

THE EFFECT OF HEMP BYPRODUCT SUPPLEMENTATION ON BEEF QUALITY

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**Title**

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North Dakota State University's regulations and meets the accepted  
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**MASTER OF SCIENCE**

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## ABSTRACT

The supplementation of hempseed cake (hemp byproduct) could be considered an alternative protein and fiber source for ruminants such as cattle. Hempseed cake might be a successful alternative feed source due to cattle's digestive abilities. Yet, the physiological effects caused by cannabinoids in hemp (cannabidiol [CBD] and (-)- $\Delta^9$ -tetrahydrocannabinol [THC]) are of concern. However, hemp with much less than 0.3% THC on a dry matter basis can remain a potential alternative feed ingredient. The objective of this study was to evaluate the significance of hempseed cake inclusion in a late finishing ration on carcass characteristics, meat quality characteristics, retail shelf-life, proximate analysis, and fatty acid profile of muscle food obtained from commercial beef heifers.

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## LIST OF ABBREVIATIONS

ACP.....	Acyl Carrier Protein
AMSA.....	American Meat Science Association
AOAC.....	Association of Official Analytical Chemists
C14:1.....	Myristoleic Acid
C17:0.....	Heptadecaenoic Acid
C17:1.....	Cis-10-Heptadecaenoic Acid
C18:2.....	Linolelaidic Acid
C18:3.....	$\gamma$ -linolenic Acid
C20:0.....	Arachidic Acid
C20:1.....	Cis-11-Eicosenoic Acid
C20:4.....	Arachidonic Acid
C22:5.....	Docosapentaeonoic Acid
CBC.....	Cannabichromene
CBD.....	Cannabidiol
CBDA.....	Cannabidiolic Acid
CBDV.....	Cannabidivarin
CBG.....	Cannabigerol
CBN.....	Cannabinol
CIE L*a*b*.....	International Commission on Illumination, L* for luminance, a* for red-green axis, and b* for blue-yellow axis.
<i>cis</i> .....	Relates to a fatty acid in a configuration with two hydrogen atoms adjacent to the double bond are on the same side of the chain that creates a bent chain
CLA.....	Conjugated Linoleic Acid
CON.....	Controlled Diet

CR.....	Color Reader
D-OR.....	Democratic Representative from Oregon
D-VT.....	Democratic Representative from Vermont
DDGS.....	Dried Distillers Grain with Solubles
DEA.....	United States Drug Enforcement Administration
DP.....	Dressing Percentage
FDA.....	United States Food and Drug Administration
g.....	Gram(s)
h.....	Hour(s)
HCW.....	Hot Carcass Weight
HEMP.....	Hempseed Cake Diet
IH.....	Industrial Hemp
kg.....	Kilograms
KPH.....	Kidney, Pelvic, and Heart Fat
µg/g.....	Microgram divided by Gram
N.....	Sample size
NC.....	North Carolina
ND.....	Not Detected
NJ.....	New Jersey
OH.....	Ohio
P.....	Probability of obtaining test results at least as extreme as the results actually observed.
PDIFF.....	Requests the p-values for differences.
pH.....	Potential of Hydrogen or Power of Hydrogen
ppm.....	Parts Per Million
PVC.....	Polyvinyl Chloride

QG.....	United States Quality Grade
R-KY.....	Republican Representative from Kentucky
R-TX.....	Republican Representative from Texas
REA.....	Ribeye Area
SAS.....	Statistical Analysis System
SEM.....	Standard Error of the Mean
THC.....	(-)- $\Delta^9$ -tetrahydrocannabinol
THCA.....	Tetrahydrocannabinolic Acid
<i>trans</i> .....	Unsaturated (monounsaturated or polyunsaturated) fatty acids with a double bond that creates a straight chain.
TR.....	Trace Amounts
Trt.....	Treatment
USDA.....	United States Department of Agriculture
w/w.....	Weight for Weight
WBSF.....	Warner-Bratzler shear force
YG.....	United States Yield Grade

## CHAPTER 1. LITERATURE REVIEW

### 1.1. Introduction

*Cannabis sativa*, or commonly known as hemp, has medicinal and industrial uses for humans, and livestock. Developed countries have begun to prioritize economic policies for cultivation and processing of hemp due to its many uses (Oseyko et al., 2019). The main end products from hemp are hemp byproducts (fiber, food products, medicine, and oilseed) and cannabinoids; cannabidiol (CBD) and (-)- $\Delta^9$ -tetrahydrocannabinol (THC) (Oseyko et al., 2019). Each end product plays a significant role in the steadily expanding market for hemp. Additionally, hemp has been observed to have a rich concentration of essential fatty acids and is a viable protein source as an alternative ingredient in human and livestock diets. This alternative feed ingredient can be a replacement to soybeans and (or) barley (Oseyko et al., 2019) in commercial feeds.

The ability to design a low cost but efficient diet for finishing cattle is an important aspect to the beef industry. However, due to the delegitimization of hemp, government restrictions have created a limited list of approved ingredients, without the consideration of hemp. These restrictions have caused producers and feedlot managers to search for alternative feed ingredients that do not reduce the efficiency of feed utilization. The search for an alternative feed ingredient has led individuals to industrial hemp. Industrial hemp contains both types of cannabinoids, but the THC concentration is much lower than the United States Department of Agriculture (USDA) recommended 0.3% (dry matter basis) and remains an attractive feed alternative. There is a possibility for industrial hemp to be utilized as an alternative feed ingredient because industrial hemp has a fiber and protein concentration that is comparable to

other more traditionally used feedstuffs. Additionally, industrial hemp can serve as a cheaper alternative to soybeans and barley (Gibb et al., 2005).

## **1.2. History**

Hemp has been utilized in industrial settings for centuries, but it was not until 1937 when President Roosevelt signed the Marihuana Tax Act into law. This law established the beginning of regulating and controlling cannabis production in the United States. This act placed a tax on all cannabis sales which led to farmers identifying alternative crops, because farmers could not produce hemp without producing marijuana. This act remained in effect until 1970, when President Nixon signed the Controlled Substances Act. This act made marijuana a Schedule 1 controlled substance which placed it in the same classification as heroin and cocaine. At this time, the distinction of cannabis products was not yet defined (Keller, 2013; Kleinhenz et al., 2020).

In 2005, Representative Ron Paul (R-TX) drafted the first federal legislation for legalizing hemp which evolved into the Industrial Hemp Farming Act. This act was the first piece of legislation that introduced the removal of restrictions on the cultivation of non-psychoactive industrial hemp, defined industrial hemp separately from marijuana, and assigned authority to states to regulate the cultivation of industrial hemp. By 2007, Roger Johnson, the North Dakota Agriculture Commissioner granted the first two state hemp farming licenses to Dave Monson and Wayne Hauge. Later at the Federal level, Senators Wyden (D-OR), Paul (R-KY), Merkley (D-OR), and Sanders (D-VT) introduced the Industrial Hemp Act of 2012 which improved upon Representative Paul's 2005 hemp bill by providing further detail regarding the exclusion of industrial hemp from the definition of marijuana. This act defined industrial hemp

to mean *Cannabis sativa L.* and any part of the plant, with less than 0.3% of (-)- $\Delta^9$ -tetrahydrocannabinol (THC) on a dry weight basis (Keller, 2013).

When President Obama signed the Farm Bill of 2014 into law, it was the first time that a bill had defined industrial hemp. Additionally, this bill authorized universities and state departments of agriculture to establish pilot programs that researched hemp (Johnson, 2019). By 2018, President Trump signed an updated Farm Bill that not only legalized hemp but designated the USDA as the federal regulators of all hemp production. The USDA published an Interim Final Rule in the Federal Register that established the federal hemp production regulations. The Interim Final Rule allowed for states and tribes to submit hemp regulations plans to the USDA so that farmers could apply directly to the USDA for a permit to conduct pilot programs and research on the cultivation of hemp (Johnson, 2019).

