21
NEAA												
Alanine	233	177	220	214	253	224	208	249	13	0.02	ND	0.11
Asparagine	48.8	45.4	64.0	51.1	54.4	48.3	57.2	60.2	3.7	0.33	Q	0.15
Aspartic acid	14.1[c]	7.1[a]	11.8[b]	14.0[c]	13.2[bc]	11.7[b]	7.1[a]	14.3[c]	1.4	0.80	Q	< 0.01
Glutamine	363[a]	330[a]	429[bc]	375[a]	363[a]	365[a]	377[ab]	454[c]	19	0.26	L	< 0.01
Glutamic acid	70.1	46.8	49.2	56.4	66.7	48.3	42.8	65.3	3.8	0.95	Q	0.07
Glycine	405	341	326	241	380	363	315	297	22	0.58	L	0.18
Proline	94.8[c]	77.2[ab]	119.9[de]	118.9[e]	102.5[cd]	70.3[a]	91.2[abc]	93.7[c]	9.5	0.01	L	0.03
Serine	113.8	95.1	125.9	111.8	117.2	100.9	117.5	123.8	10.4	0.57	L	0.65
Tyrosine	62.8[bc]	56.2[ab]	101.7[e]	79.7[d]	65.6[bc]	51.1[a]	70.0[bcd]	65.4[bc]	6.9	< 0.01	Q	0.03
Total NEAA	1,405	1,175	1,447	1,261	1,415	1,283	1,286	1,422	89	0.54	ND	0.17
Total AA	2,468	2,093	2,889	2,714	2,514	2,196	2,441	2,788	90	0.54	L	0.28

[1]Treatments consisted of 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.

[2]Control and Hemp treatment means by day sharing the same superscript do not differ ($P \leq 0.05$).

[3]Standard error of the mean (SEM) for the treatment by day (Trt x Day) interaction. Significant ($P < 0.05$) linear (L) and quadratic (Q) day effect denoted with L and Q. Not different (ND) indicates non-significant linear or quadratic effect.

Plasma glucose concentration was not different ($P = 0.17$) between treatments, but was affected quadratically ($P < 0.01$) by day with glucose concentration increasing until day 42 and then plateauing (Figure 1). Joy et al. (2017) also reported an increase and plateau (quadratic effect) as the finishing period progressed. An interaction between treatment and day was observed for PUN ($P < 0.01$) because of variability in the magnitude of differences between treatments over time, as heifers fed the HEMP diet had greater ($P < 0.05$) PUN on all collection days. The observed greater ($P \leq 0.05$) PUN in heifers fed the HEMP diet was likely because the HEMP diet had roughly 1 percentage unit more CP in the diet. Furthermore, both treatment diets likely provided excess MP relative to requirements which leads to greater urea production (Jennings et al., 2018). However, the PUN results suggest that the protein in hempseed cake has at similar digestibility as the protein in dried corn distillers grains plus solubles in the tested diets, resulting in the observed greater PUN concentrations. Ruminal degradability of protein within hempseed cake has been reported to be high (71-74%; Karlsson et al., 2012; Karlsson and Martinsson, 2011), while others have reported low ruminal degradability of hempseed meal (Mustafa et al., 1999). Concentrations of PUN linearly increased ($P < 0.01$) throughout the feeding period. This response was expected as MP requirements decrease over the feeding period resulting in greater PUN concentrations (Simpfendorfer, 1974).

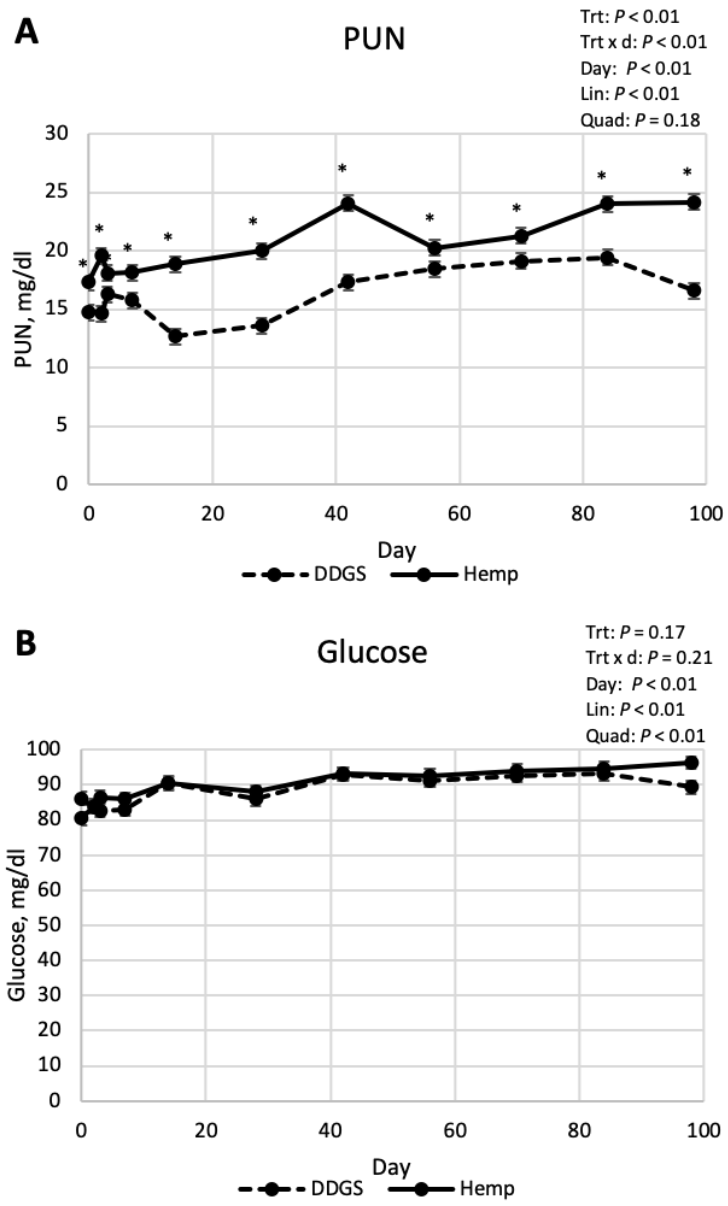

Figure 2-1. Plasma urea N (PUN; Panel A) and glucose (Panel B) of heifers fed diets containing dried corn distillers grains plus solubles (DDGS) or hempseed cake (HEMP). Means within day that differ between treatments are denoted with an * ($P < 0.05$).

2.5. Conclusions

Heifers fed the HEMP diet had reduced final BW, ADG, G:F, and HCW. The lack of difference in DMI between treatments suggests that diets containing hempseed cake is readily consumed by finishing heifers. Greater plasma lysine concentration on d 56 and 98 compared to

d 0 and 7, and greater PUN concentration in heifers fed the HEMP diet suggests that the hempseed cake protein is readily digested and utilized by the growing animal. Because diets were formulated to exceed MP requirements, the observed decrease in ADG and G:F may have been because of differential utilization of the non-protein component in hempseed cake. Acid detergent fiber concentrations are greater in hempseed cake than DDGS, which potentially results in decreased overall digestibility, and possibly growth performance. Further understanding of how hempseed cake influences cattle growth performance is necessary to better define the nutritional quality of hempseed cake for use in cattle diets. Data on total tract digestibility, post-ruminal nutrient flow, ruminal function, and immune function are needed to better understand how hempseed cake can best be utilized in cattle diets. Although hempseed cake may have lower NE_m and NE_g concentrations and potentially result in marginally lower growth performance than dried corn distillers grains plus solubles when adequate metabolizable protein is supplied, it could be a viable alternative feed source for ruminants depending on availability and cost.

2.6. Literature Cited

AOAC. 1990. Official methods of analysis. AOAC, Arlington, VA, USA.

Benson, J.A., C.K. Reynolds, D.J. Humphries, S.M. Rutter, and D.E. Beever. 2001. Effects of abomasal infusion of long-chain fatty acids on intake, feeding behavior, and milk production in dairy cows. J. Dairy Sci. 84:1182-1191. doi:10.3168/jds.S0022-0302(01)74579-1

Bergen, W. G. 2008. Measuring in vivo intracellular protein degradation rates in animal systems. J. Anim. Sci. 86:E3-E12. doi:10.2527/jas.2007-0430

Carlson, Z. E. 2017. Evaluation of protein and fiber from distillers grains plus solubles in finishing beef cattle diets. MS Thesis. University of Nebraska-Lincoln.

Chakrabarty, S., T. M. Winders, E. M. Serum, K. C. Swanson, C. R. Dahlen, M. D. Kleinhenz, and D. J. Smith. 2021. Rapid quantification of cannabinoids in bovine plasma using electrospray ionization mass spectrometry *sans* LC column. American Society for Mass Spectrometry (abstract).

Chaney, A. L., E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132. doi:10.1093/clinchem/8.2.130

Engali, S. 2012. Central and peripheral cannabinoid receptors as therapeutic targets in the control of food intake and body weight. Handb. Exp. Pharmacol. 209:357-381. doi:10.1007/978-3-642-24716-3_17

Farrance, I. 1987. Plasma glucose methods, a review. Clin. Biochem. Rev. 8:55-68.

Fawcett, J. K., and J. E. Scott. 1960. A rapid and precise method for the determination of urea. J. Clin. Path. 13:156-159. doi:10.1136/jcp.13.2.156

Fike, J. 2016. Industrial hemp: Renewed opportunities for an ancient crop. Critical reviews in plant sciences. 35:406-424. doi:10.1080/07352689.2016.1257842.

Galyean, M. 2009. The Home Page of Michael Galyean. NEm and NEg Calculations. Available: https://www.depts.ttu.edu/afs/home/mgalyean/. Accessed August 9, 2021.

Gibb, D. J., M. A. Shah, P. S. Mir, and T. A. McAllister. 2005. Effect of full-fat hemp seed on performance and tissue fatty acids of feedlot cattle. Can. J. Anim. Sci. 85:223-230. doi:10.4141/A04-078

Hammond, A. C. 1983. The use of blood urea nitrogen concentration as an indicator of protein status in cattle. Bov. Pract. 18:114-118. doi:10.21423/bovine-vol1983no18p114-118

Herrera-Saldana, R., and J. T. Huber. Influence of varying protein and starch degradabilities on performance of lactating cows. J. Dairy Sci. 72:1477–1483. doi:10.3168/jds.S0022-0302(89)79257-2.

Hessle, A., M. Eriksson, E. Nadau, T. Turner, and B. Johansson. 2008. Cold-pressed hempseed cake as a protein feed for growing cattle. Acta Agric. Scand. A Anim. Sci. 58:136-145. doi:10.1080/09064700802452192

Jennings, J. S., B. E. Meyer, P. J. Guiroy, and N. A. Cole. 2018. Energy costs of feeding excess protein from corn-based by-products to finishing cattle. J. Anim. Sci. 96:653-669. doi: 10.1093/jas/sky021

Joy, F., J. J. McKinnon, S. Hendrick, P. Gorka, and G. B. Penner. 2017. Effect of dietary energy substrate and days on feed on apparent total tract digestibility, ruminal short-chain fatty acid absorption, acetate and glucose clearance, and insulin responsiveness in finishing feedlot cattle. J. Anim. Sci. 95:5606-5616. doi: 10.2527/jas2017.1817

Karlsson, L., M. Finell, and K. Martinsson. 2010. Effects of increasing amounts of hempseed cake in the diet of dairy cows on the production and composition of milk. Animal 4:1854-1860. doi:10.1017/S1751731110001254

Karlsson, L., and K. Martinsson. 2011. Growth performance of lambs fed different protein supplements in barley-based diets. Livest. Sci. 138:125-131. doi:10.1016/j.livsci.2010.12.010

Karlsson, L., M. Ruiz-Moreno, M. D. Stern, and K. Martinsson. 2012. Effects of temperature during moist heat treatment on ruminal degradability and intestinal digestibility of protein and amino acids in hempseed cake. Asian-Australas. J. Anim. Sci. 25:1559-1567. doi:10.5713/ajas.2012.12213

Kleinhenz, M. D., G. Magnin, S. E. Ensley, J. J. Griffen, J. Goeser, E. Lynch, J. F. Coetzee. 2020. Nutrient concentrations, digestibility, and cannabinoid concentrations of industrial hemp plant components. Appl. Anim. Sci. 36:489-494. doi:10.15232/aas.2020-02018.

Lapierre, H. D. Pacheco, R. Berthiaume, D. R. Ouellet, C. G. Schwab, P. Dubreuil. G. Holtrop, and G. E. Lobley. 2006. What is the true supply of amino acids for a dairy cow? J. Dairy Sci. 89:E1-E14. doi:10.3168/jds.S0022-0302(06)72359-1

Leizer, C., Ribnicky, D., Poulev, A., Dushenkov, S. and Raskin, I. 2000. The composition of hemp seed oil and its potential as an important source of nutrition. J. Nutr. Funct. Med. Foods 2:35–53. doi:10.1300/J133v02n04_04

Lemley, C. O., L. E. Camacho, A. M. Meyer, M. Kapphahn, J. S. Caton, K. A. Vonnahme. 2013. Dietary melatonin supplementation alters uteroplacental amino acid flux during intrauterine growth restriction in ewes. Animal. 7:1500-1507. doi:10.1017/S1751731113001006

Mark, T., J. Shepherd, D. Olson, W. Snell, S. Proper, S. Thornsbury. 2020. Economic viability of industrial hemp in the United States: A review of state pilot programs. United States Department of Agriculture.

Montanholi, Y. R., K. C. Swanson, R. Palme, F. S. Schenkel, B. W. McBride, D. Lu, and S. P. Miller. 2010. Assessing feed efficiency in beef steers through feeding behavior, infrared thermography and glucocorticoids. Animal 4:692– 701. doi:10.1017/S1751731109991522

Mustafa, A. F., J. J. McKinnon, and D. A. Christensen. 1998. The nutritive value of hemp meal for ruminants. Can. J. Anim. Sci. 79:91-95. doi:10.4141/A98-031

Nair, J., G. Penner, P. Yu, H. A. Lardner, T. McCallister, D. Damiran, and J. J. McKinnon. 2015. Evaluation of canola meal derived from *Brassica juncea* and *Brassica napus* seed as an energy source for feedlot steers. Can. J. Anim. Sci. 95:599-607. doi:10.4141/CJAS-2015-055

NASEM. 2016. Nutrient requirements of beef cattle. 8th rev. ed. The National Academies Press, Washington, DC. doi:10.17226/19014

NRC. 1996. Nutrient Requirements of Beef Cattle: Eight Revised Edition. The National Academies Press. Washington, DC.

Salazar, C., J. M. Armenta, and V. Shulaev. 2012. An UPLC-ESI-MS/MS assay using 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate derivatization for targeted amino acid analysis: application to screening of arabidopsis thaliana mutants. Metabolites. 2:398-428. doi:10.3390/metabo2030398.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Loest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico and Texas Tech University survey. J. Anim. Sci. 94:2648-2663. doi: 10.2527/jas.2016-0282

Simpfendorfer, S. 1974. Relationship of body type, size, sex, and energy intake to the body composition of cattle. Ph.D. Dissertation. Cornell University, Ithaca, NY.

Swanson, K. C., Z. E. Carlson, M. C. Ruch, T. C., Gilbery, S. R. Underdahl, F. E. Keomanivong, M. L. Bauer, and A. Islas. 2017. Influence of forage source and forage inclusion level on growth performance, feeding behavior, and carcass characteristics in finishing steers. J. Anim. Sci. 95:1325-1334. doi:10.2527/jas2016.1157

USDA, Agricultural Marketing Service. 2021. Establishment of a domestic hemp production program, final rule. 7CFR Part 990. Fed. Resist. 86:5596–5691

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds. S0022-0302(91)78551-2

CHAPTER 3. EVALUATION OF THE EFFECTS OF HEMPSEED CAKE ON RUMINAL FERMENTATION PARAMETERS, NUTRIENT DIGESTIBILITY, NUTRIENT FLOW, AND NITROGEN BALANCE IN FINISHING STEERS

3.1. Abstract

There is interest in feeding industrial hemp production byproducts to livestock as demand for hemp oil for human use increases. While hempseed cake has recently been evaluated in finishing diets fed to growing heifers, little is known about the digestibility and ruminal fermentation parameters associated with this feedstuff. A nutrient balance experiment using crossbred steers (n = 5; initial BW = 542 kg, SD = 40 kg) in a Youden square design was conducted to evaluate the effects of feeding hempseed cake (HEMP) or dried corn distillers grains plus solubles (DDGS; each included at 20% of diet DM in respective treatments) in comparison to a dry-rolled corn-based treatment (CON) on organic matter (OM) intake, ruminal fermentation parameters, nutrient digestibility, nutrient flow and nitrogen (N) balance. Organic matter tended ($P = 0.07$) to increase and OM total tract digestibility decreased ($P = 0.03$) in steers fed the HEMP diet compared to steers fed the DDGS and CON diets. Total tract apparent N digestibility was greatest ($P < 0.01$) in steers fed the HEMP diet, while total tract apparent digestibility in all other nutrients was not influenced ($P \geq 0.13$) by treatment. Furthermore, apparent ruminal digestibility of OM was greater ($P < 0.01$) in steers fed the HEMP or CON diets than in steers fed the DDGS diet, and neutral detergent fiber, acid detergent fiber (ADF), and true ruminal digestibility of N was greatest ($P \leq 0.04$) in steers fed the HEMP diet than in the other diets. Ruminal total amino acid degradation was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS and CON diets. Total ruminal VFA concentration was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS and CON diets. A treatment

by hour interaction ($P = 0.01$) was observed for ruminal ammonia concentration, with steers fed the HEMP diet being greater than steers fed the CON diet at all hours, and greater than in steers fed the DDGS diet at all hours besides 1, 7, 15, and 21. Ruminal fluid pH was not influenced ($P = 0.93$) by treatment. Steers fed the HEMP diet had greater ($P < 0.01$) N retention (g/d) than steers fed the DDGS diet, which was greater ($P < 0.01$) than steers fed the CON diet, suggesting that feeding hempseed cake improved utilization of N compared to the other diets when fed to finishing steers. Taken together, these results suggest that although ruminal digestibility of all nutrients is greater in steers fed the HEMP diet, the greater ADF concentration in hempseed cake negatively influences total tract apparent OM digestibility when fed to finishing steers.

3.2. Introduction

Currently, corn distillers grains is the most common byproduct fed in finishing diets in the US and has similarities in nutrient composition to hempseed cake (CP, NDF, ether extract; Samuelson et al., 2016; Table 1). In chapter 2, we evaluated the effects of feeding hempseed cake on finishing heifer growth performance, carcass characteristics, feeding behavior and blood metabolites, but nutrient digestibility and flow data is needed to better understand the effects of feeding hempseed cake. The digestibility of a feedstuff is important to understand to better formulate diets to meet animal nutrient requirements. In ruminant nutrition, CP extent and site of digestion is critical to know in order to meet MP requirements. Although a lot of research has investigated the nutrient digestibility of dried corn distillers grains, relatively little is known about the site and extent of nutrient digestion in hempseed cake. The objective of this experiment was to determine the influence of feeding a diet containing hempseed cake in comparison to diets containing dried corn distillers grains plus solubles or a control diet

containing no byproduct on organic matter (OM) intake, ruminal fermentation parameters, nutrient digestibility, nutrient flow and nitrogen balance when fed to finishing steers.

3.3. Materials and Methods

All animal care and management practices were approved by the North Dakota State University Institutional Animal Care and Use Committee.

3.3.1. Animals, Experimental Design, and Dietary Treatments

Five ruminally and duodenally cannulated crossbred steers (n = 5; initial body weight [BW] = 542 kg, SD = 40 kg) were used in a 3-period Youden square design consisting of three periods, three treatments, and five steers assigned randomly to one of three treatment sequences (one or two steers per period per treatment) to evaluate the effect of hempseed cake on organic matter (OM) intake, total tract nutrient digestion, nitrogen balance, and ruminal fermentation parameters. For each period, steers were individually housed in slatted floor pens during a 12-day treatment adaptation. Steers were then moved to 1.1 x 2.2 m individual tie stall stanchions for each 7-day collection period. Six steers were initially assigned randomly to one of three treatment sequences, but one steer was removed because of issues adapting to the collection stanchions. The control (CON) treatment contained 75% dry-rolled corn, 20% corn silage, and 5% supplement (DM-basis). The dried corn distillers grains plus solubles treatment (DDGS) contained 55% dry-rolled corn, 20% corn silage, 20% dried corn distillers grains plus solubles, and 5% supplement (DM-basis). The hempseed cake treatment (HEMP) contained the same ingredients as the DDGS treatment except hempseed cake replaced DDGS (DM-basis; Tables 3-1 and 3-2). The 20% inclusion level for dried corn distillers grains plus solubles and hempseed cake was selected as this is a common inclusion level for similar byproducts feeds used in practice (Samuelson et al., 2016). Diets were formulated to meet or exceed ruminally degradable

and metabolizable protein, mineral and vitamin requirements (NASEM, 2016). Supplement was formulated to provide 40 mg/kg of monensin (Rumensin, Elanco Animal Health, Greenfield, IN, USA) and 1% urea (DM-basis) as well as minerals and vitamins.

Table 3-1. Composition of diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatments[1]		
Ingredient, % of diet DM	Con	DDGS	Hemp
Corn grain	75	55	55
DDGS	0	20	0
Hempseed cake	0	0	20
Corn silage	20	20	20
Supplement	5	5	5
Fine ground corn	1.57	1.57	1.57
Limestone	2	2	2
Salt	0.1	0.1	0.1
Urea	1	1	1
Chromic oxide	0.25	0.25	0.25
Vitamin premix[2]	0.01	0.01	0.01
Trace mineral premix[3]	0.05	0.05	0.05
Rumensin-90[4]	0.02	0.02	0.02

[1]Treatments were Control (Con), distillers grains plus solubles (DDGS), and hempseed cake (Hemp).
[2]Contained 48,510 kIU/kg vitamin A and 4,630 kIU/kg vitamin D.
[3]Contained 3.62% calcium (Ca), 2.56% copper (Cu), 16% zinc (Zn), 6.5% iron (Fe), 4% manganese (Mn), 1,050 mg/kg iodine (I) and 250 mg/kg cobalt (Co).
[4]Formulated to supply monensin (Rumensin-90, Elanco Animal Health, Greenfield, IN) at 40 mg/kg.

Table 3-2. Nutrient composition of diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatments[1]			Byproducts[2]	
	Con	DDGS	Hemp	DDGS	Hemp
Dry matter %	69.7	69.0	71.2	92.1	94.2
Nutrient analysis, % of DM[3]					
Organic matter	94.7	93.5	94.2	93.4	93.9
Starch	54.3	45.4	39.1	-	-
Crude protein	10.3	14.9	17.7	29.8	35.0
Ether extract	2.89	3.59	4.36	9.84	9.45
NDF	22.8	27.3	32.9	47.3	49.5
ADF	9.3	10.8	19.2	14.4	33.8
Sulfur	0.12	0.22	0.19	-	-
Calcium	0.54	0.59	0.44	0.26	0.11
Phosphorus	0.34	0.47	0.58	1.01	1.31
Calcium : Phosphorus	1.62	1.24	0.76	0.25	0.08
Dietary gross energy, kcal/g	4,212	4,356	4,437	-	-
Amino acids, % of DM					
Arginine	0.32	0.47	1.28	-	-
Histidine	0.19	0.29	0.37	-	-
Isoleucine	0.28	0.44	0.58	-	-
Leucine	0.82	1.29	1.16	-	-
Lysine	0.27	0.37	0.53	-	-
Methionine	0.17	0.23	0.32	-	-
Phenylalanine	0.36	0.54	0.67	-	-
Threonine	0.29	0.44	0.52	-	-
Tryptophan	0.06	0.09	0.14	-	-
Valine	0.38	0.58	0.74	-	-
Alanine	0.59	0.85	0.80	-	-
Aspartic acid	0.51	0.78	1.30	-	-
Cysteine	0.17	0.25	0.25	-	-
Glutamic acid	1.26	1.94	2.41	-	-
Glycine	0.32	0.46	0.63	-	-
Proline	0.60	0.94	0.78	-	-
Serine	0.31	0.47	0.61	-	-
Tyrosine	0.21	0.35	0.42	-	-

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet. Treatment nutrient analyses for the complete diet.
[2]Byproduct nutrient analyses for the individual byproducts (DDGS and Hemp).
[3]Average of diet samples taken each period.

Prior to trial initiation, steers were adapted to a high-concentrate diet over a 16-day period. Periods were 21 days with 12-day adaptation period to the treatment offered. Diet transitions were done across a 7-day period by offering a blend of old diet and new diet until the old diet was phased out by day 7. Dietary treatments and water were offered *ad-libitum*, and feed refusals were weighed back daily and adjustments for feed offered were made on a dry-matter basis using a protocol designed to make feed adjustments uniformly between steers. For example, if less than 0.45 kg of feed was refused, the feed call was increased by 0.45 kg. If 0.45-1.36 kg of feed were refused, the feed call was the same as the previous day, and if more than 1.36 kg of feed were refused, the feed call was reduced by 0.45 kg. Each period consisted of feed, orts, ruminal pH, fecal and urine collections (day 13-19 of each period for all but orts which was day 12-18), rumen fluid and duodenal fluid collections (day 15-17), rumen contents sample for bacterial isolation (day 20), and endotoxin challenge using lipopolysaccharide (LPS; day 21; data not shown).

3.3.2. Sample Collections

Diets were mixed twice a week in a stationary ribbon mixer (model HD-5, Davis Precision Horizontal Batch Mixer; H.C. Davis Sons Manufacturing Co., Inc., Bonner Springs, KS) and stored in 200 L barrels. Barrels were stored in a cooler at 4 °C to ensure diet quality was maintained. Chromic oxide (Hall Technologies, St. Louis, MO, USA) was used as an external marker, and included at 0.25% of diet DM to determine nutrient flow.

Diet samples (50 g) were collected at 0800 daily on day 13-19 of each collection period and composited. Orts were weighed at 0830 and sampled daily (10% of weight) on day 12-18 of each collection period and composited. Composites were stored at -20 °C until later analysis. Steers were fitted with fecal collection bags on day 13-19 of each collection period. A harness

was used for fecal bag attachment. Fecal bags were removed twice daily (0700 and 1800), and feces were mixed by hand to ensure a representative sample. A sample (5% of weight, as-is basis) was collected each time fecal bags were removed, composited and stored at -20 °C until later analysis. Urine was collected on day 13-19 of each collection period using a urine funnel and vacuum pump system. Urine funnels were attached with adjustable belting to allow for steer movement while keeping the funnel in place. Tubing ran from the funnel spout to a series of two 20 L containers (Nalgene polypropylene heavy-duty vacuum carboy, Thermo Scientific, Waltham, MA, USA) that were connected to a vacuum pump (Gast DOA-P704-AA High-Capacity Vacuum Pump, Cole Parmer, Vernon Hills, Chicago, IL) that pulled urine from the funnel to the carboys. Carboys contained 250 mL of 6 *N* HCL to maintain urine pH < 3. Every other day or whenever the containers were full (whatever came first), urine was weighed, sampled (2% of weight, as-is basis), composited and stored at -20 °C until later analysis.

Ruminal pH was monitored throughout the experiment using 5 indwelling data transmission boluses that were inserted into the reticulum via ruminal cannula at the beginning of the experiment (SmaXtec Premium Bolus; Animal Care GmbH, Graz, Austria). Calibration of pH probes was done during bolus initiation before placing in each steer. The boluses connect to a wireless base station and upload data multiple times per day. Reticulorumen pH data were collected every 10 minutes, obtained using SmaXtec messenger computer software, and logged in Excel (Microsoft Office 2007; Microsoft Corp., Redmond, WA). Data were then averaged and analyzed by hour and for daily minimum, average, and maximum pH readings. Ruminoreticulum pH data from the 7-day collection period were used in the analyses.

Ruminal fluid and duodenal digesta (approximately 200 mL) were collected into whirl-pak bags (532 mL; Nasco, Fort Atkinson, WI, USA) on day 15-17 of each period. Ruminal fluid

was collected using a metal strainer connected to a vacuum pump (Gast DOA-P704-AA High-Capacity Vacuum Pump, Cole Parmer) from various spots below the fiber mat in the rumen. Duodenal digesta was collected using closed T-shaped duodenal cannulas sealed with a plug, and when removed digesta flows out and can be collected. Samples were taken at 0000, 0600, 1200, and 1800 on day 15; 0400, 1000, 1600, and 2200 on day 16; and 0200, 0800, 1400, and 2000 on day 17 to account for every other hour across a 24-hr period. Ruminal fluid and duodenal digesta samples were stored at -20 °C until the end of the experiment. Duodenal fluid was thawed, composited (100 mL from each sample), freeze dried (VirTis Co., Gardiner, NY, USA) and subsampled for analysis.

On day 20 of each period, a 4 kg sample of ruminal contents was collected for bacterial isolation from several locations within the rumen of each steer to ensure both the liquid and fiber phases were represented in the sample. The contents were then mixed with 2 liters of a solution containing 3.7% formaldehyde and 0.9% NaCl and stored at - 20 °C until the end of the experiment. Contents were thawed and blended using a commercial, heavy-duty blender (model 37BL19CB6, Waring Products division, New Hartford, CT, USA), strained through four layers of cheese cloth, and freeze died prior to chemical analysis.

3.3.3. Sample Analyses

Feed, orts, and fecal samples were dried for 48 h at 60 °C in a forced air oven (Grieve SB-350, The Grieve Corporation Round Lake, IL, USA), ground to pass through a 1 mm screen (Wiley mill, model No. 3; Thomas Scientific, Swedesboro, NJ, USA), while duodenal samples were freeze dried. Rumen fluid samples were centrifuged at 2000 × g for 20 min, and the liquid portion was filtered through a 0.45 μm filter and analyzed for ammonia using the UV/VIS spectrophotometry method (Broderick and Kang, 1980). Bacterial isolation was accomplished

by centrifuging samples in 250 mL bottles at 500 × g for 20 minutes to remove protozoa and feed particles. The supernatant was removed and centrifuged at 30,000 × g for an additional 20 min to pellet bacteria, which was frozen and lyophilized before being analyzed. Feed, orts, fecal, duodenal, and bacterial samples were analyzed for DM and ash using standard procedures (AOAC, 1990). Feed, orts, fecal and duodenal samples were analyzed for neutral detergent fiber (NDF) and acid detergent fiber (ADF; Robertson and Van Soest, 1981) using an Ankom fiber analyzer (Ankom technology Corp, Fairport, NY, USA). Purine concentrations were measured in the duodenal digesta and bacterial samples using the UV/VIS spectrophotometry method (Zinn and Owens, 1986) to determine post-ruminal bacterial flow. Feed, orts, duodenal, and fecal samples were analyzed for starch concentrations (Hall et al., 2000). Feed, orts, duodenal, bacterial, fecal, and urine samples were analyzed for nitrogen (N) concentration using the Kjeldahl method. Total calcium and phosphorus concentrations were measured in the feed (AOAC, 1990). Lipid concentration was measured in feed, orts, duodenal and fecal samples using a method adapted from Folch et al. (1957). Chromium was measured in the feed, fecal, ruminal and duodenal samples using the UV/VIS spectrophotometry method of Fenton and Fenton (1979). Ruminal VFA concentrations were determined using gas chromatography (Hewlett Packard 5890A Series I GC, Wilmington, DE, USA) and separated on a capillary column (Nukol, Supelco, Bellefonte, PA< USA) using 2-ethyl butyric acid as the internal standard.

Feed, bacteria, and duodenal samples were analyzed for individual amino acid concentrations using high-performance liquid chromatography at the University of Missouri Agricultural Experiment Station Chemistry Laboratory (AOAC, 1990). Total amino acids, total essential amino acids and total non-essential amino acids were calculated by summing the amino

acid concentrations within each category. Essential amino acids consisted of histidine, arginine, threonine, lysine, methionine, valine, isoleucine, leucine, phenylalanine, and tryptophan. Non-essential amino acids consisted of glutamic acid, glycine, aspartic acid, serine, alanine, cysteine, proline, and tyrosine. Total amino acids consisted of all essential and non-essential amino acids listed previously.

3.3.4. Calculations

Nutrient and energy total tract apparent digestibility was calculated by subtracting fecal concentration of the nutrient from the amount of nutrient consumed, and dividing by nutrient intake. Total nutrient and energy flow to the small intestine was calculated based on the ratio of nutrients to chromium in the duodenal digesta as compared with intake (Merchen, 1988). Purines were used as a microbial marker to calculate microbial organic matter (OM) and N leaving the abomasum. Total tract Cr recovery ranged from 76-85%. Ruminal OM disappearance was calculated as OM intake minus the difference between microbial OM and total OM reaching the duodenum. Feed N escape to the small intestine was calculated by subtracting bacterial N from total N. Duodenal amino acid flow (g/d) from the feed were calculated by subtracting bacterial amino acid flow from total amino acid flow in the duodenum. Bacterial amino acid flow was estimated using amino acid concentrations and the ratio of DM to chromium in the duodenal digesta.

3.3.5. Statistical Analysis

Nutrient digestibility, pH range, and energy data were analyzed using the MIXED procedure in SAS 9.4 (SAS institute Inc., Cary, NC, USA) as a 3 x 5 Youden square, and all 5 steers received each treatment. The model included period and treatment as fixed effects, and steer as a random effect. One experimental unit was removed from the amino acid flow data

because it was an outlier, with amino acid ruminal bypass percentages exceeding 100% for all amino acids. Volatile fatty acid, ammonia, and average pH data were analyzed as repeated measures using the MIXED procedure of SAS, with hour as the repeated variable. Four covariance structures (autoregressive 1, compound symmetry, Toeplitz, and unstructured) were compared for each repeated measure analysis, and compound symmetry was selected based on having the lowest fit statistics. Steer within period was considered the experimental unit. Treatment and hour (for VFA, ammonia, and average pH) were included in the model as fixed effects and treatment by day interactions were tested. Pairwise comparisons (least significant difference approach) were used to analyze differences among treatment means when the treatment *P*-value was significant. Linear and quadratic effects of hour were tested using contrast coefficients that were generated using PROC IML procedure of SAS for all repeated measures analyses. Treatment differences were considered different when $P \leq 0.05$, and tending to be different when $P > 0.05$ and $P \leq 0.10$.

3.4. Results

3.4.1. Ruminal Fermentation Parameters

A treatment by hour interaction ($P = 0.01$) was observed for ruminal ammonia concentration (Figure 3-1). Ruminal ammonia concentration was greater ($P \leq 0.04$) in steers fed the HEMP diet than in steers fed the CON treatment at all hours, and greater than in steers fed the DDGS diet at all hours besides 1, 7, 15, and 21. Total ruminal VFA concentration was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS or CON diets. A treatment by hour interaction ($P < 0.01$) was observed for acetic acid, butyric acid, and isobutyric acid because the magnitude of change between treatments across hour differed (data not shown). Isovaleric acid was greater ($P = 0.03$; Table 3-3) in steers fed the DDGS and CON diets than in

steers fed the HEMP diet. All other VFA's, and the acetic acid to propionic acid ratio were not influenced ($P \geq 0.32$) by dietary treatment. Average, minimum, and maximum pH were not influenced ($P \geq 0.38$) by treatment.

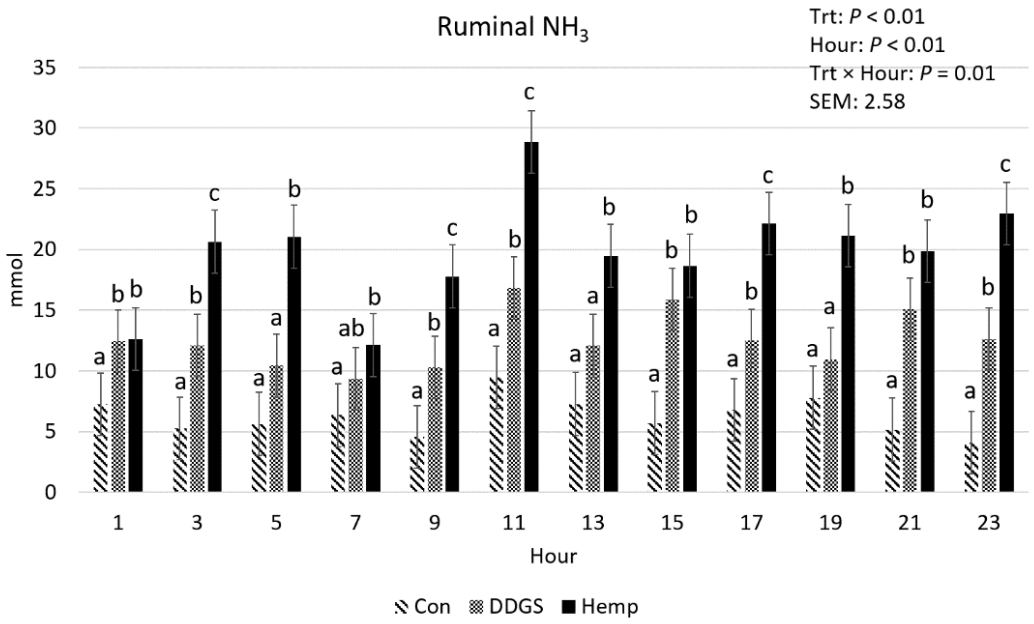

Figure 3-1. Ruminal NH_3 concentration in steers fed diets containing 0% byproduct (CON), 20% dried corn distillers grains plus solubles (DDGS), or 20% hempseed cake (Hemp; DM-basis). Means within hour that differ are denoted by differing letters ($P < 0.05$).