### **1.3. Comparison of Hemp Byproducts, Cannabidiol (CBD), and (-)- $\Delta^9$ -tetrahydrocannabinol (THC)**

A person, place, or item is often classified according to similar qualities and/or characteristics. Hemp byproducts, CBD, and THC are all derived from the same plant, *Cannabis sativa*, or commonly known as hemp (Small, 2017; Johnson, 2019). Cannabis has been classified as a narcotic and has been widely criminalized in Western countries since World War II. Due to the delegitimization of hemp, individuals have deemed hemp and cannabinoids with subclassifications of “narcotics” which include psychoactive, psychotropic, psychotomimetic, and hallucinogenic. Psychoactive is a broad term that applies to an alteration of sensation, mood, consciousness, or other behavioral and psychological actions. Psychoactive, psychotropic, and psychotomimetic are additional “mood-altering” terms associated with cannabis. These terms are affiliated with hemp due to the cannabinoids THC and CBD that can cause an inebriant and

euphoric sensation. The hallucinogenic term refers to a mental and (or) physiological sensation that exists within the mind to individuals who ingest the hallucinogenic substance. The hallucinogenic term is the least appropriate term to apply to hemp because hemp is not known to cause a hallucinogenic state (Keller, 2013; Small, 2017; Johnson, 2019).

The terms hemp and marijuana have been mistakenly interpreted as synonyms but have drastically different meanings. This has caused great confusion. Those seeking industrial application for hemp have gone through great extremes to separate the two terms. The term industrial hemp has been created to identify hemp for non-euphoric drug uses (fiber, food products, medicine, and oilseed). Hemp byproducts that are cultivated for fibers are collected from the stalk, or main stem of the plant. Hemp byproducts utilized for oilseed, are labeled as oilseed hemp, or hemp seed. In order to be labeled as industrial hemp, the product must contain no more than 0.3% THC on a dry matter basis (Small, 2017).

The various varieties of hemp plants are grouped into four different categories: wild, fiber, oilseed, and psychotomimetic. The fiber, oilseed, and psychotomimetic categories are all cultivars (a cultivated variety of the plant species that was selected and cultivated by humans; (Johnson, 2019). A brief description of each categorical grouping of *C. sativa* is as follows.

1. Wild. Wildly grown hemp plants have grown outside cultivation and are therefore able to reproduce in nature without human assistance.
2. Fiber. Because of its strength, durability, and water-resistant properties, this byproduct of hemp is one of the oldest sources of textile fiber.
3. Oilseed. The oil classification is more complex and will be explained in greater detail below.



4. Psychotomimetic. The psychotomimetic properties of hemp are associated with THC. In order to be classified as industrial hemp, most western countries have mandated that the THC content be less than 0.3% on a dry matter basis. The primary hurdle facing the commercial utilization of hemp has been the expanding industrial cultivation of cannabis for marijuana. This has led to the development of legislation that prohibits the cultivation of hemp because the concentration of THC often exceeds the content limit set by the USDA (Small, 2017).

The oilseed category has four subcategories: essential oil, hashish oil, liquid hemp, and vegetable oil. Essential oil is from the glandular secretory trichomes of the hemp plant and is a compilation of complex organic (hydrocarbon) chemicals that promote fast evaporation. Therefore, essential oil is primarily used in a diffuser or humidifier because it has the ability to evaporate quickly and blend with the mist being created by the diffuser or humidifier. The essential oil market is relatively insignificant compared to hashish oil and vegetable oil. Hashish oil is rich in THC solvent-extracts and is a highly concentrated form of marijuana, however, there is a highly concentrated CBD form that is referred to as liquid hemp. Both hashish oil and liquid hemp are commonly used for vaping, or recreational smoking. Vegetable oil is mainly made up of triglycerides that are non-volatile at room temperature and commonly used as a cooking ingredient. This form of vegetable oil can replace canola oil, or other variations of vegetable oil when frying, or baking.

The oil extracted for vegetable oil is obtained from hemp seeds. The seed is found in the fruit wall or pericarp; the protective hull or shell. The seed is typically filled by an embryo that is rich in oils, proteins, and carbohydrates. The oil is extracted from the seeds by a mechanical press. However, due to the location of the seed, the THC levels are more tightly regulated in

hemp seed oil because of the potential contamination when extracting the seeds from the plant. If not carefully extracted, the seed can come into contact with resin that is secreted by the epidermal glands on the leaves. Additionally, if the perigonal bracts are not thoroughly removed, the seed can be deemed toxic (Ross et al., 2000). The perigonal bracts contain the highest concentration of THC and are specialized leaves that act as a cover for the seed (Small, 2017). The acceptable levels of THC for consumer products are determined by the US Food and Drug Administration (FDA). The FDA determined that THC for hemp seed oils must range from 0.005 to 10 ppm. The US Department of Agriculture (USDA) sets the regulations for cannabis production. The potential toxicity comes from the THC concentration being more than 10 ppm or greater than a 5% dry matter weight (Orr and Starodub, 1999; Small and Marcus, 2002; Small, 2017). It has been suggested that cannabis with a THC level greater than 1.0% can be considered marijuana because of the potential psychotomimetic effect or intoxicating properties. Marijuana is developed by the combination of flowers and small twigs (branching system of the flowers). The branching system of flowers is referred to as buds. Buds are desired for marijuana because of the rich concentration of THC (Small, 2017).

#### **1.4. Research of Hemp for Livestock Use**

There is limited research regarding the impact of feeding industrial hemp or hemp byproducts on the growth performance characteristics of ruminant livestock. There is even less research that characterizes the nutrient concentrations and digestibility of industrial hemp and its byproducts. Those who have evaluated the inclusion of hemp and hemp byproducts in livestock diets have assumed that they would be better suited for ruminants because of the ruminants' ability to utilize high fiber feedstuffs as a nutrient source (Kleinhenz et al., 2020). Aside from an abundance of fiber, hemp seed contains approximately 30% oil that could serve as an energy

source for growing cattle (Leizer et al., 2000; Gibb et al., 2005). Furthermore, hemp seed oil is considered a complete nutritional source due to the seed oil containing all the essential amino (phenylalanine, valine, tryptophan, threonine, isoleucine, methionine, histidine, leucine, and lysine) and fatty acids (Leizer et al., 200). Kleinhenz et al. (2020) analyzed the hemp components of *Cannabis sativa* to determine the nutrient concentration and concentration of cannabinoids. Nutrient analysis was conducted on seven different plant components; 1) whole industrial hemp plants (no roots), 2) leaves, 3) stalks, 4) hemp flowers, 5) seed heads, 6) chaff, and 7) extracted female flowers. Kleinhenz et al. (2020) found the nutrient concentration and fiber digestibility was dependent on the plant part tested (Table 1.1). The whole plant and stalk tested lowest for crude protein (whole plant = 6.9%; stalk = 5.3%), and minerals (whole plant = 3.1%; stalk = 2.5%) in comparison to the flowers, leaves, and seed heads. Furthermore, the whole plant and stalks had the higher levels of fiber concentration, but was not as readily digested as leaves, hemp flowers, seed heads, chaff, and extracted female flowers due to the whole plant and stalks primarily used for the production of rope, paper, and fabric products. Additionally, it should be noted that the stalk has the lowest concentration of cannabinoids. Kleinhenz et al. (2020) indicated that hemp plants that contained a higher concentration of fat were a poor source of energy because the excessive intake of fat hinders the digestibility of fiber and therefore, would only act as a filler within the ration. However, the fiber content protects degradation in the rumen, and can be highly digestible throughout the remaining gastrointestinal tract, thus possessing the potential to serve as a valuable source of rumen by-pass protein (Owens et al., 2014). Additionally, Bull et al. (1965) and Haskins et al. (1969) implied that fiber incorporated in feed rations promoted rumination and salivation, therefore, aiding in the health and functionality of the rumen.