Table 3-3. Ammonia (NH_3), volatile fatty acid (VFA), and pH means of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatment[1]				*P*-Value[2]		
	Control[3]	DDGS[3]	Hemp[3]	SEM	Trt	Hour	Trt × hr
NH_3, mmol	6.3[a]	12.8[b]	19.8[c]	1.0	< 0.01	< 0.01	0.01
Total VFA, mmol	113[a]	120[a]	130[b]	3	< 0.01	0.53	0.96
VFA, mol/100 mol							
Acetic	48.9	51.4	52.8	1.78	0.32	0.03	< 0.01
Propionic	19.4	19.3	17.7	1.72	0.74	0.79	0.26
Butyric	21.3	19.0	19.5	1.21	0.41	< 0.01	< 0.01
Isobutyric	2.01[ab]	1.86[a]	2.11[b]	0.07	0.05	< 0.01	< 0.01
Valeric	3.24	3.40	3.67	0.54	0.85	< 0.01	0.92
Isovaleric	5.23[a]	4.99[a]	4.19[b]	0.27	0.03	< 0.01	0.78
Acetic:Propionic	2.90	2.86	3.03	0.28	0.91	0.53	0.16
pH							
Average	5.96	5.96	5.99	0.13	0.93	< 0.01	0.99
Minimum	5.49	5.50	5.60	0.12	0.48	-	-
Maximum	6.50	6.38	6.47	0.13	0.38	-	-

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Standard error of the mean (SEM) for the treatment by day (Trt x Day) interaction.
[3]Control, DDGS, and Hemp treatment means sharing the same superscript do not differ ($P \leq 0.05$).

3.4.2. Nutrient Digestibility

Total tract Cr recovery did not differ among treatments ($P = 0.11$). Organic matter intake tended to be greater ($P = 0.07$) in steers fed the HEMP diet than in steers fed the DDGS or CON diets (Table 3-4). Duodenal flow of total, feed, and bacterial OM was not different ($P \geq 0.11$) among treatments. Ruminal OM disappearance was greater ($P = 0.05$) in steers fed the HEMP diet than in steers fed the DDGS diet, with steers fed the CON diet intermediate and not different from either diet. Fecal excretion of OM was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS or CON diets. Apparent ruminal OM digestibility was greater ($P < 0.01$) in steers fed the HEMP and CON diets than in steers fed the DDGS diet. True ruminal OM digestibility was not different ($P = 0.19$) among treatments. Post-ruminal apparent OM digestibility (as a percent of OM intake) was greater ($P = 0.01$) in steers fed the DDGS diet than

in steers fed the CON diet, which was greater ($P < 0.01$) than steers fed the HEMP diet. Post-ruminal apparent OM digestibility (as a percent of OM entering the duodenum) was greater ($P < 0.01$) in steers fed the DDGS and CON diets than in steers fed the HEMP diet. Total tract apparent OM digestibility was greater ($P = 0.02$) in steers fed the CON or DDGS diets than in steers fed the HEMP diet.

Table 3-4. Organic matter (OM) and dietary energy intake and digestibility of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatments[1]				
Organic Matter	Con[2]	DDGS[2]	Hemp[2]	SEM	*P*-Value
OM intake, kg/d	8.46	8.69	10.71	0.78	0.07
Duodenal flow, kg/d					
Feed	2.59	2.96	2.97	0.37	0.75
Bacterial	1.32	1.51	1.59	0.14	0.11
Total	3.77	4.45	4.49	0.45	0.33
Ruminal disappearance, kg/d	4.92^{ab}	4.27^{a}	6.20^{b}	0.62	0.05
Apparent digestibility, % of intake	55.3^{a}	49.2^{b}	58.2^{a}	1.7	< 0.01
True digestibility, % of intake	70.6	67.2	73.1	2.1	0.19
Fecal excretion, kg/d	1.69^{a}	1.91^{a}	2.80^{b}	0.23	< 0.01
Post-ruminal apparent digestibility					
% of intake	23.9^{a}	28.9^{b}	15.9^{c}	1.3	< 0.01
% of entering duodenum	52.8^{a}	56.7^{a}	38.0^{b}	2.4	< 0.01
Total tract apparent digestibility, %	80.1^{a}	78.2^{a}	74.0^{b}	1.5	0.02
Dietary Energy[3]					
GEI, Mcal/d	37.7^{a}	40.6^{a}	50.4^{b}	3.5	0.03
GE duodenum, Mcal/d	20.2	24.3	24.1	2.5	0.31
GE fecal, Mcal/d	8.47^{a}	9.82^{a}	13.90^{b}	1.0	< 0.01
DEI, Mcal/d	29.2	30.8	36.5	2.6	0.08
DEI, Mcal/OM intake, kg	3.46	3.55	3.42	0.08	0.24
Digestibility					
Apparent ruminal, % of intake	46.6^{a}	40.1^{b}	52.4^{c}	1.4	< 0.01
Post-ruminal, % of intake	30.2^{a}	35.4^{b}	20.3^{c}	1.1	< 0.01
Post-ruminal, % of entering duodenum	56.0^{a}	59.3^{a}	42.6^{b}	2.4	< 0.01
Total tract apparent digestibility, %	77.5^{a}	76.0^{a}	72.6^{b}	1.4	0.03

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Control, DDGS, and Hemp treatment means sharing the same superscript do not differ ($P \leq 0.05$).
[3]Dietary gross energy intake (GEI), GE in duodenum (duod), and digestible energy intake (DEI).

Dietary gross energy (GE) intake and fecal GE excretion (Mcal/d) was greater ($P \leq 0.03$) in steers fed the HEMP diet than in steers fed the DDGS or CON diets, and duodenal GE (Mcal/d) did not differ ($P = 0.31$) among treatments (Table 3-4). Digestible energy (DE) intake (Mcal/d) tended to be greater ($P = 0.08$) in steers fed the HEMP treatment than in steers fed the DDGS and CON treatments, and DE intake (Mcal) per unit of DMI (kg) was not different ($P = 0.24$) among treatments. Apparent ruminal digestibility of GE (as a percent of GE intake) was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the CON diet, which was greater ($P < 0.01$) than in steers fed the DDGS diet. Post-ruminal apparent GE digestibility (as a percent of GE intake) was greater ($P < 0.01$) in steers fed the DDGS diet than in steers fed the CON diet, which was greater ($P < 0.01$) than in steers fed the HEMP diet. Post-ruminal apparent GE digestibility (as a percent of GE entering the duodenum) was greater in steers fed the DDGS or CON treatments than in steers fed the HEMP treatment ($P < 0.01$). Total tract apparent GE digestibility was greater ($P = 0.03$) in steers fed the CON and DDGS diets than in steers fed the HEMP diet.

Nitrogen intake was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS diet, which was greater ($P < 0.01$) than in steers fed the CON diet (Table 3-5). Duodenal flow of total, feed and bacterial N was not different ($P \geq 0.18$) among treatments. Ruminal N disappearance was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the CON or DDGS diets. Apparent ruminal N digestibility was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS diet, which was greater ($P = 0.02$) than in steers fed the CON diet. True ruminal N digestibility was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS or CON diets. Post-ruminal apparent N digestibility (as a percent of N intake) was greater ($P < 0.01$) in steers fed the CON diet than in steers fed the DDGS diet, which

was greater ($P < 0.01$) than in steers fed the HEMP diet. Post-ruminal apparent N digestibility (as a percent of N entering the duodenum) was greater ($P < 0.01$) in steers fed the DDGS or CON diets than in steers fed the HEMP diet. Bacterial efficiency (g of bacterial N/kg of OM truly fermented) tended to be greater ($P = 0.06$) in steers fed the DDGS diet compared to steers fed the HEMP or CON diets.

Table 3-5. N intake, digestibility, bacterial efficiency, and N balance of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatments[1]				
Item	Con[2]	DDGS[2]	Hemp[2]	SEM	*P*-Value
N intake, g/d	151[a]	217[b]	313[c]	17	< 0.01
Post-ruminal flow, g/d					
Feed	95	130	108	12	0.18
Bacterial	108	121	125	13	0.34
Total	202	251	232	21	0.21
Ruminal disappearance, g/d	-50.3[a]	-33.6[a]	81.0[b]	9.0	< 0.01
Apparent digestibility, % of intake	-34.9[a]	-16.0[b]	26.9[c]	0.06	< 0.01
True digestibility, % of intake	38.7[a]	40.3[a]	65.9[b]	0.05	< 0.01
Post-ruminal apparent digestibility					
% of N intake	104.5[a]	90.2[b]	51.6[c]	5.4	< 0.01
% of N entering duodenum	77.4[a]	77.6[a]	70.8[b]	1.3	< 0.01
Bacterial efficiency, g bacterial N/kg of OM truly fermented	9.5	12.4	8.7	1.3	0.06
N excretion, g/d					
Feces	44.4[a]	56.0[ab]	68.2[b]	5	0.01
Urine	81.7[a]	111.8[b]	154.9[c]	10.1	< 0.01
Total	126[a]	168[b]	223[c]	14	< 0.01
N excretion, % of N intake					
Feces	29.8[a]	25.7[b]	21.5[c]	1.2	< 0.01
Urine	55.8	52.2	49.0	3.2	0.33
Total tract apparent digestion					
g/d	107[a]	161[b]	245[c]	11	< 0.01
% of N intake	70.2[a]	74.3[b]	78.5[c]	1.2	< 0.01
N retained					
g/d	25.1[a]	49.4[b]	90.2[c]	7.7	< 0.01
% of N intake	14.4[a]	22.1[ab]	29.4[b]	4.3	0.04

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Control, DDGS, and Hemp treatment means sharing the same superscript do not differ ($P \leq 0.05$).

Total N excretion (sum of fecal and urinary N) and urinary N (g/d) was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS diet, which was greater ($P \leq 0.03$) than in steers fed the CON diet. Fecal N (g/d) was greater ($P = 0.01$) in steers fed the HEMP treatment than in steers fed the CON treatment, with steers fed the DDGS diet intermediate and not different from either diet. Fecal N excretion (as a percent of N intake) was greater ($P = 0.01$) in steers fed the CON diet than in steers fed the DDGS diet, which was greater ($P = 0.01$) than in steers fed the HEMP diet, and urinary N excretion (as a percent of N intake) did not differ among treatments ($P = 0.33$). Total tract apparent N digestion (g/d and as a percent of N intake) was greater ($P \leq 0.02$) in steers fed the HEMP diet than in steers fed the DDGS diet, which was greater ($P \leq 0.02$) than in steers fed the CON diet lowest in steers fed the CON diet. Furthermore, retention of N (g/d) was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS diet, which was greater ($P = 0.02$) than in steers fed the CON diet, and retention of N (as a percent of N intake) was greater ($P = 0.04$) in steers fed the HEMP diet than in steers fed the CON treatment, with steers fed the DDGS treatment intermediate and not different from either diet.

Intake and ruminal disappearance of NDF was greater ($P < 0.01$) in steers fed the HEMP than in steers fed the DDGS diet, which was greater ($P \leq 0.02$) than in steers fed the CON diet, and duodenal flow of NDF did not differ ($P = 0.11$) among treatments (Table 3-6). Fecal NDF excretion was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS or CON treatments. Apparent ruminal NDF digestibility was greater ($P = 0.04$) in steers fed the HEMP diet than in steers fed the CON diet, with steers fed the DDGS diet intermediate and not different from either diet. Post-ruminal apparent NDF digestibility (as a percent of NDF intake, and as a percent of NDF entering the duodenum) was greater ($P < 0.01$) in steers fed the DDGS

or CON diets than in steers fed the HEMP diet, and NDF total tract apparent digestibility did not differ ($P = 0.12$) among treatments. Intake, duodenal flow, ruminal disappearance, fecal output, and apparent ruminal digestion of ADF (as a percent of ADF intake) was greater ($P \leq 0.01$) in steers fed the HEMP diet than in steers fed the DDGS or CON diets (Table 3-6). Post-ruminal apparent ADF digestibility (as a percent of ADF intake, and as a percent of ADF entering the duodenum) was greater ($P < 0.01$) in steers fed either the DDGS or CON diets than in steers fed the HEMP diet. Total tract apparent digestibility of ADF did not differ ($P = 0.38$) among treatments.

Table 3-6. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) intake and digestibility of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatments[1]				
NDF, kg/d	Con[2]	DDGS[2]	Hemp[2]	SEM	*P*-Value
Intake	1.95[a]	2.53[b]	3.64[c]	0.19	< 0.01
Duodenal flow	1.30	1.55	1.75	0.23	0.11
Ruminal disappearance, kg/d	0.59[a]	0.97[b]	1.89[c]	0.10	< 0.01
Apparent digestibility, % of intake	28.2[a]	37.9[ab]	51.4[b]	6.8	0.04
Fecal excretion, kg/d	0.85[a]	1.03[a]	1.70[b]	0.12	< 0.01
Post-ruminal apparent digestibility					
% of intake	26.2[a]	21.3[a]	2.1[b]	4.0	< 0.01
% of entering duodenum	29.9[a]	33.1[a]	-2.0[b]	5.1	< 0.01
Total tract apparent digestibility, %	56.7	59.4	53.3	2.6	0.12
ADF, kg/d					
Intake	0.80[a]	1.00[a]	2.09[b]	0.09	< 0.01
Duodenal flow	0.56[a]	0.68[a]	0.91[b]	0.07	0.01
Ruminal disappearance, kg/d	0.19[a]	0.32[a]	1.19[b]	0.07	< 0.01
Apparent digestibility, % of intake	21.8[a]	30.3[a]	56.0[b]	4.7	< 0.01
Fecal excretion, kg/d	0.46[a]	0.52[a]	1.12[b]	0.06	< 0.01
Post-ruminal apparent digestibility					
% of intake	15.8[a]	17.6[a]	-8.9[b]	3.6	< 0.01
% of entering duodenum	16.1[a]	24.0[a]	-27.4[b]	7.0	< 0.01
Total tract apparent digestibility, %	42.8	48.6	46.6	3.6	0.38

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Control, DDGS, and Hemp treatment means sharing the same superscript do not differ ($P \leq 0.05$).

Starch intake, duodenal flow, ruminal disappearance, fecal output, apparent ruminal digestibility, post-ruminal apparent digestibility (as a percent of starch intake and as a percent of starch entering the duodenum), and total tract apparent digestibility did not differ ($P \geq 0.12$) among treatments (Table 3-7). Lipid intake was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS diet, which was greater ($P = 0.01$) than in steers fed the CON diet (Table 3-7). Duodenal flow of lipid was greater ($P = 0.03$) in steers fed the HEMP or DDGS diets than in steers fed the CON diet. Ruminal disappearance of lipid was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the CON diet, which was greater ($P = 0.01$) than in steers fed the DDGS diet. Fecal lipid excretion was greater ($P = 0.04$) in steers fed the HEMP diet than in steers fed the DDGS or CON diets. Apparent ruminal lipid digestion (as a percent of lipid intake) was greatest ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS or CON diets, and post-ruminal lipid digestibility (as a percent of lipid intake) was greater ($P = 0.03$) in steers fed the DDGS diet than in steers fed the CON diet, which was greater than in steers fed the HEMP diet. Post-ruminal apparent lipid digestibility (as a percent of lipid entering the duodenum) was greater ($P = 0.03$) in steers fed the DDGS diet than in steers fed the HEMP or CON diets. Total tract apparent lipid digestibility did not differ ($P = 0.14$) among treatments.

Table 3-7. Starch and Lipid intake and digestibility of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatments[1]				
Starch, kg/d	Con[2]	DDGS[2]	Hemp[2]	SEM	*P*-Value
Intake	4.80	4.28	4.57	0.43	0.61
Duodenal flow	0.44	0.47	0.59	0.08	0.40
Ruminal disappearance, kg/d	4.65	3.85	3.95	0.43	0.39
Apparent digestibility % of intake	91.0	89.4	87.1	1.2	0.12
Fecal excretion, kg/d	0.21	0.21	0.31	0.05	0.21
Post-ruminal apparent digestibility					
% of intake	4.00	5.55	6.33	1.1	0.24
% of entering duodenum	38.8	51.7	49.5	9.7	0.36
Total tract apparent digestibility, %	95.7	95.0	93.3	1.0	0.18
Lipid, kg/d					
Intake	0.28[a]	0.36[b]	0.52[c]	0.03	< 0.01
Duodenal flow	0.33[a]	0.46[b]	0.43[b]	0.03	0.03
Ruminal disappearance, kg/d	-0.06[a]	-0.10[b]	0.09[c]	0.01	< 0.01
Apparent digestibility % of intake	-21.6[a]	-28.5[a]	18.2[b]	3.7	< 0.01
Fecal excretion, kg/d	0.030[a]	0.033[a]	0.044[b]	0.004	0.04
Post-ruminal apparent digestibility					
% of intake	110[a]	119[b]	73[c]	3	< 0.01
% of entering duodenum	90.6[a]	92.9[b]	89.8[a]	0.9	0.03
Total tract apparent digestibility, %	89.4	90.9	91.5	0.8	0.14

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Control, DDGS, and Hemp treatment means sharing the same superscript do not differ ($P \leq 0.05$).

3.4.3. Amino Acid Intake, Duodenal Flow, and Digestibility

Ruminal bacterial amino acid concentration was not different ($P \geq 0.06$) among treatments (Table 3-8). Post-ruminal total amino acid and essential amino acid concentration was greater ($P \leq 0.05$) in steers fed the DDGS diet than in steers fed the HEMP diet, with steers fed the CON diet intermediate and not different from either diet, and post-ruminal non-essential amino acid concentration was greater ($P = 0.04$) in steers fed the DDGS than in steers fed the CON diet, which was greater ($P = 0.04$) than in steers fed the HEMP diet (Table 3-8). Histidine, methionine, alanine, and serine post-ruminal concentrations were greater ($P \leq 0.01$) in steers fed

the DDGS or CON diets than in steers fed the HEMP diet, and leucine, phenylalanine, glutamic acid, and proline post-ruminal concentrations were greater ($P \leq 0.04$) in steers fed the DDGS diet than in steers fed the CON diet, which was greater ($P \leq 0.03$) than in steers fed the HEMP diet. Post-ruminal cysteine and tyrosine concentration was greater ($P \leq 0.01$) in steers fed the DDGS diet than in steers fed the HEMP or CON diets. Post-ruminal threonine and valine tended to be greater ($P \leq 0.07$) in steers fed the DDGS diet than in steers fed the HEMP diet, with steers fed the CON diet intermediate and not different from either diet, and all other post-ruminal amino acid concentrations did not differ ($P \geq 0.17$) among treatments.