Table 1.1. Summary of nutrient composition (expressed as a percentage of dry matter) of hemp plants, hemp leaves, seed heads, chaff from seed harvest and cleaning, and extracted hemp flower obtained from Kleinhenz et al. (2020).

<b>Plant Components of Hemp (%)</b>							
<b>Outcome</b>	<b>Whole Plant</b>	<b>Leaves</b>	<b>Stalk</b>	<b>Hemp Flower</b>	<b>Seed Heads</b>	<b>Chaff</b>	<b>Extracted Flower</b>
Dry Matter	70.3	88.9	64.8	90.9	89.8	92.9	96.6
Fat	2.7	8.9	1.2	12.5	13.2	4.6	3.2
Ash	8.8	21.2	6.3	14.1	16.6	24.9	25.7
Sugar	2.7	5.9	2.0	5.0	2.8	6.3	4.7
Starch	0.2	0.9	0.1	0.7	0.7	1.2	0.6
Crude Protein	6.9	13.0	5.3	21.2	23.0	20.0	24.5
NDF	81.6	44.7	84.4	52.5	53.2	27.9	30.9
ADF	60.8	20.8	64.6	26.1	29.6	18.0	18.1
Calcium	1.4	4.3	1.0	2.3	2.6	5.7	3.6
Phosphorus	0.3	0.4	0.3	1.1	0.7	0.4	0.4
Magnesium	0.2	0.5	0.2	0.4	0.5	0.5	0.5
Potassium	1.1	3.3	0.9	2.4	1.3	1.9	2.4
Sulfur	0.1	0.4	0.1	0.4	0.3	0.2	0.3

The content of cannabinoids is the distinguishing factor that separates the classification of industrial hemp from marijuana. This is also the limiting factor in the large-scale utilization of hemp as a livestock feed. Kleinhenz et al. (2020) reported the components of hemp that contained various concentrations of cannabinoid (Table 1.2). The flowers and leaves contained the highest concentrations of CBD and cannabidiolic acid (CBD precursor) and the highest levels of THC and (-)- $\Delta^9$ -tetrahydrocannabinolic acid A (THC precursor). It should be noted that THC and the THC precursor were found in all the samples, but all the concentrations were less than 0.3% on a dry matter basis. Due to the THC concentration being less than 0.3%, the plant materials could be identified as industrial hemp and not marijuana.

Table 1.2. Cannabinoid concentration of hemp plants, hemp leaves, hemp flower, seed heads, chaff from seed harvest after cleaning, and extracted hemp flower summarized from Kleinhenz et al. (2020).

Cannabinoid Concentration of Plant Sample							
Cannabinoid	Whole Plant	Leaves	Stalks	Hemp Flower	Seed Heads	Cleanin gs	Extracted Flowers
Cannabinol (µg/g)	9.0	31.0	4.0	27.0	11.0	7.0	21.0
Δ9-tetrahydrocannabinol (µg/g)	186.0	573.0	31.0	664.0	275.0	158.0	301.0
Δ9-tetrahydrocannabinolic acid A (µg/g)	626.0	4609.0	119.0	3379.0	1228.0	458.0	16.0
Δ8-tetrahydrocannabinol (µg/g)	ND*	ND*	ND*	ND*	ND*	ND*	ND*
Cannabichromene (µg/g)	192.0	417.0	49.0	513.0	68.0	140.0	ND*
Cannabidiol (µg/g)	721.0	3347.0	132.0	3509.0	262.0	463.0	8062.0
Tetrahydrocannabivarin (µg/g)	30.0	2.0	ND*	1.0	303.0	2.0	ND*
Cannabidiolic acid (µg/g)	4,870.0	36,920.0	1,705.0	32,900.0	3,184.0	5,309.0	1,960.0
Cannabigerolic acid (µg/g)	519.0	1788.0	362.0	1938.0	285.0	654.0	154.0
Cannabichromenic (µg/g)	851.0	4041.0	500.0	2916.0	411.0	663.0	ND*
Cannabigerol (µg/g)	67.0	293.0	28.0	230.0	23.0	79.0	ND*

\* ND\* = not detected.

\*\* TR = trace amounts.

#### 1.4.1. Hemp as a Livestock Feedstuff

Hemp seed has the ability to be a valuable source of energy in feedlot diets, due to the fact that it contains approximately 30% oil (Leizer et al., 2000) with 80% of that oil composed of polyunsaturated fatty acids (Deferne and Pate, 1996). The inclusion of hemp byproducts as a fiber source fed to ruminant livestock species in grow-finish applications has great potential, especially if inclusion does not negatively impact carcass characteristics. That said, there have been very few controlled studies that have evaluated feeding of hemp to meat-producing animals. Gibb et al. (2005) fed dietary hemp seed for 166 days, setting treatments at a hemp seed inclusion rate of 0, 9, and 14% as a substitution of steam-rolled barley grain and barley silage in

beef finishing ration (Table 1.2). The authors indicated that feeding hemp seed did not affect the carcass weight, dressing percentage, backfat, or ribeye area (Table 1.3).

Table 1.3. Comparison of beef carcass characteristics between a control diet and hemp seed diets fed a 9 or 14% inclusion rate as a substitution for steam-rolled barley grain and barley silage in beef finishing ration (summarized from Gibb et al., 2005).

Harvest Characteristic	Hemp seed inclusion rate in treatment diets			SEM	Treatment	P Values 0 vs. 9 and 14% <sup>y</sup>
	0%	9%	14%			
Hot Carcass Weight (kg)	325.7	323.0	330.2	9.6	0.87	0.94
Dressing Percentage (%)	56.8	57.3	57.7	0.49	0.47	0.27
Backfat (mm)	14.7	14.1	16.3	0.97	0.26	0.66
Ribeye Area (cm <sup>2</sup> )	79.5	78.9	96.3	12.6	0.54	0.60

The nutrient analysis of the complete finishing ration revealed that the fat content increased from 1.8% relative to the control diet (0% hemp inclusion) versus 5.9% increase relative the hemp seed inclusion rate of 14%. The increased concentration of crude fat in dietary rations that incorporated hemp seed was also noted by Kleinhenz et al. (2020). The dietary inclusion of additional crude fat led to *de novo* synthesis of fatty acids into fatty carcass tissues resulting in differences in the concentrations of fatty acids found in beef brisket fat. Gibb et al. (2005) reported brisket fat concentrations of C17:0 (heptadecaenoic acid), C17:1 (*cis*-10-heptadecaenoic acid), and C20:1 (*cis*-11-eicosenoic acid) decreased as hemp seed inclusion increased while C14:1 *cis* (myristoleic acid) C18:2 *trans, trans* (linolelaidic acid) C18:3 ( $\gamma$  – linolenic acid), C20:0 (arachidic acid), C20:4 (arachidonic acid), C22:5 (docosapentaenoic acid), and CLA *cis* - 9, *trans* – 11 (conjugated linoleic acid) increased as hemp seed dietary inclusion increased (Table 1.4). The differences in the concentration of fatty acids in the brisket fat could be explained by the presence of these specific fatty acids present in the diet. Another explanation could be attributed to the influence of desaturase enzyme activity. Desaturase enzymes convert dietary saturated fatty acids to monounsaturated or polyunsaturated fatty acids