Table 3-8. Amino acid (AA) concentration in the diet, bacterial content, and duodenal content of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Source[1]									
	Bacterial					Duodenal				
Item, % DM	Con	DDGS	Hemp	SEM	*P*-Val	Con	DDGS	Hemp	SEM	*P*-Val
Essential AA										
Arginine	1.95	1.69	1.76	0.10	0.24	1.01	1.02	1.06	0.05	0.65
Histidine	0.75	0.65	0.65	0.04	0.23	0.49^{a}	0.52^{a}	0.43^{b}	0.02	0.01
Isoleucine	2.31	2.05	2.12	0.10	0.25	1.14	1.19	1.10	0.03	0.17
Leucine	3.09	2.79	2.76	0.14	0.27	2.11^{a}	2.37^{b}	1.79^{c}	0.06	< 0.01
Lysine	2.35	1.96	2.04	0.11	0.10	1.65	1.63	1.57	0.06	0.60
Methionine	0.76	0.65	0.74	0.04	0.22	0.41^{a}	0.42^{a}	0.39^{b}	0.01	0.01
Phenylalanine	1.90	1.70	1.69	0.09	0.26	1.12^{a}	1.21^{b}	1.03^{c}	0.03	0.01
Threonine	2.15	1.91	1.97	0.10	0.28	1.11	1.16	1.05	0.03	0.07
Tryptophan	0.44	0.38	0.36	0.02	0.06	0.25	0.25	0.23	0.01	0.33
Valine	2.61	2.29	2.35	0.12	0.22	1.32	1.38	1.23	0.04	0.06
Total EAA	18.3	16.1	16.5	0.8	0.21	10.2^{ab}	11.1^{a}	9.9^{b}	0.3	0.05
Non-Essential AA										
Alanine	3.00	2.58	2.64	0.14	0.16	1.52^{a}	1.64^{a}	1.36^{b}	0.05	< 0.01
Aspartic acid	4.41	3.86	3.99	0.20	0.22	2.16	2.22	2.12	0.07	0.61
Cysteine	0.52	0.50	0.50	0.03	0.78	0.34^{a}	0.41^{b}	0.33^{a}	0.01	0.01
Glutamic acid	5.03	4.46	4.54	0.24	0.27	3.30^{a}	3.64^{b}	3.03^{c}	0.11	< 0.01
Glycine	2.17	1.91	2.01	0.09	0.22	1.28	1.29	1.24	0.05	0.50
Proline	1.54	1.38	1.38	0.07	0.27	1.21^{a}	1.41^{b}	1.04^{c}	0.04	< 0.01
Serine	1.54	1.36	1.37	0.07	0.23	0.95^{a}	1.01^{a}	0.87^{b}	0.03	< 0.01
Tyrosine	0.16	0.03	0.05	0.04	0.13	0.97^{a}	1.06^{b}	0.91^{a}	0.03	0.02
Total NEAA	18.4	16.1	16.5	0.8	0.20	11.7^{a}	12.7^{b}	10.9^{c}	0.3	0.01
AA	36.7	32.2	32.9	1.65	0.20	22.3^{ab}	23.8^{a}	20.8^{b}	0.61	0.02

[1]Control, DDGS, and Hemp treatment means within source sharing the same superscript do not differ ($P \leq 0.05$).

Total, essential and non-essential amino acid intake was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS diet, which was greater ($P < 0.01$) than in steers fed the CON diet (Table 3-9). Furthermore, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, aspartic acid, glutamic acid, glycine, serine, and tyrosine intakes were greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS diet, which was greater ($P \leq 0.02$) than in steers fed the CON diet. Leucine, alanine, cysteine, and proline intake was greater ($P \leq 0.02$) in steers fed the DDGS or HEMP diets than in steers fed the CON diets, and arginine intake was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS and CON diets. Duodenal flow of total and bacterial amino acids (g/d) was not influenced ($P \geq 0.08$) by treatment (Table 3-10). Total and non-essential amino acid post-ruminal flow from feed tended to be greater ($P \leq 0.09$) in steers fed the DDGS diet than in the HEMP or CON diets, while essential amino acid post-ruminal flow from feed was not influenced ($P = 0.11$) by treatment. Histidine, leucine, alanine, cysteine, glutamic acid, and proline post-ruminal flow (g/d) from feed was greater ($P \leq 0.05$) in steers fed the DDGS diet than in steers fed the HEMP or CON diets.

Feed amino acids that bypassed ruminal degradation were calculated as a percent of total feed amino acid intake (Table 9). Steers fed the CON or DDGS diets amino acid ruminal bypass percent was 66.4 and 68.9% respectively, which was greater ($P < 0.01$) than the ruminal amino acid bypass percent in steers fed the HEMP treatment (36.8%). This trend among treatments was observed for all individual amino acids, with several exceptions. Cysteine bypass percent which was greater ($P < 0.01$) in steers fed the DDGS diet than in steers fed the HEMP diet, with steers fed the CON diet intermediate and not different from either diet, and lysine and methionine bypass percent was greater ($P \leq 0.03$) in steers fed the DDGS diet than in steers fed the CON

diet, which was greater ($P \leq 0.02$) than in steers fed the HEMP diet. Alanine bypass percent was greater ($P < 0.01$) in steers fed DDGS than in steers fed the HEMP or CON diets. Lysine, glycine, and tyrosine values were inflated by methods used to isolate bacteria from ruminal samples, leading to ruminal bypass percentages near or exceeding 100%.

Table 3-9. Amino acid (AA) intake, Feed AA duodenal flow, and ruminal bypass (Bypass) % of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Source[1]													
	Intake					Feed AA duodenal flow					Bypass %				
Item, g/d	Con	DDGS	Hemp	SEM	*P*-Val	Con	DDGS	Hemp	SEM	*P*-Val	Con	DDGS	Hemp	SEM	*P*-Val
Essential AA															
Arginine	27.7^{a}	44.2^{a}	145.0^{b}	7.4	< 0.01	15.7	24.4	26.8	3.2	0.07	50.2^{a}	55.8^{a}	19.0^{b}	4.7	< 0.01
Histidine	16.8^{a}	27.3^{b}	41.5^{c}	3.5	< 0.01	11.5^{a}	16.2^{b}	12.0^{a}	1.6	0.05	60.9^{a}	58.9^{a}	29.1^{b}	8.0	< 0.01
Isoleucine	25.3^{a}	41.0^{b}	65.6^{c}	5.1	< 0.01	18.5	27.1	22.5	3.7	0.19	62.5^{a}	65.0^{a}	35.0^{b}	6.5	< 0.01
Leucine	75^{a}	119^{b}	131^{b}	15	< 0.01	52.3^{a}	78.5^{b}	49.1^{a}	8.8	0.02	62.0^{a}	64.6^{a}	37.9^{b}	6.6	< 0.01
Lysine	23.2^{a}	34.5^{b}	59.9^{c}	4.2	< 0.01	44.6	49.6	49.1	4.5	0.64	167^{a}	143^{b}	84^{c}	8.9	< 0.01
Methionine	14.6^{a}	21.7^{b}	35.8^{c}	2.7	< 0.01	6.4	11.0	8.2	1.5	0.07	38.4^{a}	50.3^{b}	23.7^{c}	5.0	< 0.01
Phenylalanine	32.2^{a}	50.6^{b}	75.8^{c}	6.8	< 0.01	24.3	34.4	26.7	4.2	0.12	67.2^{a}	67.1^{a}	35.5^{b}	7.3	< 0.01
Threonine	25.5^{a}	40.6^{b}	58.4^{c}	5.0	< 0.01	17.9	26.3	22.8	2.8	0.08	61.0^{a}	64.3^{a}	39.9^{b}	6.8	< 0.01
Tryptophan	5.28^{a}	8.44^{b}	15.92^{c}	1.03	< 0.01	4.80	6.26	6.26	0.76	0.28	79.3^{a}	73.3^{a}	39.7^{b}	6.5	< 0.01
Valine	33.7^{a}	54.1^{b}	84.1^{c}	6.5	< 0.01	20.7	31.5	25.9	3.6	0.08	53.7^{a}	57.8^{a}	31.4^{b}	6.1	< 0.01
Total EAA	279^{a}	442^{b}	713^{c}	57	< 0.01	217	305	249	33	0.11	67.9^{a}	68.4^{a}	35.5^{b}	5.6	< 0.01
Non-Essential AA															
Alanine	52.6^{a}	79.1^{b}	90.7^{b}	9.7	< 0.01	23.1^{a}	41.1^{b}	28.3^{a}	6.6	0.03	37.9^{a}	51.2^{b}	32.0^{a}	5.7	< 0.01
Aspartic acid	45.5^{a}	72.6^{b}	147.0^{c}	9.5	< 0.01	33.4	48.2	45.7	6.1	0.18	63.6^{a}	65.8^{a}	31.5^{b}	6.9	< 0.01
Cysteine	15.4^{a}	23.5^{b}	28.4^{b}	2.9	< 0.01	8.3^{a}	13.0^{b}	9.1^{a}	1.8	0.04	46.7^{ab}	55.0^{a}	33.0^{b}	6.0	< 0.01
Glutamic acid	114^{a}	180^{b}	273^{c}	24	< 0.01	74^{a}	117^{b}	85^{a}	16	0.03	57.1^{a}	64.7^{a}	31.9^{b}	4.6	< 0.01
Glycine	28.2^{a}	43.2^{b}	71.3^{c}	5.2	< 0.01	30.3	37.0	32.5	6.4	0.63	97.2^{a}	82.9^{a}	44.9^{b}	9.0	< 0.01
Proline	54.8^{a}	87.6^{b}	88.5^{b}	11.5	0.02	33.5^{a}	51.7^{b}	32.1^{a}	7.4	0.02	52.5^{a}	58.0^{a}	37.1^{b}	5.0	< 0.01
Serine	27.9^{a}	43.7^{b}	69.4^{c}	5.8	< 0.01	20.3	29.6	23.4	3.3	0.08	64.8^{a}	67.1^{a}	34.1^{b}	7.1	< 0.01
Tyrosine	19.2^{a}	32.5^{b}	47.4^{c}	4.6	< 0.01	43.3	55.2	48.5	5.9	0.25	192^{a}	172^{a}	104^{b}	11	< 0.01
Total NEAA	357^{a}	562^{b}	606^{c}	72	< 0.01	266	393	305	44	0.07	65.1^{a}	69.2^{a}	38.0^{b}	4.6	< 0.01
AA	636^{a}	1,004^{b}	1,528^{c}	129	< 0.01	483	698	554	77	0.09	66.4^{a}	68.9^{a}	36.8^{b}	5.2	< 0.01

[1]Control, DDGS, and Hemp treatment means within source sharing the same superscript do not differ ($P \leq 0.05$).

Table 3-10. Total duodenal amino acid (AA) flow and bacterial AA flow (bacterial) of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Source[1]									
	Total					Bacterial				
Item, g/d	Con	DDGS	Hemp	SEM	*P*-Val	Con	DDGS	Hemp	SEM	*P*-Val
Essential AA										
Arginine	46.6	54.6	57.5	6.1	0.40	30.9	30.2	30.6	4.5	0.99
Histidine	23.4	27.8	23.4	2.7	0.26	12.0	11.7	11.4	1.9	0.97
Isoleucine	56.1	64.0	59.4	7.0	0.62	37.6	36.9	36.9	5.5	0.99
Leucine	102	128	97	14	0.11	49.6	50.0	48.1	7.1	0.97
Lysine	82.2	84.7	84.9	8.1	0.96	37.6	35.1	35.8	5.3	0.92
Methionine	19.9	22.8	21.0	2.7	0.63	13.4	11.8	12.8	2.0	0.78
Phenylalanine	54.2	64.9	56.1	7.0	0.36	29.9	30.5	29.5	4.3	0.97
Threonine	53.1	60.7	57.1	6.2	0.59	35.3	34.3	34.3	5.2	0.99
Tryptophan	11.7	13.1	12.6	1.4	0.72	6.94	6.86	6.34	1.00	0.85
Valine	62.3	72.6	66.9	7.2	0.49	41.6	41.1	41.0	6.1	0.99
Total EAA	512	594	536	61	0.49	295	288	287	43	0.99
Non-Essential AA										
Alanine	71.4	87.5	74.3	9.8	0.31	48.4	46.4	46.0	7.4	0.97
Aspartic acid	104	117	115	12	0.66	70.6	69.3	69.6	10.6	0.99
Cysteine	17.2	22.1	17.8	2.1	0.11	8.86	9.03	8.71	1.51	0.98
Glutamic acid	157	197	164	22	0.24	82.8	80.2	79.2	12.8	0.98
Glycine	65.5	71.4	67.5	10.8	0.88	35.2	34.3	35.0	5.3	0.99
Proline	58.3	76.5	56.0	8.2	0.08	24.8	24.7	23.9	3.5	0.97
Serine	45.2	53.9	47.3	5.8	0.36	24.9	24.4	23.9	3.7	0.98
Tyrosine	46.6	56.0	49.2	5.7	0.34	3.31	0.78	0.72	0.91	0.10
Total NEAA	565	682	592	76	0.37	299	289	287	45	0.98
AA	1,076	1,276	1,128	137	0.42	594	578	574	88	0.98

3.5. Discussion

An evaluation of the effects of hempseed cake on ruminal fermentation parameters, digestibility and flow of nutrients, and N balance has not been explored in beef cattle finishing diets. We observed that steers fed hempseed cake had greater ruminal VFA and ammonia concentration, tended to have increased OM intake and decreased OM total tract digestibility, had greater N digestibility and retention, and diets containing hempseed cake were more ruminally available than dried corn distillers grains plus solubles or dry-rolled corn diets.

3.5.1. Ruminal Fermentation Parameters

Ruminal ammonia concentration can be influenced by rate of ruminal CP digestibility as well and OM supply (Russell et al., 1992). The interaction observed for ruminal ammonia could be resulting from differences in N concentration in the feed, feed intake, and site of digestion (Bach et al., 2005), with steers fed the HEMP diet having greater CP digestion in the rumen as well as greater CP intake compared to DDGS and CON treatments. If ruminal CP and carbohydrate digestion are not synchronized, fluctuations in ruminal ammonia (and VFA) concentrations can result (Tamminga, 1977). An increase in total VFA concentration was observed for the HEMP treatment, in part because of the increase in apparent ruminal OM digestibility compared to steers fed the DDGS diet as well as greater OM disappearance compared to the DDGS and CON diets, which can lead to greater VFA concentration (Bach et al., 2005). Although a treatment by hour interaction for acetic acid, butyric acid, and isobutyric acid was observed, the average concentrations for acetic acid and butyric acid were not different, suggesting that rate of ruminal digestion and ruminal digestibility of individual nutrients could have potentially influenced VFA production (Tamminga, 1977; Fance and Dijkstra, 2005). Isobutyric acid average concentration may have been influenced by differing branched chain amino acid intake observed in this trial (Bach et al., 2005). The lack of effect of dietary treatment on ruminal pH (average, minimum, and maximum) is somewhat surprising considering the dietary differences in carbohydrate composition (starch, NDF, ADF, etc.), particularly when comparing the HEMP diet to the CON diet. However, starch intake was not influenced by treatment and steers fed HEMP diets had a tendency for greater intake and ruminal disappearance of OM, likely contributing to the lack of response in ruminal pH to dietary treatment.

3.5.2. Nutrient Digestibility

The observed tendency for greater OM intake in steers fed the HEMP treatment suggests hempseed cake inclusion did not limit feed intake and that perhaps OM intake may increase to compensate for lower OM and GE total tract apparent digestibility (Krehbeil et al., 2006). Furthermore, the greater fiber concentration may increase passage rate in finishing diets (Fox and Tedeschi, 2002). Although an increase in OM intake was not necessarily expected (Chapter 2), greater DMI has been observed when feeding hempseed cake in dairy and lamb diets (Karlsson et al., 2010; Karlsson and Martinsson, 2011). Furthermore, the observed increase in intake of CP, NDF, ADF and lipid in steers fed the HEMP diet is largely because of the greater concentration of these nutrients within hempseed cake as well the tendency for greater OM intake. Greater apparent ruminal OM digestibility in steers fed the HEMP and CON diets than in steers fed the DDGS diet, as well as greater true ruminal N digestibility, total VFA concentration, and ruminal ammonia concentration in steers fed the HEMP diet compared to steers fed the DDGS or CON diets suggests that hempseed cake may be more available for microbial degradation. Greater ruminal degradation may be because of the greater ruminally available nitrogen observed in hempseed cake, as this can increase microbial fermentation (Russell et al., 1982).

Dietary GE intake differences among treatments is largely driven by OM intake, although lipid concentration differences could be influencing GE intake as well. The HEMP diet had the lowest total tract apparent OM digestibility, but digestible energy intake per kg of OM intake did not differ among treatments, likely because of the greater lipid concentration as well as greater kcal/g of feed in the HEMP diet. Greater GE apparent ruminal digestibility and reduced post-

ruminal apparent and true GE digestibility further supports the site of digestion results, indicating more ruminal availability in steers fed the HEMP diet than in steers fed the DDGS or CON diets

Nutrients in the HEMP treatment (N, NDF, ADF, and lipid) have greater apparent ruminal digestibility than nutrients in DDGS and CON, and OM apparent ruminal digestibility is greater in HEMP and CON diets than DDGS, resulting in reduced nutrient flow to the duodenum as a percent of nutrient intake for steers fed the HEMP diet. Furthermore, post-ruminal apparent digestibility (as a percent of intake, and as a percent of nutrient entering the duodenum) of OM, N, NDF, ADF and lipid was lowest in steers fed the HEMP treatment compared to the DDGS and CON treatments. Total tract apparent OM digestibility was 6.5% lower for the HEMP diet compared to the DDGS and CON diets, likely in part because of the greater ADF concentration in hempseed cake, which has the lowest digestibility of the nutrients measured in this experiment. Although total tract ADF digestibility was not different among treatments, the HEMP treatment contained 48% more ADF than the DDGS and CON treatments, resulting in greater ADF flow to the small intestine and decreased total tract apparent OM digestibility. The reduction in total tract apparent OM digestibility was not proportionate to the greater concentration of ADF in the HEMP diet, but apparent ruminal ADF digestibility was greater in steers fed the HEMP diet compared to the CON and DDGS diets, partially explaining the disproportionate reduction in total tract apparent OM digestibility. Starch intake being similar among treatments largely results from overall OM intake differences among treatments more so than dietary starch concentration differences. Total tract apparent starch digestibility is similar to what others have observed when dry-rolled corn is the main starch-containing ingredient consumed in similar finishing rations (Rodenhuis et al., 2018). Lipid intake was greatest in steers fed the HEMP treatment largely resulting from overall OM intake differences among

treatments more so than dietary lipid concentration differences, as steers fed the HEMP diet had 19% and 21% greater OM intake than steers fed DDGS and CON diets, respectively. In general, lipids bypass microbial degradation (Doreau and Chilliard, 1997), which is supported by the post-ruminal flow observed, with variability potentially resulting from marker estimations of post-ruminal digesta flow.

True ruminal N digestibility was 41% and 45% greater for the HEMP diet compared to DDGS and CON diets, respectively. Although post-ruminal N digestibility (as a percent of N entering the duodenum) was reduced for the HEMP diet by approximately 10% compared to the DDGS and CON diets, the improvement in apparent ruminal N digestibility was greater than the reduction in post-ruminal apparent N digestibility, therefore improving total tract apparent N digestibility of the HEMP diet compared to the DDGS and CON diets by 5.4% and 11%, respectively. These results suggest that the ruminally degradable protein (RDP) fraction of hempseed cake CP is greater than the RDP fraction of CP for dried corn distillers grains plus solubles and dry-rolled corn, which is useful information when formulating diets to meet RDP and MP requirements. This is further supported by the individual amino acid flow (g/d) data observed in this trial, where less feed amino acids bypassed ruminal degradation for the HEMP treatment. Dietary amino acid bypass (ruminally undegraded total amino acids) was 35.4% for the HEMP diet, which is similar to in situ ruminally undegradable protein of hempseed cake evaluated in lactating cows (Karlsson et al., 2012) and in vivo in growing lambs (Karlsson and Martinsson, 2011). Karlsson et al. reported slightly greater ruminal degradability of individual amino acids than what was found in the present experiment, likely because amino acid ruminal degradation and bypass percentage in the present experiment was for the entire diet and not for a single ingredient. Dietary bypass amino acid percentages for the CON and DDGS diets were

68.0% and 68.5%, which are slightly greater than the values reported in NASEM (2016) for dry-rolled corn and dried corn distillers grains plus solubles. Again, this is likely because the reported values are of the diet, not individual ingredient, however bypass concentrations can be influenced by dilution rate as well as the other components of the diet (Orskov and Mcdonald, 1979).

The greater amount of NDF and ADF ruminal disappearance, as well as their greater apparent ruminal digestibility in steers fed the HEMP treatment could be contributing to the reduced bacterial efficiency in steers fed the HEMP treatment, as bacteria that degrade structural carbohydrates grow slower, reducing the bacterial efficiency (Russell, 1992). Furthermore, greater hour zero PUN concentrations observed in the LPS portion of this trial (data not shown) in steers fed the HEMP diet may suggest greater loss of ruminal N as ammonia being absorbed through the rumen wall, which can negatively influence bacterial efficiency (Bach et al., 2005)

3.5.3. Nitrogen Balance

Although ruminal N disappearance in steers fed the HEMP diet was greater than in steers fed the DDGS or CON diets, total N excretion was greatest in steers fed the HEMP diet. The increased N excretion was likely because of the 31 and 52% greater N intake in steers fed the HEMP diet than in steers fed the DDGS and CON diets, respectively and N intake and N excretion are positively correlated (Broderick, 2003; Waldrip et al., 2013). Interestingly, urinary N is more positively correlated than fecal N with N intake, likely because N digestibility is not influenced by level of N intake (Waldrip et al., 2013), unless N is limiting. In the present experiment, more N was excreted in the urine than in the feces for all treatments, which differs from what Salim et al. (2016) observed when feeding whole corn- and dried corn distillers grains plus solubles-based diets to finishing steers. In the present experiment, N retention was greatest

in steers fed the HEMP treatment, which may suggest that N or energy were limiting in the CON and DDGS diets, however, steers fed the HEMP and DDGS to have similar N retention as a percent of N intake, and steers fed the CON diet was reduced compared to steers fed the HEMP diet. An increase in N retention has been observed in finishing steers evaluating the effects of inclusion of wet corn distillers grains plus solubles at 30% of the diet DM (Hales et al., 2012), and in growing lambs fed increasing levels (10.5%, 12.5% and 15%, DM-basis) of dietary CP (Cole, 1999). However, Vasconcelos et al. (2007) fed increasing levels (11.5%, 13%, and 14.5%, DM-basis) of dietary CP and did not observe any influence on N retention. This could be because MP requirements were met, or that cattle have changing RDP intake and RUP degradability requirements throughout a finishing period (Klopfenstein et al., 2002). Taken together, these data indicate that N retention increases with N intake, but may depend on MP and energy requirements, and may help explain why N retention was greatest in steers fed the HEMP diet in this experiment.

3.5.4. Post-Ruminal Amino Acid Flow

Because steers fed the HEMP diet had the greatest amino acid intake, and lowest ruminal bypass percent, duodenal flow of total amino acids from feed did not differ among treatments. Despite differences in amino acid intake and rumen degradability of amino acids, bacterial amino acid flow to the small intestine was not influenced by treatment, suggesting that bacterial yield was similar among treatments. Individual amino acid dietary concentration differences as well as bypass percent differences is important to consider when formulating diets to meet RDP and MP requirements, and these results suggest that hempseed cake may be a better source of RDP than dried corn distillers grains plus solubles or dry-rolled corn.

3.6. Conclusions

Overall, the HEMP diet had a lower total tract OM digestibility than the DDGS or CON diets, likely because of the greater ADF concentration of hempseed cake compared to the other test feed ingredients. Although OM total tract digestibility is reduced, ruminal OM digestibility was greater in steers fed the HEMP or CON diets than in steers fed the DDGS diet, and ammonia and total VFA concentration were greater in steers fed the HEMP diet than in steers fed the DDGS or CON diets. Although greater ruminal disappearance of OM, N, NDF, and ADF in the HEMP diet was observed, duodenal flow of these nutrients was similar among treatments likely because of the tendency for greater OM intake, however, post-ruminal digestibility was lowest for the HEMP diet. Although OM total tract digestibility was lowest in steers fed the HEMP diet, digestible energy intake was greatest, largely because of the tendency for greater OM intakes in steers fed the HEMP diet. Feed amino acid degradability differences suggest that hempseed cake has greater ruminal degradability than dried corn distillers grains plus solubles or dry-rolled corn. This information could be used when formulating diets containing hempseed cake to meet or exceed RDP and MP requirements. An increase in N retention in steers fed the HEMP diet is interesting from a production standpoint as this could imply more muscle protein synthesis, however, when fed to finishing heifers HCW and ribeye area were not increased (Chapter 2). Further research exploring the greater N retention in steers fed the HEMP diet is warranted.

3.7. Literature Cited

AOAC. 1990. Official methods of analysis. AOAC, Arlington, VA, USA.

Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88:E9–E21. doi:10.3168/jds.S0022-0302(05)73133-7.

Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370–1381. doi:10.3168/jds.S0022-0302(03)73721-7.

Broderick, G.A., and Kang, J.H. 1980. Automated simultaneous determination ofammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63:64–75. doi:10.3168/jds.S0022-0302(80)82888-8. PMID:7372898.

Doreau, M., and Y. Chilliard. 1997. Digestion and metabolism of dietary fat in farm animals. Br. J. Nutr. 78:S15–S35. doi:10.1079/bjn19970132.

Fenton, T.W., and Fenton, M. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59: 631–634. doi:10.4141/cjas79-081.

France, J., and J. Dijkstra. 2005. Volatile fatty acid production. In: Dijkstra, J., Forbes, J.M., France, J. (Eds.), Quantitative Aspects of Ruminant Digestion and Metabolism, 2nd ed. CAB International, Wallingford, pp. 157–175. doi: 10.1079/9780851998145.0157.

Folch, J., Lees, M., and Stanley, G. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–509. PMID:13428781.

Gibb, D. J., M. A. Shah, P. S. Mir, and T. A. McAllister. 2005. Effect of full-fat hemp seed on performance and tissue fatty acids of feedlot cattle. Can. J. Anim. Sci. 85:223–230. doi:10.4141/A04-078.

Hall, M. B. 2000. Starch gelatinization and hydrolysis method. Page 29 in Neutral Detergent Soluble Carbohydrates, Nutritional Relevance and Analysis—A Laboratory Manual. Dept. of Anim. Sci., Univ. of Florida, Gainesville. Hao.

Hessle, A., M. Eriksson, E. Nadeau, T. Turner, and B. Johansson. 2008. Cold-pressed hempseed cake as a protein feed for growing cattle. Acta Agric. Scand. A Anim. Sci. 58:136–145. doi:10.1080/09064700802452192.

Karlsson, L., M. Finell, and K. Martinsson. 2010. Effects of increasing amounts of hempseed cake in the diet of dairy cows on the production and composition of milk. Animal. 4:1854–1860. doi:10.1017/S1751731110001254.

Karlsson, L., and K. Martinsson. 2011. Growth performance of lambs fed different protein supplements in barley-based diets. Livest. Sci. 138:125–131. doi:10.1016/j.livsci.2010.12.010.

Karlsson, L., M. Ruiz-Moreno, M. D. Stern, and K. Martinsson. 2012. Effects of temperature during moist heat treatment on ruminal degradability and intestinal digestibility of protein and amino acids in hempseed cake. Asian-Australasian J. Anim. Sci. 25:1559–1567. doi:10.5713/ajas.2012.12213.

Kleinhenz, M. D., G. Magnin, S. M. Ensley, J. J. Griffin, J. Goeser, E. Lynch, and J. F. Coetzee. 2020a. Nutrient concentrations, digestibility, and cannabinoid concentrations of industrial hemp plant components. Appl. Anim. Sci. 36:489–494. doi:10.15232/aas.2020-02018.

Kleinhenz, M. D., G. Magnin, Z. Lin, J. Griffin, K. E. Kleinhenz, S. Montgomery, A. Curtis, M. Martin, and J. F. Coetzee. 2020b. Plasma concentrations of eleven cannabinoids in cattle following oral administration of industrial hemp (Cannabis sativa). Sci. Rep. 10:1–7. doi:10.1038/s41598-020-69768-4.

Merchen, N.R. 1988. Digestion, absorption and excretion in ruminants. Page 172 in D.C. Church, ed. The ruminant animal—digestive physiology and nutrition. Prentice-Hall, Englewood Cliffs, NJ.

Mertens, D. R. 1994. Regulation of forage intake. In: Fahey G. C., M. Collins, D. R. Mertens, L. E. Moser, editors. Forage quality, evaluation, and utilization. ASA. CSSA, SSSA, Madison; p. 450-493.

Mustafa, A. F., J. J. McKinnon, and D. A. Christensen. 1999. The nutritive value of hemp meal for ruminants. Can. J. Anim. Sci. 79:91–95. doi:10.4141/A98-031.

NASEM. 2016. Nutrient requirements of beef cattle. 8th rev. ed. The National Academies Press, Washington, DC. doi:10.17226/19014.

Nagaraja, T. G., C. J. Newbold, C. J. van Nevel, and D. I. Demeyer. 1997. Manipulation of ruminal fermentation. Rumen Microb. Ecosyst. 523–632. doi:10.1007/978-94-009-1453-7_13.

Orskov, E. R., and I. Mcdonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499–503. doi:10.1017/S0021859600063048.

Rodenhuis, M. A., F. E. Keomanivong, M. L. Bauer, and K. C. Swanson. 2018. Effect of grain type and dried distillers grains plus solubles oil concentration on site of digestion in cattle fed finishing diets. Can. J. Anim. Sci. 98:368–375. doi:10.1139/cjas-2017-0164.

Robertson, J.B., and Van Soest, P.J. 1981. The detergent system of analysis and its application to human foods. Pages 123–158 in W.P.T. James and O. Theander, eds. The analysis of dietary fiber. Marcell Dekker, New York, NY.

Russell, J., J. D. O'Connor, C. J. Sniffen, D. G. Fox, and W. Chalupa. 1992. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. J. Anim. Sci. 70:3551–3561. doi:10.2527/1992.70113551x.

Salim, H., K. M. Wood, J. P. Cant, and K. C. Swanson. 2016. Influence of feeding increasing levels of dry or modified wet corn distillers grains plus solubles in whole corn grain-based finishing cattle diets on pancreatic α-amylase and trypsin activity. J. Anim. Sci. 96:294–298. doi:10.1139/cjas-2015-0150.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico state and Texas tech university survey. J. Anim. Sci. 94:2648–2663. doi:10.2527/jas.2016-0282.

Tamminga, S. 1977. Protein Degradation in the Forestomachs. J. Anim. Sci. 49:1615–1630.

Waldrip, H. M., R. W. Todd, and N. A. Cole. 2013. Prediction of nitrogen excretion by beef cattle: A meta-analysis. J. Anim. Sci. 91:4290–4302. doi:10.2527/jas.2012-5818.

Zinn, R. A., and F. N. Owens. 1986. a Rapid Procedure for Purine Measurement and Its Use for Estimating Net Ruminal Protein Synthesis. Can. J. Anim. Sci. 66:157–166. doi:10.4141/cjas86-017.

CHAPTER 4. EVALUATION OF THE EFFECTS OF HEMPSEED CAKE ON IMMUNE PARAMETERS IN RESPONSE TO AN LPS CHALLENGE IN FINISHING STEERS

4.1. Abstract

There is interest in the effects that cannabinoids have on the immune system in response to infection. Hempseed cake, a byproduct of industrial hemp and subsequent hempseed oil extraction processes, contains cannabinoids and fatty acids that could be potentially therapeutic for livestock. While hempseed cake has recently been evaluated in finishing diets fed to cattle on growth performance and nutrient digestibility in two separate studies, little is known about the extent to which hempseed cake can influence immune response parameters of finishing steers. An endotoxin (lipopolysaccharide; LPS) challenge experiment using crossbred steers (n = 5; initial BW = 542 kg, SD = 40 kg) was conducted to evaluate the effects of hempseed cake treatment (HEMP) and dried corn distillers grains plus solubles treatment (DDGS; each included at 20% of diet DM in respective treatments) in comparison to a dry-rolled corn-based negative control treatment (CON) on plasma glucose, non-esterified fatty acids (NEFA), urea nitrogen (PUN), rectal temperature, twelve cytokine (IFNγ, IL-1α, IL-6, IL-8, IL-10, IL-36RA, IP10, MCP-1, MIP-1α, MIP-1β, TNFα, and VEGF-A), and amino acid concentrations at five timepoints after administration of LPS. Steers were administered LPS at 0.25 μg/kg BW in a solution diluted to 3 mL, and blood was collected via jugular venipuncture before and 1, 2, 4 and 6-hours post-bolus injection. Data were analyzed as repeated measures using the MIXED procedure of SAS, with hour as the repeated variable, and pre-bolus values was used as a covariate. Plasma glucose, NEFA, and rectal temperature were not influenced by treatment ($P \geq 0.13$), while a treatment by hour interaction ($P = 0.04$) was observed for PUN. Pre-bolus PUN was greater ($P < 0.01$) in steers fed the HEMP than in steers fed the DDGS diet, which was

greater ($P < 0.01$) than in steers fed the CON diet. Plasma IL-1α, IL-36RA, and TNF-α were lower ($P \leq 0.02$), while IL-10 and MIP-1α tended ($P \leq 0.10$) to be lower in steers fed the HEMP diet than in steers fed the DDGS or CON diets. All other cytokines were not influenced by treatment ($P \geq 0.15$). Plasma isoleucine, leucine, and tryptophan concentrations were greater ($P \leq 0.04$) in steers fed the DDGS diet than in steers fed the HEMP and CON diets. Plasma aspartic acid and glycine concentrations were greater ($P \leq 0.02$) for steers fed the DDGS and CON diets than in steers fed the HEMP diet, while tyrosine ($P < 0.01$) was greater in steers fed the DDGS diet than in steers fed the HEMP diet, with steers fed the CON diet being intermediate and not different from either diet. Total plasma amino acid concentrations were not influenced ($P = 0.13$) by treatment. Overall, these data suggest that hempseed cake has potential to influence inflammation by altering cytokine production, but more research is needed to further understand animal growth performance and health implications.

4.2. Introduction

Maintaining animal health and immune function is critical for optimal animal performance and welfare (Gonzalez et al. 2008), and as a result, health challenges are economically challenging for producers (Quimbly et al., 2001). Although beef production has been maintained with fewer cattle on feed (Capper, 2011), morbidity has increased over time (Vogel et al., 2015). Furthermore, with sub-therapeutic feeding of medically-important antibiotics in animal agriculture becoming more restricted, and consumer demand to produce cattle without antimicrobials, exploring alternative approaches to maintain or improve animal health is gaining interest (Burdick Sanchez et al., 2020; Drouillard, 2018). Finishing cattle are exposed to various types of stress that can lead to inflammation and subsequently reduced performance, resulting from transport, dietary changes, or environmental stress among others

(Khafipour et al., 2009; Sanz-Fernandez et al., 2008). Inflammation can have negative effects on animal growth performance, gain to feed, and reproduction among others, largely because of the energetic cost of the immune response (Kvidera et al., 2016). Inflammation can occur in response to immunoactivation triggered by detection of invading pathogens, such as bacterial endotoxin lipopolysaccharide (LPS), by innate immune cells, ultimately leading to the upregulation of pro-inflammatory cytokines (Rosadini et al., 2017). The production of these cytokines stimulates an immune response, characterized by increasing body temperature among other indicators (Broom, 2007). Approaches to positively influence animal health are of interest, and while components of hemp byproducts have been shown to have therapeutic benefits when fed to mice (Oh et al., 2010; Ribeiro et al., 2015), little is known in beef cattle.

Chakrabartay et al. (2021) observed that cannabinoids are present in finishing cattle fed hempseed cake, including cannabidiol (CBD), suggesting potential therapeutic effects in beef cattle. There has been a dramatic increase in interest exploring the effects of cannabinoids, primarily cannabidiol, on the immune system and inflammation (Burtsein, 2015). Although the effects of CBD on inflammation are not fully understood (Pellati et al., 2018) and very little is known about effects in cattle, an increasing amount of data suggests that it has some influence on inflammation in various species (Pagano et al., 2016; Ribiero et al., 2015). An LPS challenge mimics the symptoms caused by bacterial infection (Waldron et al. 2003) and is a common experimental method to induce an immune response, but there has not been any research exploring the effects of hempseed cake in response to an LPS challenge in beef cattle. The objectives of this experiment were to evaluate the effects of hempseed cake on plasma glucose, plasma urea nitrogen (PUN), non-esterified fatty acids (NEFA), cytokine, and amino acid concentrations as well as rectal temperature in response to an LPS challenge in finishing steers.

4.3. Materials and Methods

All animal care and management practices were approved by the North Dakota State University Institutional Animal Care and Use Committee.

4.3.1. Animals, Experimental Design, and Dietary Treatments

Five ruminally and duodenally cannulated crossbred steers (n = 5; initial body weight [BW] = 542 kg, SD = 40 kg) were used in a 3-period Youden square design consisting of three periods, three treatments, and five steers assigned randomly to one of three treatment sequences (one or two steers per period per treatment) to evaluate the effect of hempseed cake on plasma glucose, PUN, NEFA, cytokine, and amino acid concentrations as well as rectal temperature in response to an LPS challenge. For each period, steers were individually housed in slatted floor pens during a 12-day treatment adaptation. Steers were then moved to 1.1 x 2.2 m individual tie stall stanchions for each 7-day collection period (data from this portion of the period not shown). Six steers were initially assigned randomly to one of three treatment sequences, but one steer was removed because of issues adapting to experimental procedures. The control (CON) treatment contained 75% dry-rolled corn, 20% corn silage, and 5% supplement (DM-basis). The dried corn distillers grains plus solubles treatment (DDGS) contained 55% dry-rolled corn, 20% corn silage, 20% dried corn distillers grains plus solubles, and 5% supplement (DM-basis). The hempseed cake treatment (HEMP) contained the same ingredients as the DDGS treatment except hempseed cake replaced DDGS (DM-basis; Tables 4-1 and 4-2). The 20% inclusion level for dried corn distillers grains plus solubles and hempseed cake was selected as this is a common inclusion level for similar byproducts feeds used in practice (Samuelson et al., 2016).