through a series of dihydrogen reactions. Hemp seed oil has a higher concentration of polyunsaturated fatty acids (linoleic acid,  $\alpha$ -linolenic acid,  $\gamma$  – linolenic acid, and stearidonic acid) compared to common household vegetable oils. Hemp seed oil has been reported to contain a concentration of up to 80% polyunsaturated fatty acids (Bielecka et al., 2014). There have been two multifunctional classes of desaturases found in plants, one is soluble, and the other is membrane bound (Shanklin and Cahoon, 1998). The linoleic acid,  $\alpha$ -linolenic acid,  $\gamma$  – linolenic acids are synthesized in the stroma of plastids by soluble  $\Delta^9$  stearoyl-ACP desaturase, which contributes to the development of complex membrane lipids (Ohlrooge and Browse, 1995). Continual desaturation of the fatty acids into membrane lipids is completed by membrane bound desaturases (Ohlrooge and Browse, 1995). Through the entire desaturase cycle, linoleic acid will be desaturated to  $\alpha$ -linolenic acid. The production of  $\gamma$  – linolenic acid is from the desaturation of linoleic acid and stearidonic acid. Furthermore,  $\Delta^{12}$  desaturase is the main contributor to transforming oleic acid to linoleic acid by adding a double bond at the  $\Delta^{12}$  position (Nayeri and Yarizade, 2014). Also,  $\Delta^{15}$  desaturase converts linoleic acid to  $\alpha$ -linolenic acid in endoplasmic reticulum of plants (Soltani et al., 2020). This desaturation process can be a potential explanation as to why there is an increased concentration of monounsaturated and polyunsaturated fatty acids in hemp byproducts.

Table 1.4. Comparison of the fatty acid profile (expressed as a total percentage of crude fat content on a w/w basis) of beef brisket fat tissue between a control diet and hemp seed diets fed at 9 or 14% inclusion rate as a substitute for steam-rolled barely grain and barley silage in a beef finishing ration (summarized from Kleinhenz et al., 2020).

Fatty Acid, %	Hemp seed inclusion rate in treatment diets			SEM	P Values	
	0%	9%	14%		Treatment <sup>x</sup>	0 vs. 9 and 14% <sup>y</sup>
C14:0	3.43	3.31	3.49	0.25	0.87	0.94
C14:1 cis	1.89	2.23	1.92	0.22	0.50	0.49
C16:0	26.72	25.00	24.08	1.12	0.32	0.15
C16:1 trans	0.25	0.21	0.22	0.02	0.32	0.15
C16:1 cis	7.55	8.16	7.55	0.74	0.75	0.71
C17:0	1.59 <sup>a</sup>	1.27 <sup>b</sup>	1.24 <sup>b</sup>	0.10	0.04	0.01
C17:1	1.85 <sup>a</sup>	1.59 <sup>b</sup>	1.36 <sup>c</sup>	0.06	<0.001	<0.001
C18:0	7.23	7.15	8.00	0.54	0.52	0.62
C18:1 trans - 9	1.83	1.74	1.74	0.59	0.99	0.90
C18:1 cis - 9	44.07	45.39	41.32	1.57	0.22	0.72
C18:2 trans, trans	0.22 <sup>b</sup>	0.45 <sup>a</sup>	0.43 <sup>a</sup>	0.04	0.00	0.01
C18:2 cis, cis	1.49	1.58	1.40	0.13	0.66	0.99
C18:3	0.32	0.41	0.41	0.04	0.19	0.07
C20:0	0.05 <sup>b</sup>	0.06 <sup>b</sup>	0.08 <sup>a</sup>	0.02	0.03	0.05
C20:1	0.23	0.19	0.17	0.02	0.18	0.08
C20:4	0 <sup>b</sup>	0.03 <sup>a</sup>	0.02	0.01	0.02	0.01
C22:5	0.00	0.02	0.02 <sup>ab</sup>	0.01	0.15	0.06
CLA cis - 9, trans - 11	0.41	0.48	0.53	0.07	0.50	0.28
CLA trans - 10, cis - 12	0.04	0.06	0.04	0.01	0.28	0.42
Saturated	39.82	37.47	37.82	1.69	0.58	0.31
Unsaturated	60.15	65.52	62.16	1.69	0.58	0.31
Saturated/Unsaturated	0.67	0.60	0.63	0.04	0.54	0.60

\*Different superscripts within a row differ at  $P < 0.05$

The diet consumed by a ruminant animal is the main factor that affects the overall flavor of the meat. Development of (intermuscular fat, intramuscular fat, muscle, and other tissues) are influenced by dietary components, which affect the flavor. Furthermore, the type and concentration of fatty acids consumed play a large role in the development of beef flavor (Larick et al., 1990; Melton, 1990). Ruminants are predominately fed a forage-based diet that contains a higher concentration of n-3 polyunsaturated fatty acids, mainly linolenic acid. Beef steaks containing a higher concentration of linolenic acid have been reported to score lower in consumer taste panels (Moloney et al., 2000). While ruminants fed a diet consisting mainly of



grain contain more n-6 polyunsaturated fatty acids (oleic acid and linoleic acid) and score higher in taste panel analysis of “overall like” of beef flavor (Elmore et al., 2004; Calkins and Hodgen, 2007).

### **1.5. Fatty Acid Profile**

Leizer et al. (2000) reported that consumption of linoleic acid has been associated with anticancer, anti-inflammatory, and anti-thrombotic properties in mammals, as well as possess the ability to aid in the increase of metabolic rates and promotion of fat burning. Hempseed oil has been identified as a good source of  $\alpha$  – linolenic acid, processing a concentration between 12 – 23% of the total fatty acid composition.

Hemp seed that underwent a cold press extraction technique recorded no detectable  $\Delta$ 9-tetrahydrocannabinol. This is important because the presence of  $\Delta$ 9-tetrahydrocannabinol greater than 0.3% (dry matter basis) would result in hemp oil being reclassified as marijuana and thus not applicable for livestock feed. Leizer et al. (2000) evaluated the fatty acid content of feed-grade hemp seed oil and identified linoleic acid (C18:2) and  $\gamma$  – linolenic acid (C18:3) as the major omega-6 and omega-3 polyunsaturated fatty acids detected at a ratio of 3:1 (Table 1.5). It is interesting to note that the 3:1 omega-6 to omega-3 ratio is optimal for human nutrition according to Deferne and Pate (1996). Additionally, the study stated that the presence of  $\gamma$ -linolenic acid made hemp seed oil more nutritionally desirable than comparable oils available for human consumption (canola oil, olive oil, coconut oil, avocado oil, sunflower oil, and peanut oil).  $\gamma$ -linolenic acid is derived from linoleic acid, but the conversion of linoleic acid to  $\gamma$ -linolenic acid can be slow in mammals due to physiological stress, ageing, or pathology (Deferne and Pate, 1996). Therefore, the direct availability of supplemental  $\gamma$ -linolenic acid makes hemp

seed oil superior to other seed based oils. The concentration of linoleic acid has been reported between 52 – 62% of the total fatty acid composition (Table 1.5).

Table 1.5. Fatty acids and other volatiles present in hemp seed oil macro composition summarized from Leizer et al. 2000

<b>Components</b>	<b>Results</b>
<i>Fatty Acids</i>	
	(% w/w)
Linoleic Acid	52-62
$\alpha$ - Linolenic Acid	12-23
Oleic Acid	8-13
Palmitic Acid	5-7
Stearic Acid	1-2
$\gamma$ - Linolenic Acid	3-4
Eicosanoic Acid	0.39-0.79
Eicosenoic Acid	0.51
Eicosadienoic Acid	0.00
<i>Natural Products</i>	
Cannabidiol	10 mg/kg
$\Delta$ 9 - tetrahydrocannabinol	ND
Myrcene	160 mg/L
$\beta$ - caryophyllene	740 mg/L
$\alpha$ - tocopherol	TR
$\gamma$ - tocopherol	468 mg/L
Methyl salicylate	TR

\*ND = not detectable

\*\*TR = trace amounts

The findings of Leizer et al. (2000; Table 1.5), indicate that 75% of hemp seed oil is comprised of essential fatty acids (linoleic acid,  $\alpha$  - linolenic acid, oleic acid, palmitic acid,  $\gamma$  - linolenic acid, stearic acid, eicosanoic acid, eicosenoic acid, and eicosadienoic acid). Even with that being said, there is still a need to determine how the higher concentrations of monounsaturated and polyunsaturated fatty acids (oleic acid and linoleic acid) found in hemp byproducts could potentially alter the aroma and/or taste of meat when livestock are fed a ration that includes hemp byproduct(s). Dinh et al. (2021) reported that ruminants contain more

saturated fatty acids and monounsaturated fatty acids than polyunsaturated fatty acids within the fat tissues due to the dehydrogenation that occurs in the rumen. Through the thermal oxidation of fatty acids that occurs during the cooking process, fat derived flavor compounds are formed and result in a specific aromatic character. Those flavor compounds consist of non-volatiles that manifest within lipids. The source of those volatiles thus contributes to the flavor profile of cooked meat products (Arshad et al, 2018). The common fatty acids in red meat consist of myristic (saturated), palmitoleic (saturated), stearic (saturated), palmitoleic (monounsaturated), oleic (monounsaturated), linoleic (polyunsaturated), linolenic (polyunsaturated), and arachidonic (polyunsaturated) acid (Dinh et al., 2021). The increased passage of polyunsaturated fatty acids in the rumen is affected by the increased inclusion of forage and distillers grains (Klopfenstein et al., 2008; Schingoethe et al., 2009) that resulted in an increased concentration of polyunsaturated fatty acids in intermuscular and intramuscular fat. Compounds (aldehydes, ketones, carboxylic acids, alcohols, lactones, and alkylfurans) derived from lipids result from the oxidation of fatty acids during the cooking process that generate the favorable aromas and cooked flavor profiles (Nawar, 1984; Mottram, 1998; Song et al., 2011; Amaral et al., 2018; Dominguez et al., 2019). Elmore et al. (1999) increased the polyunsaturated fatty acid content in the muscle of steers supplemented linseed and fish oil; which are rich in  $\alpha$ -linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid. The muscle samples were cooked and due to the increased polyunsaturated fatty acids, there were more undesirable lipid oxidation compounds that created a less desirable (fishy) cooked aroma. Arshad et al. (2018) indicated that ruminants finished on grass have a higher concentration of  $\alpha$ -linolenic acid than ruminants finished on grain-based diets, and the concentration of  $\alpha$ -linolenic acid contributes to the flavor profile of cooked meat products. It is vital to understand that the ability to experience flavor is complex and unique to

each individual. There is a dual olfactory system (orthonasal olfaction and retro nasal olfaction) within the human brain. The orthonasal olfaction occurs during sniffing (breathing in) or when food is ingested. Retro nasal olfaction utilizes the systems of the brain when breathing out, or when food is inside the oral cavity (mouth). Retro nasal stimulation happens due to volatile molecules being released from food caused by the chewing movements of the mouth (Sun and Halpern, 2005). The perception of flavor or taste pathway, moves from the nucleus of the singular tract in the brainstem to the hypothalamus, and then to the taste nuclei of the somatosensory thalamus, which then reaches the primary taste cortex.

Having an increased concentration of polyunsaturated fatty acids can enhance the overall aroma and flavor perceptions of cooked meat but identifying the specific concentration of polyunsaturated fatty acids to achieve that enhanced flavor profile is imperative, because it can be assumed that too much polyunsaturated fatty acids can create an undesirable aroma and/or flavor profile for certain individuals.

## **1.6. Implications**

Gibb et al. (2005) demonstrated that there were no negative impacts on carcass composition or quality with the inclusion of hemp seed in the beef finishing ration. Additionally, Kleinhenz et al. (2020) indicated that there were small (or no) changes in the fatty acid profiles of beef brisket tissues. While C17:0 and C17:1 both increased in tissue and C18:2 *trans*, *trans* doubled with the dietary supplementation of hemp seed, the overall content was still less than 1% of total fat.

Research evaluating the inclusion of hemp and hemp byproducts in ruminant feed rations remains in its infancy. There are few research projects that have examined the impact of feeding hemp and (or) hemp byproducts on the resulting meat products consumed by humans. Therefore,

the objective of the current research was to evaluate the impact of the inclusion of hemp seed cake in a late finishing ration fed to commercial beef heifers on carcass parameters, mechanical beef palatability attributes, color stability, and fatty acid composition. Therefore, we hypothesize that the inclusion of hemp seed cake (20% of total diet) in a balanced beef cattle feedlot finishing ration (for either 22, 23, 26, or 30 days) will not have a negative impact on these economically important parameters.

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## **CHAPTER 2. THE EFFECT OF HEMP BYPRODUCT SUPPLEMENTATION ON BEEF QUALITY**

### **2.1. Abstract**

Hemp byproducts possess cellulose-containing plant material; therefore, it is assumed that hemp byproducts could be an alternative fiber source for ruminants, primarily cattle. The objective of this study was to evaluate the impact of 30d step-up inclusion of hempseed cake into a late finishing ration on the meat quality attributes of 22 to 24-month-old crossbred heifers. The heifers were randomly assigned to 1 of 2 treatments associated with different duration of supplement withdrawal (d 0, 1, 4, or 8) prior to harvest. No significant differences were observed for harvest and quality characteristics, Minolta (CIE L\*, a\*, b\*, chroma, and hue angle) color scores, fatty acid profile, or proximate analysis results across treatment (inclusion of hempseed cake) or treatment <sup>x</sup> withdrawal day. Therefore, hempseed cake does not negatively or positively impact beef quality.

### **2.2. Introduction**

Worldwide utilization of hemp dates back centuries. During the 1930s, the U. S. Drug Enforcement Agency (DEA) regulated the growth and cultivation of hemp due to tetrahydrocannabinol (THC) (Kleinhenz et al., 2020). In 2014, the Agriculture Improvement Act gave permission to states to conduct research focusing on the use of industrial hemp (IH) (Public Law 113-79). Industrial hemp is cultivated from the seeds found on the stalk of *Cannabis sativa* and must contain less than 0.3% THC. In 2018, the amendment to the Agriculture Improvement Act removed IH as a drug monitored by the U. S. DEA. Instead, IH was prompted to be observed as a potential agricultural commodity (USDA, Agricultural Marketing Service, 2019).



Hemp byproducts have been found to have cellulose-containing plant material, and therefore, it is assumed that hemp byproducts could be an alternative fiber source for ruminant animals, primarily cattle (Kleinhenz et al., 2020). Due to limited data on the impact of IH on livestock species and the potential adulterant from THC, the US Food and Drug Administration has not approved the use of IH as a feed ingredient for livestock feeds (Kleinhenz et al., 2020).

We hypothesize that the inclusion of hempseed cake in a balanced beef cattle feedlot finishing ration would not have a significant impact on beef quality. Therefore, the objective of this study was to evaluate the impact of inclusion of hempseed cake in a late finishing ration on the meat quality attributes of meat obtained from commercial crossbred heifers.

## **2.3. Materials and Methods**

### **2.3.1. Animals and Feeding Treatments**

All procedures regarding the use and care of animals in this study were reviewed and approved by the North Dakota State University Institutional Animal Care and Use Committee (IACUS #A21010). Crossbred heifers 22 to 24 months of age (N = 32) were randomly assigned to one of two treatment groups. Treatments were a complete balanced ration (National Research Council 2000) containing either dried distillers grain with solubles (DDGS) or hempseed cake (HEMP), fed in five phases. The first four phases lasted 5 to 6d for each phase and the fifth phase, started the 22d of the feeding trial, and lasted until each assigned CON and HEMP group completed the withdrawal period (d 0, 1, 4, or 8). A phase fed diet is a nutritional strategy that is designed to modify the quantity of a feed ingredient over a certain amount of time.