Table 4-1. Composition of diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatments[1]		
Ingredient, % of diet DM	Con	DDGS	Hemp
Corn grain	75	55	55
DDGS	0	0	0
Hempseed cake	0	20	20
Corn silage	20	20	20
Supplement	5	5	5
Fine ground corn	1.57	1.57	1.57
Limestone	2	2	2
Salt	0.1	0.1	0.1
Urea	1	1	1
Chromic Oxide	0.25	0.25	0.25
Vitamin premix[2]	0.01	0.01	0.01
Trace mineral premix[3]	0.05	0.05	0.05
Rumensin-90[4]	0.02	0.02	0.02

[1]Treatments were Control (Con), distillers grains plus solubles (DDGS), and hempseed cake (Hemp).
[2]Contained 48,510 kIU/kg vitamin A and 4,630 kIU/kg vitamin D.
[3]Contained 3.62% calcium (Ca), 2.56% copper (Cu), 16% zinc (Zn), 6.5% iron (Fe), 4% manganese (Mn), 1,050 mg/kg iodine (I) and 250 mg/kg cobalt (Co).
[4]Formulated to supply monensin (Rumensin-90, Elanco Animal Health, Greenfield, IN) at 40 mg/kg.

Table 4-2. Nutrient composition of diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatments[1]		
	Con	DDGS	Hemp
Dry Matter %	69.7	69.0	71.2
Nutrient Analysis, % of DM[2]			
Organic Matter	94.7	93.5	94.2
Starch	54.3	45.4	39.1
Crude Protein	10.3	14.9	17.7
Ether Extract	2.89	3.59	4.36
NDF	22.8	27.3	32.9
ADF	9.3	10.8	19.2
Sulfur	0.12	0.22	0.19
Calcium	0.54	0.59	0.44
Phosphorus	0.34	0.47	0.58
Calcium : Phosphorus	1.62	1.24	0.76
Dietary Gross Energy, kcal/g	4,212	4,356	4,437
Amino Acids, % of DM			
Arginine	0.32	0.47	1.28
Histidine	0.19	0.29	0.37
Isoleucine	0.28	0.44	0.58
Leucine	0.82	1.29	1.16
Lysine	0.27	0.37	0.53
Methionine	0.17	0.23	0.32
Phenylalanine	0.36	0.54	0.67
Threonine	0.29	0.44	0.52
Tryptophan	0.06	0.09	0.14
Valine	0.38	0.58	0.74
Alanine	0.59	0.85	0.80
Aspartic acid	0.51	0.78	1.30
Cysteine	0.17	0.25	0.25
Glutamic acid	1.26	1.94	2.41
Glycine	0.32	0.46	0.63
Proline	0.60	0.94	0.78
Serine	0.31	0.47	0.61
Tyrosine	0.21	0.35	0.42

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Average of diet samples taken each period.

Diets were formulated to meet or exceed ruminally degradable and metabolizable protein, mineral and vitamin requirements (NASEM, 2016). Supplement was formulated to provide 40 mg/kg of monensin (Rumensin, Elanco Animal Health, Greenfield, IN, USA) and 1% urea (DM-basis) as well as vitamins and minerals. Prior to trial initiation, steers were adapted to a high-concentrate diet over a 16-day period. Periods were 21 days with 12-day adaptation period to the treatment offered. Diet transitions were done across a 7-day period by offering a blend of old diet and new diet until the old diet was phased out by day 7. Dietary treatments and water were offered *ad-libitum*, and feed refusals were weighed back daily and adjustments for feed offered were made accordingly (for more information on feed adjustment protocols, see chapter 3). Each period consisted of feed, orts, rumen pH, fecal and urine collections (day 13-19 of each period for all but orts which was day 12-18; data not shown), rumen fluid and duodenal fluid collections (day 15-17; data not shown), rumen contents sample for bacterial isolation (day 20; data not shown), and LPS challenge (day 21).

4.3.2. Collections of Feed Samples

Diets were mixed twice a week in a stationary ribbon mixer (model HD-5, Davis Precision Horizontal Batch Mixer; H.C. Davis Sons Manufacturing Co., Inc., Bonner Springs, KS) and stored in 200 L barrels. Barrels were stored in a cooler at 4 °C to ensure diet quality was maintained. Diet samples (50 g) were collected at 0800 daily on day 13-19 of each collection period and composited. Composites were stored at -20 °C until later analysis.

4.3.3. LPS Challenge

On day 21 at 0800 after completion of the nutrient balance collection of each period, a lipopolysaccharide (LPS) challenge was conducted. Lipopolysaccharide (*Escherichia coli* O55:B5; Sigma Aldrich, St. Louis, MO) was dissolved in sterile saline at a concentration of 50

μg/mL and passed through a 0.2 μm sterile syringe filter (Thermo Scientific, Watham, MA). Approximately 3 mL of LPS solution was administered to provide 0.25 μg/kg of BW LPS into the jugular vein via jugular venipuncture. This dosage was selected based on other literature illustrating an immune response at this dosage (Littlejohn et al., 2019; Burdick Sanchez et al., 2020). Baseline blood samples were collected via jugular venipuncture using tubes containing sodium heparin (Becton Dickinson, Rutherford, NJ) immediately before (pre-bolus, time hour 0), and 1, 2, 4, and 6 hours after LPS injection. Plasma was isolated by centrifugation (3,000 × g at 4 °C) and stored at -80 °C until analyses. Plasma samples were analyzed for glucose, PUN, NEFA, and 12 cytokine (IFNγ, IL-1α, IL-6, IL-8, IL-10, IL-36RA, IP10, MCP-1, MIP-1α, MIP-1β, TNFα, and VEGF-A) concentrations at each hour of blood collection. Furthermore, plasma samples were analyzed for individual amino acid concentrations. Feed was withheld for the duration of the 6-hour data collection period.

4.3.4. Feed Sample Analyses

Feed samples were dried for 48 h at 60 °C in a forced air oven (Grieve SB-350, The Grieve Corporation Round Lake, IL, USA), and ground to pass through a 1 mm screen (Wiley mill, model No. 3; Thomas Scientific, Swedesboro, NJ, USA). Feed samples were analyzed for DM and ash using standard procedures (AOAC, 1990), neutral detergent fiber (NDF) and acid detergent fiber (ADF; Robertson and Van Soest, 1981) using an Ankom fiber analyzer (Ankom technology Corp, Fairport, NY, USA). Feed samples were analyzed for starch concentrations (Hall et al., 2000). Feed samples were analyzed for nitrogen (N) concentration using the Kjeldahl method. Total calcium and phosphorus concentrations were measured in the feed (AOAC, 1990; procedure numbers 968.08, 957.17, and 920.39). Lipid concentration was measured in feed samples using a method adapted by Folch et al. (1957).

Feed samples were analyzed for individual amino acid concentrations using high-performance liquid chromatography at the University of Missouri Agricultural Experiment Station Chemistry Laboratory (AOAC, 1990). Total amino acids, total essential amino acids and total non-essential amino acids were calculated by summing the amino acid concentrations within each category. Essential amino acids (EAA) consisted of histidine, arginine, threonine, lysine, methionine, valine, isoleucine, leucine, phenylalanine, and tryptophan. Non-essential amino acids (NEAA) consisted of glutamic acid, glycine, aspartic acid, serine, alanine, cysteine, proline, and tyrosine. Total amino acids (AA) consisted of all EAA and NEAA listed previously.

4.3.5. Blood Analyses

Plasma glucose concentration was analyzed using the hexokinase/glucose-6-phosphate dehydrogenase method (Farrance, 1987) using the Infinity glucose hexokinase kit (Thermo Trace, Louisville, KY, USA). Plasma urea N was determined using the urease/Berthelot procedure (Chaney and Marbach 1962; Fawcett and Scott 1960) using the QuantiChrom urea assay kit (BioAssay Systems, Hayward, CA, USA). Plasma NEFA concentrations were determined using the modified methods of Eisemann et al. (1988) with a commercial enzymatic kit (HR Series NEFA-HR; Fujifilm Waco Diagnostics, Mountain View, CA, USA). Plasma cytokines concentrations were determined using the MILLIPLEX Bovine Cytokine/Chemokine 15-plex kit (BCYT1-33 K; EMD Millipore Corporation, Billerica, MA, USA). Plasma amino acid concentrations were analyzed by reversed phase ultra-performance liquid chromatography after pre-column derivatization of amino acids with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Salazar et al., 2012; Lemley et al., 2013) and using an ethylene bridged hybrid C_{18} column (2.1 x 150 mm; 1.7 μm; Waters Corp., Milford, MA, USA). Total amino acids, EAA and NEAA were calculated by summing the amino acid concentrations within each category for

each heifer. Essential amino acids consisted of histidine, arginine, threonine, lysine, methionine, valine, isoleucine, leucine, phenylalanine, and tryptophan. Non-essential amino acids consisted of asparagine, glutamic acid, glutamine, glycine, aspartic acid, cysteine, serine, alanine, proline, and tyrosine. Total amino acids consisted of all essential and non-essential amino acids listed previously.

4.3.6. Statistical Analysis

Pre-bolus data were analyzed using the MIXED procedure in SAS 9.4 (SAS institute Inc., Cary, NC, USA) as a 3 x 5 Youden square. The model included period and treatment as fixed effects, and steer was included in the model as a random effect. All plasma metabolites, rectal temperatures, plasma cytokines, and plasma amino acid data were analyzed as repeated measures using the MIXED procedure of SAS, with hour as the repeated variable. Four covariance structures (autoregressive 1, compound symmetry, Toeplitz, and unstructured) were compared for each repeated measure analysis, with compound symmetry ultimately being selected based on lowest fit statistics. Steer within period was considered the experimental unit. Each specific variable's pre-bolus value collected during the LPS challenge served as a covariate. The lowest-detection limit (7.78 pg/ml) for IL-6 concentration was used for three steers pre-bolus values because concentrations were below the detection limit. All pre-bolus values were analyzed separately from the post-bolus values. Treatment and hour (for plasma metabolites, rectal temperatures, plasma cytokines, and plasma amino acids) were included in the model as fixed effects and treatment by day interactions were tested. Pairwise comparisons (least significant difference approach) were used to analyze differences among treatment means when the treatment *P*-value was significant. Linear and quadratic effects of hour were tested using contrast coefficients that were generated using PROC IML procedure of SAS for all repeated

measures analyses. Linear and quadratic effects were tested beginning at hour 1 with pre-bolus values used as a covariate. Treatment differences were considered significant when $P \leq 0.05$, and tendencies between $P > 0.05$ and $P \leq 0.10$.

4.4. Results

4.4.1. LPS – Response Parameters

Pre-bolus PUN concentration was greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the DDGS diet, which was greater ($P < 0.01$) than in steers fed the CON diet, and all other LPS response measures were not different ($P \geq 0.17$) among treatments (Table 4-3). A treatment by hour interaction ($P = 0.04$) was observed for PUN (Figure 4-1), while no other treatment by hour interactions ($P \geq 0.39$) were observed for remaining LPS response measures (Table 4-4). Glucose, NEFA and rectal temperature were not influenced ($P \geq 0.13$) by treatment. Plasma IL-1α and TNF-α concentrations decreased ($P \leq 0.02$), and IL-10 and MIP-1α tended to decreased ($P \leq 0.10$) in steers fed the HEMP diet than in steers fed the DDGS or CON diets. Furthermore, IL-36RA was lower ($P < 0.02$) in steers fed the HEMP diet than in steers fed the DDGS diet, while steers fed the CON diet was intermediate and not different from either diet, and all other cytokines were not influenced ($P \geq 0.15$) by dietary treatment. There was a time effect ($P < 0.01$) for all LPS response parameters with the exceptions of PUN, Il-1α, IL-36RA, and VEGF-A ($P \geq 0.10$; Table 4-4). Plasma glucose and PUN linearly decreased ($P \leq 0.02$) NEFA decreased quadratically ($P < 0.02$), and rectal temperature increased quadratically ($P < 0.01$) as hour increased (Table 4-5). Plasma MIP-1α, MIP-1β, TNF-α, and IL-10 peaked between hour 1 and 2 and linearly decreased ($P < 0.01$) while IFNγ, IL-6, IP-10, and MCP-1 increased quadratically ($P < 0.01$) as hour increased.

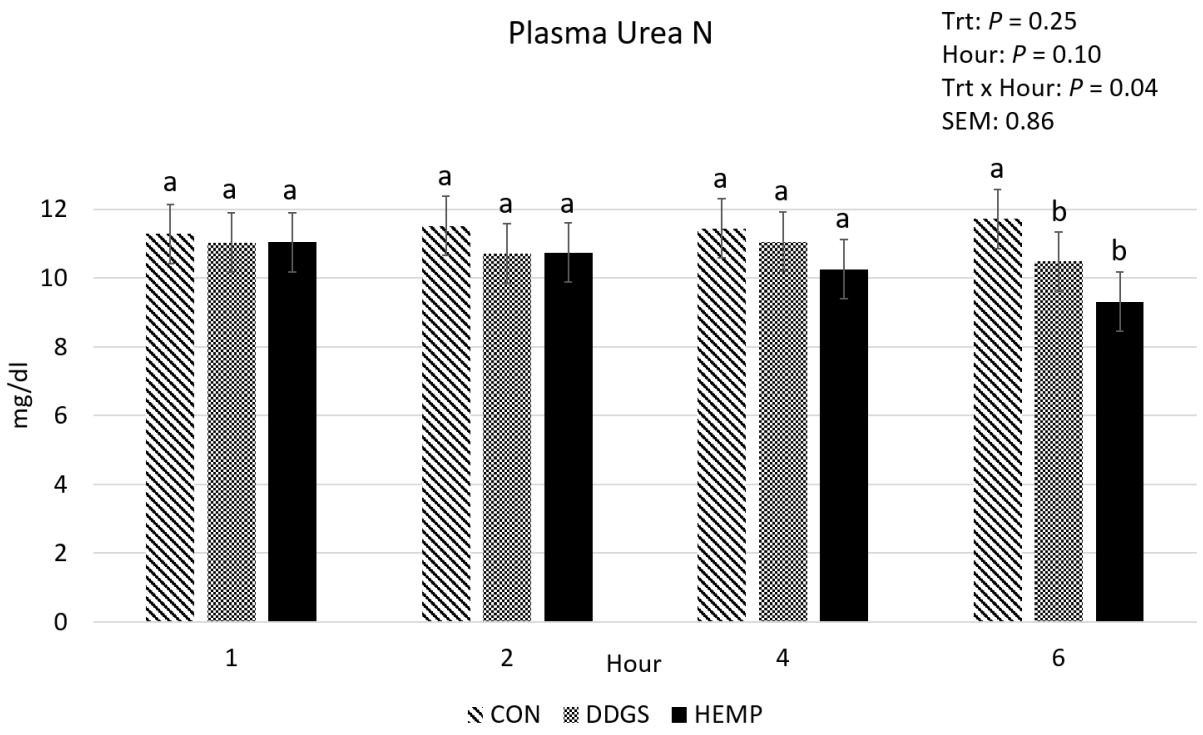

Figure 4-1. Plasma urea N concentration in steers fed diets containing 0% byproduct (CON), 20% dried corn distillers grains plus solubles (DDGS), or 20% hempseed cake (HEMP; DM-basis). Means within hour that differ are denoted by differing letters, means within treatment that differ are denoted by differing numbers ($P < 0.05$).

Table 4-3. Pre-bolus LPS response parameters of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatment[1]				
Item[2]	Con[3]	DDGS[3]	Hemp[3]	SEM	*P*-Value
Glucose, mg/dl	79.7	76.2	80.6	3.8	0.65
PUN, mg/dl	7.9[a]	11.1[b]	15.7[c]	0.8	< 0.01
NEFA, µmol/L	115	115	110	11	0.91
Temp, °C	38.9	38.8	38.7	0.1	0.17
Cytokines, pg/ml					
IFNγ	0.63	0.92	0.73	0.17	0.51
IL-1α	33.7	95.9	54.0	48.5	0.56
IL-6	27.1	30.5	67.0	36.4	0.67
IL-8	254	585	222	167	0.27
IL-10	279	1,157	426	645	0.53
IL-36RA	125	193	153	60	0.64
IP-10	257	400	298	80	0.29
MCP-1	313	696	358	275	0.49
MIP-1α	177	724	291	394	0.52
MIP-1β	66.1	47.0	57.0	9.0	0.39
TNFα	5,620	29,515	8,269	16,678	0.50
VEGF-A	32.7	84.9	51.2	38.3	0.49

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Plasma glucose, urea N (PUN), non-esterified fatty acid (NEFA), and rectal temperature (Temp).
[3]Control, DDGS, and Hemp treatment means sharing the same superscript do not differ ($P \leq 0.05$).

Table 4-4. LPS response parameters of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatment[1]				*P*-Value[2]		
Item[3]	Con[4]	DDGS[4]	Hemp[4]	SEM	Trt	Hour	Trt × Hr
Glucose, mg/dl	75.0	78.6	75.8	2.2	0.50	< 0.01	0.82
PUN, mg/dl	11.5	10.8	10.3	0.4	0.25	0.10	0.04
NEFA, µmol/L	152	109	133	13	0.13	< 0.01	0.43
Temp, °C	39.6	39.7	39.7	0.1	0.64	< 0.01	0.86
Cytokines, pg/ml							
IFNγ	1.65	1.71	1.53	0.46	0.91	< 0.01	0.97
IL-1α	61.8[a]	66.2[a]	35.3[b]	10.7	< 0.01	0.81	0.96
IL-6	2,294	2,109	2,287	613	0.96	< 0.01	0.99
IL-8	309	552	411	120	0.27	< 0.01	0.39
IL-10	1,292	1,385	910	201	0.06	< 0.01	0.92
IL-36RA	131[ab]	159[a]	111[b]	17	0.02	0.73	0.84
IP-10	1,072	1,071	906	207	0.43	< 0.01	0.82
MCP-1	1,572	1,948	1,848	371	0.39	< 0.01	0.99
MIP-1α	698	793	470	109	0.10	< 0.01	0.72
MIP-1β	6,724	6,089	5,178	2,182	0.70	< 0.01	0.94
TNFα	21,681[a]	24,535[a]	15,226[b]	2,651	< 0.01	< 0.01	0.72
VEGF-A	58.0	58.5	45.5	5.9	0.15	0.55	0.97

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Standard error of the mean (SEM) for the treatment by hour (Trt x Hr) interaction.
[3]Plasma glucose, urea N (PUN), non-esterified fatty acid (NEFA), and rectal temperature (Temp).
[4]Control, DDGS, and Hemp treatment means sharing the same superscript do not differ ($P \leq 0.05$).

Table 4-5. LPS response parameters by hour of steers during the LPS challenge.

	Hour[1]					*P*-Value	
Item[2]	1	2	4	6	SEM	Linear	Quad
Glucose, mg/dl	84.2	81.5	71.9	68.3	3.6	< 0.01	0.49
PUN, mg/dl	11.11	10.99	10.91	10.50	0.25	0.02	0.54
NEFA, µmol/L	114	106	111	195	24	< 0.01	0.02
Temp, °C	39.7	39.9	40.1	39.0	0.1	< 0.01	< 0.01
Cytokines, pg/ml							
IFNγ	0.84	1.31	2.73	1.65	0.47	0.02	< 0.01
IL-1α	50.6	60.8	53.3	53.1	9.0	0.90	0.65
IL-6	359	3,841	3,455	1,265	845	0.84	< 0.01
IL-8	240	881	212	361	153	0.25	0.34
IL-10	1,901	1,071	1,086	726	232	< 0.01	0.15
IL-36RA	132	144	134	124	14	0.48	0.50
IP-10	657	1,418	1,176	813	168	0.77	< 0.01
MCP-1	1,059	3,051	2,157	891	297	0.01	< 0.01
MIP-1α	1,067	670	478	401	174	< 0.01	0.11
MIP-1β	8,024	10,930	4,098	936	2,063	< 0.01	0.33
TNFα	26,849	23,919	15,808	15,346	3,341	< 0.01	0.21
VEGF-A	48.7	60.9	51.8	54.6	6.5	0.89	0.65

[1]Pre-bolus value used as covariate and therefore not shown.
[2]Plasma glucose, urea N (PUN), non-esterified fatty acid (NEFA), and rectal temperature (Temp).