In this study, the base (CON; Table 2.1 ) diet was comprised of a combination of 75% corn silage and corn grain. Corn grain replaced corn silage in phased increments of 10% with a phase 1 containing 65% corn silage and 10% corn grain incrementally increased to phase 5 at

20% corn silage and 55% corn grain (Table 2.1). The CON diet contained 20% DDGS consistently across each phase. This CON diet was compared to the treatment diet containing the same phase feeding strategy as CON, but with HEMP replacing DDGS across all phased feeding of corn silage/corn grain (Table 2.1). Table 2.2 presents the data comparison of nutrient compositions between the control and hempseed cake diet.

Table 2.1. Composition of final phase diet for control vs. hemp treatments

<b>Ingredient, % of diet DM</b>	<b>Treatments</b>	
	<b>Control</b>	<b>Hemp</b>
Corn grain	55	55
DDGS	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 <sup>1</sup>	0.01	0.01
Trace mineral premix <sup>2</sup>	0.05	0.05
Rumensin-90 <sup>3</sup>	0.02	0.02

<sup>1</sup>Contained 48,510 kIU/kg vitamin A and 4,630 kIU/kg vitamin D

<sup>2</sup>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).

<sup>3</sup>Formulated to supply monensin (Rumensin-90, Elanco Animal Health, Greenfield, IN) at 40 mg/kg.

Table 2.2. Nutrient composition for control vs. hempseed cake treatment

Nutrient Analyses <sup>1</sup> , %	Treatments	
	Control	Hemp
Dry Matter	66.0	65.13
Ash	5.79	6.39
Starch	43.7	43.2
Crude Protein	14.8	15.8
Ether Extract	3.47	3.38
NDF	29.1	30.4
ADF	11.4	16.3
Calcium	0.69	0.78
Phosphorus	0.44	0.53
Calcium : Phosphorus	1.56	1.48

<sup>1</sup>Average of weekly samples

The U. S. Food and Drug Administration considers THC a potential adulterant and has not approved the use of IH as a feed ingredient for livestock feeds. Therefore, it is important to determine whether or not residual THC is detectable in the eventual food product (meat) and to determine if withdrawal of IH inclusion in feed rations at a specified number of days prior to harvest eliminates or reduces the level of THC in consumable muscle tissue below the levels of 0.3% THC established by the 2014 Agriculture Improvement Act. Thus, the heifers within the HEMP treatment were randomly assigned to one of four hempseed cake withdrawal treatments set at 0, 1, 4, or 8 days withdrawal of hempseed cake prior to humane harvest. For accurate comparison, four CON heifers were harvested on the same day as four heifers from each of the four withdrawal treatment days. Because no differences were seen for THC content across HEMP withdrawal days (Swanson et al., unpublished data) the withdrawal days will be statistically analyzed as “slaughter day” whereby day of harvest and treatment by harvest day interactions will be evaluated for statistical differences. Table 2.3 illustrates the cannabinoid and cannabinoid concentrations identified in the hempseed cake utilized in the HEMP diet.

Table 2.3. Quantifications of cannabinoids in hempseed cake diet.

<b>Cannabinoid</b>	<b>Cannabinoid Concentration in Hempseed Cake</b>
Cannabichromene (CBC)	Not detected ppm
Cannabidiol (CBD)	5 ppm
Cannabidiolic Acid (CBDA)	16 ppm
Cannabidivarin (CBDV)	Not detected ppm
Cannabinol (CBN)	Not detected ppm
Tetrahydrocannabinolic acid (THCA)	Not detected ppm
$\Delta$ 8-THC (Delta-8-tetrahydrocannabinol)	Not detected ppm
$\Delta$ 9-THC (Delta-9-tetrahydrocannabinol)	Not detected ppm
Cannabigerol (CBG)	Not detected ppm

### 2.3.2. Harvest Process and Carcass Characteristics

All animals were humanely harvested under USDA federal inspection in accordance with the Humane Slaughter Act of 1978 (USDA, Food Safety and Inspection Services, 2017).

Carcasses were chilled at 2°C for a minimum of 24 hours before collection of all carcass data.

Data obtained from the carcasses were live weight, hot carcass weight (HCW), dressing percentage (DP), 12th rib ribeye area (REA), 12th rib fat depth, kidney, pelvic and heart fat (KPH expressed as a percentage of HCW), USDA yield grade (YG), USDA quality grade (QG), marbling score, and bone maturity. The live weight, HCW, and DP were established on the respective days of harvest. The DP was calculated following equation:  $(\text{HCW} / \text{Live Weight}) * 100$  (American Meat Science Association, 2016). All of the following data were collected from the left side of each carcass. The REA, fat depth, YG, marbling score, bone maturity, and QG were recorded 24h post-harvest after chilling in the cooler set at 2°C. The YG was calculated in accordance with the equation reported by the American Meat Science Association (AMSA, 2016) whereby:

$$\text{YG} = 2.50 + (2.5 \times \text{adjusted fat thickness; inches}) + (.20 \times \text{percent KPH}) + (0.0038 \times \text{HCW; pounds}) - (0.32 \times \text{REA; square inches})$$

It should be noted that marbling score was input as a numeric score for statistical analysis whereby a marbling score devoid = 0-100, practically devoid = 100-199, traces = 200-299, slight = 300-399, small = 400-499, modest = 500-599, moderate = 600-699, slightly abundant = 700-799, moderately abundant = 800-899, and abundant = 900-999.

### **2.3.3. Quality Collection and Analysis**

Three boneless steaks (*Longissimus dorsi*) were obtained from the right side of each carcass from the 11th to 13th thoracic rib. Each steak was vacuumed packaged using a Cryovac machine (Sealed Air, Charlotte, NC) and frozen at  $-3.33^{\circ}\text{C}$  until analysis. Steak 1 was evaluated for drip loss (component of overall moisture loss). Drip loss was collected from beef ribeye samples (7 to 18 grams) that were suspended in a bag for 24h at  $4^{\circ}\text{C}$ . The samples were reweighed after the completion of 24h and the drip loss was calculated following equation:  $[(\text{Initial Weight} - \text{Final Weight}) / \text{Initial Weight}] * 100$  (AMSA, 2016). Steak 2 were used to evaluate storage moisture (purge) loss, pH, color (CIE  $L^*$ ,  $a^*$ , and  $b^*$ ), cook loss, and Warner-Bratzler shear force (WBSF) (American Meat Science Association, 2016). Purge loss was collected by weighing the vacuum packaged steak and then removing the steak from the packaging and reweighing the steak. The purge loss was calculated following equation:  $[(\text{In-bag Weight} - \text{Out-bag Weight}) / \text{In-bag Weight}] * 100$  (AMSA, 2016). After steaks were removed from packaging, they were allowed a 30-minute “bloom” period at room temperature ( $19^{\circ}\text{C}$ ) prior to color analysis. Color measurements (CIE  $L^*$ ,  $a^*$ , and  $b^*$  values) were collected using a Commission Internationale de l’Eclairage (CIE)  $L^*$ ,  $a^*$ , and  $b^*$  color space were ( $L^*$  = lightness;  $a^*$  = redness;  $b^*$  = yellowness). Color measurements were obtained by Konica Minolta CR-400 Chroma Meter (Minolta Co., Ltd., Ramsey, NJ.) calibrated to a white tile with the measurements of  $Y = 083.4$ ,  $x = 0.3182$ , and  $y = 0.3251$ . Hue angle and chroma values were calculated

following formulas: hue angle = arctangent ( $b^*/a^*$ ) and chroma =  $(a^{*2} + b^{*2})^{1/2}$  (AMSA, 2012). The pH values were collected by Hanna Instruments Portable pH/Temperature Meter HI 99163 (Hanna Instruments, Woonsocket, RI.). Steak 2 was cooked on George Forman clamshell grills (Spectrum Brands, Beachwood, OH.) to an internal temperature of 65°C and re-weighed after reaching 19°C (room temperature). The cook loss was calculated following equation:  $[(\text{raw weight} - \text{cooked weight}) / \text{raw weight}] * 100$ . Warner-Bratzler shear force was conducted according to (AMSA, 2016). Briefly, 6 cores (1.27cm in diameter) were extracted from each steak after the steaks reached an internal temperature of 23°C, and each core was placed in the middle of a V-notched (60° angle) cutting blade. After the blade cut through the core, a number was displayed that indicated the kilograms of force needed to cut through the core.