No treatment by hour interactions ($P \geq 0.31$) were observed for individual plasma amino acid concentrations (Table 4-6). Plasma isoleucine, leucine, and tryptophan concentrations were greater ($P \leq 0.04$) in steers fed the DDGS diet than in steers fed the HEMP or CON diets. Plasma aspartic acid and glycine concentrations were greater ($P \leq 0.02$) in steers fed the DDGS or CON diets than in steers fed the HEMP diet, while plasma tyrosine concentration was greater ($P = 0.05$) in steers fed the DDGS diet than in steers fed the HEMP diet, with steers fed the CON diet intermediate and not different from either diet. Plasma valine and asparagine concentrations tended to be greater ($P \leq 0.09$) in steers fed the DDGS diet than in steers fed the HEMP or CON diets. There was an hour effect ($P \leq 0.02$) for all plasma amino acids concentrations (including total EAA, total NEAA and total AA), with the exceptions of arginine, histidine, aspartic acid, glutamine, and glycine (Table 6). The general trend was for plasma amino acid concentrations to

linearly decline as hour progressed, although a tendency for a quadratic decrease ($P \leq 0.10$) was observed for total EAA, total NEAA and total AA (Table 4-7). Linear decreases ($P \leq 0.02$) in plasma amino acids as hour increased were observed for histidine, lysine, methionine, phenylalanine, threonine, valine, asparagine, cysteine, glutamic acid, glycine, proline, serine, tyrosine, total EAA, total NEAA, and total AA. A quadratic decrease ($P \leq 0.03$) in plasma amino acid concentrations as hour increased was observed for both isoleucine, leucine, tryptophan, alanine, and tyrosine. Pre-bolus arginine and aspartic acid concentrations were greater ($P < 0.01$) in steers fed the HEMP diet than in steers fed the CON diet, which was greater ($P \leq 0.02$) than in steers fed the DDGS diet (Table 4-8). Pre-bolus plasma leucine concentrations were greater ($P < 0.01$) in steers fed the DDGS diet than in steers fed the CON diet, which was greater ($P = 0.02$) than in steers fed the HEMP diet, and pre-bolus lysine was greater ($P = 0.04$) in steers fed the HEMP or CON diets than in steers fed the DDGS diet. Pre-bolus plasma phenylalanine concentrations were greater ($P = 0.04$) in steers fed the CON diet than in steers fed the HEMP diet, with steers fed the DDGS diet intermediate and not different from either diet. Pre-bolus plasma glycine concentrations were greater ($P = 0.04$) in steers fed the HEMP diet than in steers fed the CON diet, with steers fed the DDGS diet intermediate and not different from either diet. All other pre-bolus amino acid concentrations were not influenced by treatment ($P \geq 0.07$).

Table 4-6. Plasma amino acid concentrations of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatment[1]				*P*-Value[2]		
Item, µM	Con[3]	DDGS[3]	Hemp[3]	SEM	Trt	Hour	Trt × Hr
Essential AA							
Arginine	44.6	47.1	51.1	6.0	0.55	0.67	0.87
Histidine	47.0	48.8	49.0	1.1	0.45	0.06	0.86
Isoleucine	46.8[a]	56.7[b]	46.2[a]	4.7	0.04	< 0.01	0.99
Leucine	95.9[a]	126.2[b]	81.0[a]	13.3	< 0.01	< 0.01	0.74
Lysine	56.4	46.2	55.8	7.0	0.26	< 0.01	0.97
Methionine	15.2	16.7	15.6	0.8	0.14	< 0.01	0.93
Phenylalanine	44.0	46.4	45.3	1.6	0.11	< 0.01	0.41
Threonine	37.9	38.6	35.2	2.7	0.29	< 0.01	0.97
Tryptophan	31.9[a]	34.8[b]	32.4[a]	0.9	< 0.01	< 0.01	0.81
Valine	141	154	146	6	0.09	< 0.01	0.97
Total EAA	559	607	567	30	0.15	< 0.01	0.99
Non-Essential AA							
Alanine	147	145	147	4.7	0.93	< 0.01	0.99
Asparagine	24.9	28.4	24.4	1.7	0.08	< 0.01	0.97
Aspartic acid	10.4[a]	10.4[a]	7.5[b]	1.2	0.02	0.31	0.79
Cysteine	2.10	1.94	2.22	0.17	0.32	< 0.01	0.31
Glutamine	321	331	309	14	0.50	0.39	0.99
Glutamic acid	29.0	31.3	32.1	2.0	0.50	0.02	0.89
Glycine	227[a]	232[a]	208[b]	8	< 0.01	0.09	0.93
Proline	51.5	54.3	52.0	3.2	0.72	< 0.01	0.98
Serine	49.8	51.5	51.8	2.0	0.71	< 0.01	0.83
Tyrosine	32.3[ab]	36.1[a]	31.2[b]	2.1	0.05	< 0.01	0.58
Total NEAA	884	939	859	25	0.05	< 0.01	0.98
AA	1,446	1,529	1,440	49	0.13	< 0.01	0.99

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Standard error of the mean (SEM) for the treatment by hour (Trt x Hour) interaction.
[3]Control, DDGS, and Hemp treatment means sharing the same superscript do not differ ($P \leq 0.05$).

Table 4-7. Plasma amino acid concentrations by hour of steers during the LPS challenge.

	Hour[1]					*P*-Value	
Item, µM	1	2	4	6	SEM	Linear	Quad
Essential AA							
Arginine	51.1	48.8	45.1	45.5	4.9	0.27	0.59
Histidine	50.5	49.4	48.1	45.1	1.3	< 0.01	0.69
Isoleucine	68.1	55.2	39.4	36.6	4.3	< 0.01	< 0.01
Leucine	127	111	86	79	6	< 0.01	0.02
Lysine	64.0	56.8	46.7	43.6	4.5	< 0.01	0.26
Methionine	18.7	17.2	14.2	13.3	0.8	< 0.01	0.09
Phenylalanine	49.2	46.9	43.4	41.6	1.3	< 0.01	0.23
Threonine	46.6	39.9	32.6	29.8	2.6	< 0.01	0.06
Tryptophan	34.0	34.8	33.7	29.8	1.0	< 0.01	< 0.01
Valine	171	160	134	123	7	< 0.01	0.21
Total EAA	680	620	523	488	29	< 0.01	0.10
Non-Essential AA							
Alanine	162	154	132	137	6.9	< 0.01	0.02
Asparagine	31.2	26.8	23.3	22.2	2.1	< 0.01	0.11
Aspartic acid	10.0	8.9	8.4	10.2	0.7	0.76	0.07
Cysteine	2.16	2.45	1.95	1.78	0.20	< 0.01	0.40
Glutamine	329	307	319	326	13	0.71	0.25
Glutamic acid	36.1	29.4	29.4	28.5	2.6	0.02	0.12
Glycine	231	228	218	212	7.6	0.01	0.86
Proline	63.4	56.9	45.9	44.1	3.5	< 0.01	0.06
Serine	55.8	54.4	48.9	45.1	3.0	< 0.01	0.96
Tyrosine	41.0	35.3	29.0	27.6	2.2	< 0.01	0.03
Total NEAA	962	903	856	855	31	< 0.01	0.08
AA	1,642	1,523	1,378	1,343	53	< 0.01	0.06

[1]Pre-bolus value used as covariate and therefore not shown.

Table 4-8. Pre-bolus plasma amino acid concentrations of steers fed diets containing no byproduct (Con), dried corn distillers grains plus solubles (DDGS), or hempseed cake (Hemp).

	Treatment[1]				
Item, µM	Con[2]	DDGS[2]	Hemp[2]	SEM	*P*-Value
Essential AA					
Arginine	60.9[a]	48.3[b]	72.7[c]	2.5	< 0.01
Histidine	55.0	55.0	53.2	3.6	0.79
Isoleucine	78.1	81.9	75.4	5.0	0.26
Leucine	136[a]	167[b]	112[c]	8	< 0.01
Lysine	89.3[a]	70.8[b]	89.4[a]	6.2	0.04
Methionine	23.0	21.3	21.6	1.6	0.60
Phenylalanine	55.9[a]	52.9[ab]	45.1[b]	3.3	0.04
Threonine	51.0	56.0	54.0	5.3	0.75
Tryptophan	35.4	31.7	36.2	2.8	0.33
Valine	174	191	176	16	0.35
Total EAA	759	775	736	43	0.38
Non-Essential AA					
Alanine	184	165	178	8	0.23
Asparagine	41.4	37.6	35.7	4.0	0.63
Aspartic acid	10.8[a]	9.1[b]	12.7[c]	0.5	< 0.01
Cysteine	2.61	2.33	1.54	0.36	0.15
Glutamine	346	309	375	16	0.07
Glutamic acid	48.4	39.6	33.9	5.2	0.17
Glycine	207[a]	225[ab]	256[b]	14.5	0.04
Proline	71.3	83.4	63.7	6.3	0.10
Serine	68.9	60.9	63.6	6.4	0.64
Tyrosine	49.6	52.0	42.5	5.6	0.33
Total NEAA	1,030	984	1,063	28	0.22
AA	1,789	1,759	1,799	50	0.80

[1]Treatments consisted of 0% byproduct (Con), 20% DDGS or 20% Hemp (DM-basis) in a finishing diet.
[2]Control, DDGS, and Hemp treatment means sharing the same superscript do not differ ($P \leq 0.05$).

4.5. Discussion

4.5.1. LPS – Response Parameters

The CBD concentration and/or the fatty acid profile of hempseed cake have been shown to influence the immune response of other species (Ribeiro et al., 2015; Pagano et al., 2016) and could potentially influence the immune response of steers. The LPS challenge model is a commonly used approach for examining treatment effects on chronic inflammation (Burdick Sanchez et al., 2020; Cangiano et al., 2019; Littlejohn et al., 2019). The LPS challenge resulted in increased production of cytokines in response to LPS is initiated when LPS is detected by LPS-binding protein (LBP) at the plasma membrane of the innate immune cell. The cluster of differentiation 14, located at the plasma membrane then transfers the LPS monomer to a heterodimer formed by myeloid differentiation protein-2 and TLR4. The interaction of LPS with this heterodimer initiates the assembly of the intracellular myddosome, which leads to NF-κB activation, stimulating inflammatory cytokine production (Kieser and Kagan, 2017; Freudenberg et al., 2008). Macrophages are an innate immune cell largely responsible for the production of TNF-α, IL-6, and other cytokines (Littlejohn et al., 2019). This process of immune system activation that can subsequently influence animal health and performance. Worth noting, production of cytokines can be beneficial to mounting an immune response, but over production can have negative consequences that occur under inflammatory conditions (Calder, 2001).

During an immune response to infection, glucose utilization increases (Lang et al., 1993), largely resulting from immune cells becoming glucose utilizers to support the energetic demand of activating the immune system (Kvidera et al., 2017). An immune response was successfully induced by LPS injection in the current experiment as indicated by decreased plasma glucose and increased rectal temperature after LPS administration. Rectal temperature changes in response to

LPS injection is not necessarily dose-dependent (Waldron et al., 2003), but an increase is typically observed for a short period of time (2-8 hours) following LPS administration (Borderas et al., 2008). The decrease in plasma glucose as hour progressed likely resulted in an increase in NEFA to compensate for energetic needs associated with less available glucose (Cangiano et al., 2019). No treatment differences were observed for pre- or post-bolus plasma glucose or NEFA concentrations in the present trial, likely because there were no differences in digestible energy intake between treatments (chapter 3), suggesting that dietary treatments did not influence these indicators of energy balance when steers were challenged with LPS. While an LPS challenge will typically result in reduced DMI (Elsasser et al., 1995), intake is only responsible for roughly 50% of the reduction in milk yield in dairy cows, with immune system glucose consumption accounting for the rest of the reduction in performance (Kvidera et al., 2017). Feed was withheld in the current experiment for the duration of the challenge that lasted 6 hours, so differences in DMI likely were not the cause of the observed decrease in plasma glucose concentrations. The observed reduction of PUN concentration at hour 6 in steers fed the DDGS and HEMP diets than in steers fed the CON diet could suggest altered nitrogen utilization among these treatments as the immune challenge progressed.

Plasma amino acid concentrations that were significantly influenced by treatment during the LPS challenge likely result from dietary treatment concentration, organic matter intake, and site of digestion differences. The linear decrease in plasma amino acid concentration as hour progresses is similar to what has been observed in dairy cows administered with LPS (Zhao et al., 2018) and suggests that amino acids may be consumed during the immune response. Amino acid metabolism and subsequent plasma concentrations can be influenced by immune status. To mount an immune response, amino acid metabolism shifts away from growth and towards

immune response processes, such as gluconeogenesis, cytokine production, acute phase protein production, and immunoglobulin synthesis (Le Floc'h et al., 2004; Coleman et al., 2020). Synthesis of acute phase proteins can account for 30% of protein synthesis in humans, indicating a substantial effect from the immune system on amino acid concentrations (Reeds et al., 1994).

The innate immune response to LPS administration resulted in an increase in plasma cytokine concentration of all measured cytokines within 1 to 4 hours, with a majority peaking at hour 2. This experiment observed that all measured cytokines, except for IFNγ, peaked at hour one or two and then declined, while temperature peaked at hour 4 and then declined, suggesting that cytokine upregulation may precede the temperature increase. The reduction in plasma IL-1α, IL-36RA, and TNF-α concentrations, and tendency for reduced MIP-1α in steers receiving the HEMP treatment suggests that hempseed cake may have anti-inflammatory characteristics in steers challenged with LPS compared to dry-rolled corn and dried corn distillers grains plus solubles. TNF-α, IL-1, and IL-6 mediate fever, intake regulation, mobilization of protein and fat, and production of acute phase proteins, suggesting that these cytokines could have potential animal growth performance, carcass and health implications (Calder, 2001). A decrease in plasma anti-inflammatory cytokine IL-36RA concentration and the tendency for a decrease in plasma concentration of anti-inflammatory cytokine IL-10 in steers fed the HEMP treatment suggests IL-1α and TNF-α reduction occurred independent of IL-10.

The anti-inflammatory properties of certain cannabinoids found in hempseed, specifically cannabidiol (CBD), could be influencing plasma IL-1α and TNF-α concentrations. Results from the experiment conducted in Chapter 4 illustrated that CBD (and other cannabinoids) from hempseed cake is absorbed into the plasma of finishing heifers (data not shown), suggesting that CBD could be influencing cattle physiological responses. The effect of CBD on inflammation

has not been evaluated extensively in cattle, but there is increasing focus on the effects of CBD on inflammation in other species (Pagano et al., 2016; Ribiero et al., 2015). While the influence of CBD on immune system and endocannabinoid system is not completely understood (Pellati et al., 2018), exogenous CBD is thought to interact with the endocannabinoid system by downregulating cannabinoid receptor 2 and orphan G-protein coupled receptor 55, while upregulating transient receptor channel subfamily V member 1 (TRPv1). This leads to a decrease in cyclooxygenase 2, inducible nitric oxide synthase, and cytokine production, ultimately reducing the inflammatory response to infection (Pellati et al., 2018). Furthermore, CBD has been suggested to restore gut permeability and serve as an antioxidant potentially through peroxisome proliferator-activated receptor gamma modulation, reducing inflammation by downregulating IL-1β, IL-2, IL-6, IL-8, TNF-α, and IFNγ and improving immune function (Pellati et al., 2018; McPartland et al., 2015). Worth noting, the CBD concentration is quite low in hempseed oil, and most oil is extracted from the seed when hempseed cake is produced, so more research is needed to determine CBD concentrations necessary to elicit effects on immune function.

Hempseed oil fatty acid composition is 53% linoleic (omega-6) and 17.5% linolenic (omega-3), whereas corn oil fatty acid composition is 56% omega-6 and 1.3% omega-3 (Pavan et al., 2007). Omega-3 fatty acids have been shown to reduce TNF-α, IL-6, and MCP-1 in mice by activating GPR120, which blocks downstream NF-κB activation and subsequent cytokine production (Oh et al., 2010). A low ratio of omega-6 to omega-3 fatty acids, similar to that found in hempseed oil, has been suggested to be optimal for human health (DiNicolantonio and O'Keefe, 2018) as omega fatty acids have been shown to decrease superoxide production, neutrophil and monocytes, and production of pro-inflammatory cytokines (Calder, 2001).

Furthermore, Farran et al. (2002) observed improved health and immune response of cattle fed diets containing high levels of linolenic acid. Additionally, alpha-linolenic acid can be a precursor for eicosapentanoic and docosahexaenoic that are known to have preventative as well as therapeutic roles in inflammation (Belluzzi, 2002). Biohydrogenation of linolenic and linoleic fatty acids within the rumen is high (Duckett et al., 2002), so further research is needed to determine the fatty acid profile from hempseed cake flowing out of the rumen to determine possible effects on inflammation.

4.6. Conclusions

The observed reduction in plasma concentrations of pro-inflammatory cytokines IL-1α and TNF-α in steers fed the HEMP diet could have positive implications on animal health and performance and suggests possibilities of situational use of hempseed cake as a feedstuff during times of elevated stress, such as at weaning, transportation, receiving, processing, or diet changes. More research is needed as to why certain cytokines were influenced by treatment while others were not to better understand the mechanisms by which feeding hempseed cake may influence immune function. Furthermore, the influence of hempseed cake on individual amino acids as well as the PUN treatment by hour response suggests potential nitrogen metabolism implications, but more research is needed to understand the implications of these results. Overall, hempseed cake offers intriguing potential as a feedstuff that improves the immune response in finishing steers experiencing stress. These initial observations require more research to better understand what is driving these responses and to gauge potential future implications of feeding hempseed cake in finishing diets.

4.7. Literature Cited

AOAC. 1990. Official methods of analysis. AOAC, Arlington, VA, USA.

Belluzzi, A. 2002. n -3 Fatty acids for the treatment of inflammatory bowel diseases. Proc. Nutr. Soc. 61:391–395. doi:10.1079/pns2002171.

Broom, M. 2007. Physiology of fever. Paediatr. Nurs. 19:40–44. doi:10.7748/paed.19.6.40.s32.

Burdick Sanchez, N. C., J. A. Carroll, P. Rand Broadway, T. S. Edrington, I. Yoon, and C. R. Belknap. 2020. Some aspects of the acute phase immune response to a lipopolysaccharide (LPS) challenge are mitigated by supplementation with a Saccharomyces cerevisiae fermentation product in weaned beef calves. Transl. Anim. Sci. 4:1–13. doi:10.1093/tas/txaa156.