#### **2.3.4. Shelf-Life Study**

The third steak was used in an 8-day mock shelf-life study to determine treatment differences in shelf stability. Each steak was placed on a conventional Styrofoam retail tray and over-wrapped with oxygen permeable PVC film and placed under constant fluorescent light at 1°C. Objective  $L^*$ ,  $a^*$ , and  $b^*$  measurements were taken at 1500h each day from the time the steaks were placed in a retail tray (day 0) through day 9. A Konica Minolta CR-410 Chroma Meter (Minolta Co., Ltd., Ramsey, NJ) was used to collect  $L^*$ ,  $a^*$ , and  $b^*$  values which were used to calculate hue angle and chroma values using the following formulas (AMSA, 2012):

$$\text{hue angle} = \text{arctangent} (b^*/a^*)$$

$$\text{chroma} = \sqrt{(a^{*2} + b^{*2})}$$

#### **2.3.5. Fatty Acid Profile**

Approximately 110 g of internal (peri-renal) and external (subcutaneous adjacent the brisket) fat was obtained from pre-rigor carcasses at slaughter. The adipose samples were placed in labeled Whirlpak™ bags and frozen for later fatty acid component analysis (AOAC Official

Method 996.06 & AOCS Official Method Ca 5b-71) at the University of Missouri Experiment Station Chemical Labs.

### **2.3.6. Proximate Analysis**

Muscle samples (100 g) were extracted from the Longissimus dorsi to determine the percentage of crude fat (AOAC 920.38.), crude protein (AOAC 984.13), moisture (AOAC 934.01), crude fiber (AOAC 978.10), and ash (AOAC 942.05). The muscle samples were sent to the University of Missouri Experiment Station Chemical Labs for proximate analysis (AOAC Official Method 984.13[A-D]).

### **2.3.7. Statistics**

Harvest, quality, fatty acid profile, and proximate analysis data were analyzed using the MIXED procedure of SAS (SAS 9.4, SAS Institute Inc., Cary, NC) with fixed effects of treatment and slaughter day for all analyses. Significance levels for all analyses were set at  $P \leq 0.05$ . Least-square means was separated using PDIF procedure in SAS 9.4. Data was also analyzed for an interaction between treatment groups and slaughter day and provided as tables.

Shelf life data were analyzed using the MIXED procedure of SAS (SAS 9.4, SAS Institute Inc., Cary, NC) with fixed effects of treatment and slaughter day for all analyses. Significance levels for all analyses were set at  $P \leq 0.05$ . Least-square means and standard errors were reported for all measured attributes and presented as figures. Repeated measures were utilized for Minolta (CIE L\*, a\*, and b\*) color scores, chroma, and hue angle.

## **2.4. Results and Discussion**

### **2.4.1. Harvest Process and Carcass Characteristics**

No differences were observed across treatment or withdrawal (harvest) days for LW ( $P = 0.81$ ), HCW ( $P = 0.77$ ), DP ( $P = 0.91$ ), YG ( $P = 0.40$ ), and QG ( $P = 0.41$ ) presented in Table

2.4. The similarities in carcass characteristics between the CON and HEMP carcasses should be noted as the inclusion of hempseed cake in the finishing ration of commercial heifers did not affect carcass characteristics in either a positive or negative manner.

It is important to note that the heifers used in the present study were considerably heavier and older (22 to 24 months of age) than conventional finishing cattle. In comparison with a similar hemp feeding trial, Gibb et al. (2005) utilized beef heifers spanning an HCW of 320 to 330kg, while the crossbred beef heifers used in the present study weighed from 399 to 432kg. Furthermore, feeder heifers used in Gibb et al., 2005 averaged a 3.18% lower DP, a 1.98cm<sup>2</sup> larger REA, and 0.07mm less 10<sup>th</sup> rib FD than observed in our present study.

Despite the physical differences between the two groups of research feeder cattle, similarities between the present study and Gibb et al. (2005) were observed for HCW, DP, REA, and FD carcass characteristics between heifers fed the control versus hempseed diet.



Table 2.4. Interactions of withdrawal days and treatment groups for harvest characteristics of crossbred beef heifers between CON and HEMP diets

Carcass Data <sup>†</sup>	Trt	Withdrawal Days				P Values	
		0	1	4	8	Trt	Trt x Withdrawal Day
LW (kg)	CON	716 ± 28.92	687 ± 28.92	685 ± 28.92	692 ± 28.92	0.42	0.81
	HEMP	665 ± 28.92	673 ± 28.92	686 ± 28.92	689 ± 28.92		
HWC (kg)	CON	432 ± 18.45	408 ± 18.45	421 ± 18.45	418 ± 18.45	0.47	0.77
	HEMP	399 ± 18.45	400 ± 18.45	420 ± 18.45	420 ± 18.45		
DP (%)	CON	60.50 ± 0.69	59.40 ± 0.69	61.39 ± 0.69	60.43 ± 0.69	0.94	0.91
	HEMP	60.08 ± 0.69	59.44 ± 0.69	61.34 ± 0.69	60.99 ± 0.69		
REA (cm <sup>2</sup> )	CON	99.68 ± 0.66	96.64 ± 0.66	96.64 ± 0.66	93.09 ± 0.66	0.34	0.16
	HEMP	86.64 ± 0.66	97.61 ± 0.66	90.52 ± 0.66	99.23 ± 0.66		
FD (cm)	CON	1.78 ± 0.09	1.60 ± 0.09	1.78 ± 0.09	1.80 ± 0.09	0.61	0.66
	HEMP	1.57 ± 0.10	1.55 ± 0.09	2.01 ± 0.09	1.49 ± 0.09		
YG	CON	3.4 ± 0.32	3.15 ± 0.32	3.45 ± 0.32	3.63 ± 0.32	0.87	0.40
	HEMP	3.5 ± 0.37	3.0 ± 0.37	3.95 ± 0.32	3.03 ± 0.32		
QG	CON	2.0 ± 0.09	2.0 ± 0.09	2.0 ± 0.09	2.0 ± 0.09	0.33	0.41
	HEMP	1.75 ± 0.09	2.0 ± 0.09	2.0 ± 0.09	2.0 ± 0.09		
MS	CON	3.90 ± 0.46	3.73 ± 0.46	4.1 ± 0.46	3.3 ± 0.46	0.55	0.29
	HEMP	3.05 ± 0.46	4.00 ± 0.46	4.55 ± 0.46	4.23 ± 0.46		
BM	CON	1.68 ± 0.28	1.0 ± 0.28	1.30 ± 0.28	1.60 ± 0.28	0.45	0.53
	HEMP	1.0 ± 0.28	1.0 ± 0.32	1.33 ± 0.28	1.63 ± 0.28		

\*Trt represents treatment; Control (CON) = 20% corn silage, 55% corn grain, 20% DDGS, Hempseed cake (HEMP) = CON diet with replacement of 20% DDGS with 20% hempseed cake.