Burstein, S. 2015. Cannabidiol (CBD) and its analogs: A review of their effects on inflammation. Bioorganic Med. Chem. 23:1377–1385. doi:10.1016/j.bmc.2015.01.059.

Calder, P. 2001. Polyunsaturated fatty acids and immunity. Lipids. 36:1007–1024. doi:10.1007/s11745-001-0812-7.

Cangiano, L. R., M. G. Zenobi, C. D. Nelson, I. R. Ipharraguerre, and N. Dilorenzo. 2019. A bioactive extract from *Olea europaea* protects newly weaned beef heifers against experimentally induced chronic inflammation. J. Anim. Sci. 97:4349-4361. doi: 10.1093/jas/skz285.

Capper, J. L. 2011. The environmental impact of beef production in the United States: 1977 compared with 2007. J. Anim. Sci. 89:4249-4261. doi: 10.2527/jas.2010-3784.

Chakrabarty, S., T. M. Winders, E. M. Serum, K. C. Swanson, C. R. Dahlen, M. D. Kleinhenz, and D. J. Smith. 2021. Rapid quantification of cannabinoids in bovine plasma using

electrospray ionization mass spectrometry *sans* LC column. American Society for Mass Spectrometry (abstract).

Chaney, A.L., and Marbach, E.P. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8: 130-132. doi: 10.1093/clinchem/8.2.130.

Coleman, D. N., V. Lopreiato, A. Alharthi, and J. J. Loor. 2020. Amino acids and the regulation of oxidative stress and immune function in dairy cattle. J. Anim. Sci. 98:S175-S193. doi: 10.1093/JAS/SKAA138.

DiNicolantonio, J. J., and J. H. O'Keefe. 2018. Importance of maintaining a low omega-6/omega-3 ratio for reducing inflammation. Open Heart. 5:1-4. doi: 10.1136/openhrt-2018-000946.

Drouillard, J. S. 2018. Current situations and future trends for beef production in the United States of America – A review. Asian-Australas. J. Anim. Sci. 31:1007-1016. doi: 10.1093/jas/skaa160.

Duckett, S. K., J. G. Andrae, and F. N. Owens. 2002. Effect of high-oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. J. Anim. Sci. 80:3353–3360. doi:10.2527/2002.80123353x.

Eisemann, J. H., G. B. Huntington, and C. L. Ferrell. 1988. Effects of dietary clenbuterol on metabolism of the hindquarters in steers. J. Anim. Sci. 66:342-353. doi: 10.2527/jas1988.662342x

Farran, T. B., M. F. Spire, J. J. Sindt, S. P. Montgomery, C. M. Coetzer, H. J. LaBrune, J. E. Minton, J. J. Higgins, T. H. Elsasser, and J. S. Drouillard. 2002. Immune response in feeder cattle fed different sources of dietary lipid. Kansas Agric. Exp. Stn. Res. Reports. 5–9. doi:10.4148/2378-5977.1677.

Farrance, I. 1987. Plasma glucose methods, a review. Clin. Biochem. Rev. 8:55-68.

Fawcett, J. K., and J. E. Scott. 1960. A rapid and precise method for the determination of urea. J. Clin. Path. 13:156-159. doi:10.1136/jcp.13.2.156

Folch, J., Lees, M., and Stanley, G. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–509. PMID:13428781.

Freudenberg, M. A., S. Tchaptchet, S. Keck, G. Fejer, M. Huber, N. Schütze, B. Beutler, and C. Galanos. 2008. Lipopolysaccharide sensing an important factor in the innate immune response to Gram-negative bacterial infections: Benefits and hazards of LPS hypersensitivity. Immunobiology. 213:193–203. doi:10.1016/j.imbio.2007.11.008.

Gonzalez, L. A., B. J. Tolkamp, M. P. Coffey, A. Ferret, and I. Kyriazakis. 2008. Changes in feeding behavior as possible indicators for the automatic monitoring of health disorders in dairy cows. J. Dairy Sci. 91:1017-1028. doi: 10.3168/jds.2007-0530.

Hall, M. B. 2000. Starch gelatinization and hydrolysis method. Page 29 in Neutral Detergent Soluble Carbohydrates, Nutritional Relevance and Analysis—A Laboratory Manual. Dept. of Anim. Sci., Univ. of Florida, Gainesville. Hao.

Khafipour, E., D. O. Krause, and J. C. Plaizer. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92:1060-1070. doi: 10.3168/jds.2008-1389.

Kieser, K. J., and J. C. Kagan. 2017. Multi-receptor detection of individual bacterial products by the innate immune system. Nat. Rev. Immunol. 17:376–390. doi:10.1038/nri.2017.25.

Kindt, T. J., R. A. Goldsby, B. A. Osborne, and J. Kuby. 2007. Kuby immunology. 6th ed. New York (NY): W.H. Freeman.

Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. S. Fernandez, and L. H. Baumgard. 2017. Glucose requirements of an activated immune system in lactating Holstein cows. J. Dairy Sci. 100:2360–2374. doi:10.3168/jds.2016-12001.

Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L. H. Baumgard. 2016. Technical note: A procedure to estimate glucose requirements of an activated immune system in steers. J. Anim. Sci. 94:4591–4599. doi:10.2527/jas.2016-0765.

Le Floc'h, N., D. Melchior, and C. Obled. 2004. Modifications of protein and amino acid metabolism during inflammation and immune system activation. Livest. Prod. Sci. 87:37-45. doi: 10.1016/j.livprodsci.2003.09.005.

Lemley, C. O., L. E. Camacho, A. M. Meyer, M. Kapphahn, J. S. Caton, and K. A. Vonnahme. 2013. Dietary melatonin supplementation alters uteroplacental amino acid flux during intrauterine growth restriction in ewes. Animal. 9:1500-1507. doi: 10.1017/S1751731113001006.

Littlejohn, B. P., N. C. B. Sanchez, J. A. Carroll, D. M. Price, R. C. Vann, T. H. Welsh, and R. D. Randel. 2019. Influence of prenatal transportation stress on innate immune response to an endotoxin challenge in weaned Brahman bull calves. Stress. 22:236–247. doi:10.1080/10253890.2018.1523895.

Luheshi, G. N. 1998. Cytokines and fever: mechanisms and sites of action. Ann. NY Acad. Sci. 856:83-89. doi: 10.1111/j.1749-6632.1998.tb08316.x.

McPartland, J. M., M. Duncan, V. Di Marzo, and R. G. Pertwee. 2015. Are cannabidiol and Δ9-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br. J. Pharmacol. 172:737–753. doi:10.1111/bph.12944.

NASEM. 2016. Nutrient requirements of beef cattle. 8th rev. ed. The National Academies Press, Washington, DC. doi:10.17226/19014

Pagano, E., R. Capasso, F. Piscitelli, B. Romano, O. A. Parisi, S. Finizio, A. Lauritano, V. Di Marzo, A. A. Izzo, and F. Borrelli. 2016. An orally active Cannabis extract with high content in cannabidiol attenuates chemically-induced intestinal inflammation and hypermotility in the mouse. Front. Pharmacol. 7:1–12. doi:10.3389/fphar.2016.00341.

Pellati, F., V. Borgonetti, V. Brighenti, M. Biagi, S. Benvenuti, and L. Corsi. 2018. Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer. Biomed Res. Int. 2018. doi:10.1155/2018/1691428.

Quimby, W. F., B. F. Sowell, J. P. G. Bowman, M. E. Branine, M. E. Hubbert, and H. W. Sherwood. 2001. Application of feeding behaviour to predict morbidity of newly received calves in a commercial feedlot. Can. J. Anim. Sci. 81:315–320. doi: 10.4141/A00-098.

Reeds, P. J., C. R. Fjeld, and F. Jahoor. 1994. Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? J Nutr. 124: 906-910. doi: 10.1093/jn/124.6.906.

Ribeiro, A., V. I. Almeida, C. Costola-De-Souza, V. Ferraz-De-Paula, M. L. Pinheiro, L. B. Vitoretti, J. A. Gimenes-Junior, A. T. Akamine, J. A. Crippa, W. Tavares-De-Lima, and J. Palermo-Neto. 2015. Cannabidiol improves lung function and inflammation in mice submitted to LPS-induced acute lung injury. Immunopharmacol. Immunotoxicol. 37:35–41. doi:10.3109/08923973.2014.976794.

Robertson, J.B., and Van Soest, P.J. 1981. The detergent system of analysis and its application to human foods. Pages 123–158 in W.P.T. James and O. Theander, eds. The analysis of dietary fiber. Marcell Dekker, New York, NY.

Rosadini, C. V., and J. C. Kagan. 2017. Early innate immune responses to bacterial LPS. Curr. Opin. Immunol. 44:14–19. doi:10.1016/j.coi.2016.10.005.

Salazar, C., J. M. Armenta, and V. Shulaev. 2012. An UPLC-ESI-MS/MS assay asing 6-aminoquinolyl-NHydroxysuccinimidyl carbamate derivatization for targeted amino acid analysis: application to screening of *Arabidopsis thaliana* mutants. Metabolites. 2:398-428. doi: 10.3390/metabo2030398.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico state and Texas tech university survey. J. Anim. Sci. 94:2648–2663. doi:10.2527/jas.2016-0282.

Sanz-Fernandez, V. M., J. B. Daniel, D. J. Seymour, S. K. Kvidera, Z. Bester, J. Doelman, and J. Martin-Tereso. 2020. Targeting the hindgut to improve health and performance of cattle. Animals. 10:1817. doi: 10.3390/ani10101817.

Waldron, M. R., T. Nishida, B. J. Nonnecke, and T. R. Overton. 2003. Effect of lipopolysaccharide on indices of peripheral and hepatic metabolism in lactating cows. J. Dairy Sci. 86:3447–3459. doi:10.3168/jds.S0022-0302(03)73949-6.

Vogel, G. J., C. D. Bokenkroger, S. C. Rutten-Ramos, and J. L. Bargen. 2015. A Retrospective evaluation of animal mortality in US feedlots: Rate, timing, and cause of death. The Bovine Practitioner, 49: 113-123. doi: 10.21423/bovine-vol49no2p113-123.

Zhao, F. F., T. Y. Wu, H. R. Wang, L. Y. Ding, G. Ahmed, H. W. Li, W. Tian, and Y. Z. Shen. 2018. Jugular arginine infusion relieves lipopolysaccharide-triggered inflammatory stress

and improves immunity status of lactating dairy cows. J. Dairy Sci. 101:5961-5970. doi: 10.3168/jds.2017-13850

CHAPTER 5. SUMMARY AND CONCLUSIONS

The renewed interest in industrial hemp largely for hempseed oil extraction will continue to generate hempseed byproducts, such as hempseed cake, that do not currently have a market that provides value for the product. Cattle are commonly fed protein byproducts, but price and availability of these byproducts influence their utilization, so finding alternative feedstuffs that cattle can efficiently use continues to be important. The crude protein, fiber and oil concentrations in hempseed cake make cattle an ideal target species to explore the effects of this product. Furthermore, finding a use for hempseed cake is in the interest of both industrial hemp and cattle producers, but first, gaining a better understanding of how hempseed cake it utilized by cattle consuming finishing diets is crucial.

A series of experiments were conducted and discussed in this dissertation to further investigate the potential uses of hempseed cake as a feedstuff in finishing cattle diets. The lack of data on feeding hempseed byproducts to ruminants created a need for this body of work in order to better understand important utilization characteristics of hempseed cake. The available data on feeding hempseed byproducts to ruminants suggest that DMI is typically not negatively influenced at dietary inclusions ranging from 9-33% (DM-basis), while growth performance (ADG and G:F) decreases, sometimes negatively influencing final BW and HCW. The consistent decrease in animal growth efficiency suggest that there could be potential digestibility differences between hempseed cake and other feedstuffs fed to ruminants. Conversely, milk production has been shown to improve when feeding hempseed cake, likely because of the CP concentration found in hempseed byproducts. Although feeding hempseed byproducts to ruminants has been evaluated in several experiments, many questions still remain and was the basis for the set of experiments conducted for this dissertation.

In experiment 1 (Chapter 2) we evaluated the effects of hempseed cake compared to dried corn distillers grains plus solubles on animal growth performance, feeding behavior, plasma metabolite concentrations, and carcass characteristics in heifers fed finishing diets. The heifers fed hempseed cake had decreased ADG, G:F, final BW, and HCW while DMI and all other carcass characteristics were not influenced by treatment. Furthermore, feeding behavior and plasma glucose was not influenced by treatment, while PUN was greater for heifers fed hempseed cake. Several individual plasma amino acid concentrations were influenced by treatment, although total plasma amino acid concentrations were not different between treatments. Heifer growth performance was negatively influenced by hempseed cake, ultimately reducing final BW and HCW. These results suggest that there may be digestibility differences between hempseed cake and dried corn distillers plus solubles because DMI was similar, while ADG and G:F were negatively influenced, potentially because of the greater ADF concentration in hempseed cake. These results illustrated the need to investigate hempseed cake digestibility to further explain these growth performance results.

In experiment 2 (Chapter 3) we evaluated the effects of feeding a diet containing hempseed cake (HEMP) in comparison to diets containing dried corn distillers grains plus solubles (DDGS) or a control diet containing no byproduct (CON) on organic matter (OM) intake, ruminal fermentation parameters, nutrient digestibility, nutrient flow and nitrogen balance when fed to finishing steers. The goal was to gain more insight into how the chemical composition of hempseed cake may influence cattle utilization of this product. Overall, total tract apparent OM digestibility was decreased, and N total tract apparent digestibility was greatest in steers fed the HEMP diet than in the other treatments. Site of digestion differences were observed, as the ruminal total VFA and ammonia concentration, nutrient digestibility, and

nutrient flow data suggest that the HEMP diet is more ruminally available for digestion than the DDGS or CON diets. Nitrogen retention was greatest as for steers fed the HEMP diet as well. Taken together, the OM digestibility results may partially explain results observed in experiment 1, and the improvement in N digestion and retention have potential growth performance and efficiency implications that would require future research focuses to address.

Experiments 1 and 2 focus on nutrition characteristics of hempseed cake, and although cattle nutrient utilization has major implications on performance it is not the only area deserving attention when evaluating this product. Animal health influences performance and profitability, and animal stress can negatively affect animal health. There are several stress-inducing aspects in cattle production systems that may have health implications, such as at weaning, transportation, receiving, processing, or diet changes, so evaluating immune response parameters of hempseed cake is of interest. Experiment 3 (Chapter 4) evaluated the effects of the HEMP diet on immune response parameters when steers were challenged with an LPS injection. The results suggest that inflammation is decreased in steers fed HEMP, indicated by a decrease in plasma TNF-α and IL-1α concentrations. These data suggest that hempseed cake has potential to reduce inflammation by influencing cytokine production, but more research is needed to further understand animal growth performance and health implications.

Taken together, this series of experiments shows intriguing potential for hempseed cake as a feedstuff for cattle fed finishing diets. Understanding hempseed cake cannabinoid residues in the tissue are needed to further understand hempseed cake metabolism in cattle as well as cattle and human safety implication, and these results are forthcoming. Although hempseed cake reduced growth performance and is less digestible than dried corn distillers grains plus solubles, these results indicate cattle still perform well with it in the diet. Furthermore, the ruminal VFA

and ammonia concentration, N digestibility, N retention, and amino acid flow results suggest that hempseed cake can influence fermentation and site of digestion, while still allowing for adequate dietary amino acid utilization. Combining these results with the observed influence on the immune system, hempseed cake shows promise as a feedstuff for cattle fed finishing diets, but more data are needed to further understand the implications of the main findings of this dissertation.

In order to feed hempseed cake to livestock, it must first gain FDA approval through the AAFCO. Briefly, the submitting party must provide the FDA and AAFCO with the following items: animal and human safety data, the analytical methodology used to cultivate that data, detailed lists of the manufacturing processes involved, toxicology results on any harmful substances in the ingredient, data to show the ingredient will be manufactured consistently, and a proposed legal definition for the product. When these items are undergoing AAFCO review, the FDA may find potential problems and require that the more formal food additive petition (FAP) procedure be followed, like if there are human safety concerns then it likely will go to the FAP process. Once the FDA-Center for Veterinary Medicine approves of the product, it then goes to the AAFCO ingredient committee approval process, then to the AAFCO general membership approval process and then if that is successful, the product is approved. The other option is gaining GRAS status. This is a provision required and regulated by the Federal Food, Drug, and Cosmetic act that regulates the FDA. This process does not require pre-market review and approval by the FDA as a food additive. Another option is to gain generally recognized as safe (GRAS) status. In order to do so, the submitting party must prove that the substance meets these criteria: the product meets the same safety standards as existing food additives, will not cause harm if used appropriately, and is recognized as "safe" by the scientific community, based on

publicly available scientific information. The product can then be placed in the market based on those conclusions. Or the FDA can make a voluntary notification and undergo a review process that is much shorter than the FDA food additive petition (FAP) process (270 days compared to several years for FAP). The product must then be recognized with a "common, or usual name", an approved food additive, or defined/listed in the AAFCO official publication. One of the caveats with this process is that GRAS ingredients are not recognized by all state feed agencies, which can create state by state differences in approval for this product. These are the processes available to bring a novel feed/drug to market. Once it is approved, the animal food additive must be used within the constraints that led to its established regulation.

Prior to gaining its approval, more research is likely needed to fill gaps in the literature. One area that is important from an animal and human safety standpoint is hempseed cake compound residues in animal tissue. While this dissertation did not report these data, withdrawal period and tissue residue data were collected during the experiment conducted in chapter 2 and will be made available in the near future. Another area that needs to be evaluated further is how hempseed cake should be utilized as a feedstuff. Feeding hempseed cake at multiple dietary concentrations (for example, 0, 10, 20, and 30% of the diet DM) to investigate an optimal inclusion rate would likely have practical implications allowing for producers and/or nutritionists to make feeding decisions based on these data. More research is needed to evaluate opportune times to use this feedstuff as well. The receiving period seems like an ideal timeframe to further investigate this feedstuff for multiple reasons based on the data presented in this dissertation. The influence hempseed cake has on the immune system may be beneficial during high stress times, such as when cattle are received at a feedlot and are undergoing multiple stressors (weaning, transportation, co-mingling, new environment, diet adaptation etc.). During this time

period, getting calves to increase their DMI is important, and hempseed cake was observed to at minimum not influence DMI, and in some instances has increased intake. Further investigating any potential influences of greater fiber concentration in hempseed cake on ruminal pH could be interesting, as cattle are undergoing dietary energy concentration adaptations during the receiving period. Lastly, the receiving period often coincides with the greatest MP requirements and most efficient N utilization by the growing animal, and hempseed cake offers greater N digestibility as well as greater N retention as observed in this dissertation. While the body of work covered in this dissertation has made great strides towards answering many previously unknown questions about hempseed cake as a ruminant feedstuff, there are many more questions yet to be answered. Further investigations addressing the gaps in knowledge discussed previously are important to gain greater understanding the effects and utilization of hempseed cake as a feedstuff in finishing cattle diets.