<sup>†</sup>Carcass Data abbreviations: LW = live weight; HCW = pre-rigor (hot) carcass weight; FD = subcutaneous fat depth measured adjacent the 10<sup>th</sup> rib; YG = USDA yield grade; QG = USDA quality grade; whereby Prime = 1.0 – 1.99, Choice = 2.0 – 2.99; MS = marbling score; whereby devoid = 0-100, practically devoid = 100-199, traces = 200-299, slight = 300-399, small = 400-499, modest = 500-599, moderate = 600-699, slightly abundant = 700-799, moderately abundant = 800-899, and abundant = 900-999; BM = bone maturity; whereby 1.0 – 1.99 = A0 to A99 maturity.

USDA Quality Grade; Prime = 1.0 – 1.99, Choice = 2.0 – 2.99

### 2.4.2. Beef Quality

To our knowledge, this is the first experiment feeding supplemental hempseed cake in a late finishing beef ration to evaluate the potential impacts of supplementation on beef quality.

Treatment effects were observed (Table 2.5) for pH ( $P = 0.03$ ), CL ( $P = 0.02$ ) and WBSF ( $P = 0.009$ ), but no differences were observed for PL, DL, Chroma, or Hue. The differences observed

between CON versus HEMP treatments for pH, cook loss, and WBSF cannot be easily rationalized from a physiological perspective.

It should be noted, that from a physiological standpoint, the pH differences at the 100<sup>th</sup> fraction may not be considered biologically different and, could also be dismissed as within the margin of error of the pH instrument. The other differences found in the meat quality characteristics could be largely driven by the slaughter day affect and completely unrelated to dietary treatment. Therefore, additional research is needed to verify the cause of differences in the meat quality characteristics comparing control diet fed beef heifers and hempseed cake diet fed beef heifers.

Table 2.5. LSMEANS ( $\pm$  standard error) for various beef quality traits obtained from crossbred feeder heifers fed a standard finishing diet (CON) vs. a finishing diet containing hempseed (HEMP) withdrawn from the diet 0, 1, 4, or 8 days prior to harvest.

Quality Traits <sup>†</sup>	Trt	Withdrawal Days				P Value	
		0	1	4	8	Trt	Trt x Withdrawal Day
pH	CON	5.41 $\pm$ 0.02	5.52 $\pm$ 0.02	5.53 $\pm$ 0.02	5.53 $\pm$ 0.02	0.20	0.03
	HEMP	5.49 $\pm$ 0.02	5.52 $\pm$ 0.02	5.52 $\pm$ 0.02	5.52 $\pm$ 0.02		
PL (%)	CON	6.47 $\pm$ 0.80	6.81 $\pm$ 0.80	4.94 $\pm$ 0.80	4.83 $\pm$ 0.80	0.69	0.33
	HEMP	4.94 $\pm$ 0.80	7.13 $\pm$ 0.80	5.74 $\pm$ 0.80	6.15 $\pm$ 0.80		
DL (%)	CON	6.07 $\pm$ 2.33	5.54 $\pm$ 2.33	4.17 $\pm$ 2.33	0 $\pm$ 2.33	0.61	0.30
	HEMP	3.47 $\pm$ 2.33	1.79 $\pm$ 2.33	2.50 $\pm$ 2.33	4.58 $\pm$ 2.33		
CL (%)	CON	16.11 $\pm$ 2.09	13.30 $\pm$ 2.09	19.37 $\pm$ 2.09	14.34 $\pm$ 2.09	0.09	0.02
	HEMP	16.96 $\pm$ 2.09	21.07 $\pm$ 2.09	14.34 $\pm$ 2.09	20.97 $\pm$ 2.09		
WBSF Avg. (N)	CON	2.36 $\pm$ 0.21	1.72 $\pm$ 0.21	2.71 $\pm$ 0.21	2.32 $\pm$ 0.21	0.009	0.56
	HEMP	2.78 $\pm$ 0.21	2.14 $\pm$ 0.21	2.82 $\pm$ 0.21	3.04 $\pm$ 0.21		

\*Trt represents treatment; Control (CON) = 20% corn silage, 55% corn grain, 20% DDGS, Hempseed cake (HEMP) = CON diet with replacement of 20% DDGS with 20% hempseed cake.

<sup>1</sup> Values within the same row with different letter indicate significance at ( $P < 0.05$ ).

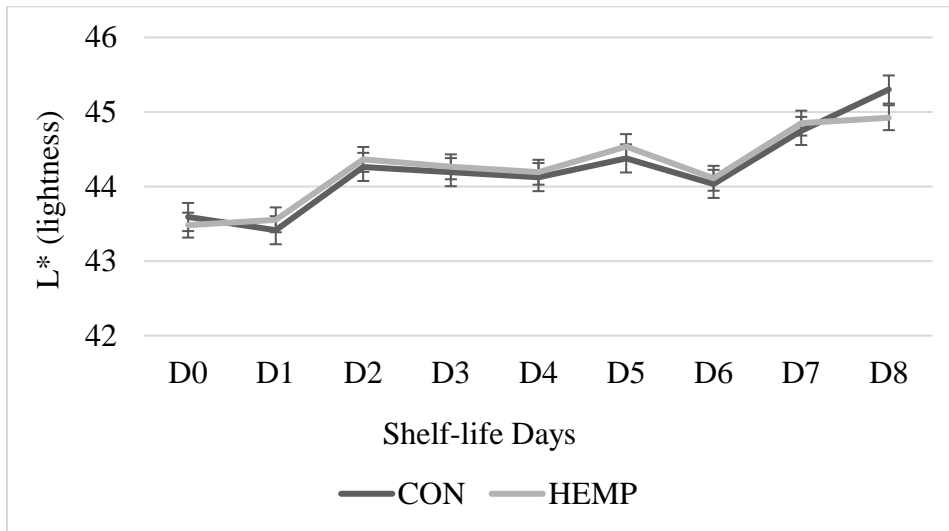
<sup>†</sup>Quality Trait abbreviations: PL = purge loss; DL = drip loss; CL = cook loss; and WBSF Avg. = Warner Bratzler Shear Force average.

### 2.4.3. Shelf-Life Study

The color of steaks is the most important observation consumers use to select a beef steak for purchase at retail (Moloney et al., 2021). Therefore, it is very important that alterations to feed rations of finishing cattle do not impact the color of the retail product. Priolo et al. (2001) reported that steaks possessing a darker color in the retail case do not end up in the shopping cart and are marked for sale at a significant discount. Hempseed inclusion in the diet of late-finishing heifers had no impact on the color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , chroma, and hue angle) over time for ribeye steak shelf stability (Figures 2.1-2.5). The present study is the first trial to examine the impact of hempseed inclusion in a late-finishing diet on beef shelf life. Thus, further research is necessary to confirm that hempseed cake inclusion does not impact retail marketability of hemp-fed beef.

Wood et al. 2004; Baublits et al. 2009; Scerra et al. (2014); and Hunt et al. (2016) have shown that beef steaks obtained from feedlot cattle that had consumed a ration that contained higher content of unsaturated fat, resulted in incorporation of more unsaturated fat in the membranes and fatty tissues present in the edible portions of muscle. Steaks containing a high concentration of unsaturated fat can be more prone to oxidative spoilage (rancidity) over time in the retail space (Enser, 2001; Simitzis and Deligeorgis, 2010). Given that hempseed possesses a higher proportion of unsaturated fat (Matthaus and Bruhl, 2008), the potential exists for more unsaturated fat incorporated into edible muscle and thus, the potential for early development of rancidity and the development of rancid odor. Despite the maintenance of an acceptable retail color over time in the retail case, by day 5, the steaks had developed an offensive odor (data not collected). If these off odors were due to early oxidative rancidity as a result of a greater proportion of unsaturated fats present in the steak tissue, the differences would have been

observed in the fatty acid profile data collected for this project. The fatty acid results and discussion are explained below, but in summary, no differences were seen across treatments for unsaturated fatty acid content. That said, further research should examine differences in potential spoilage mechanisms biologically impacted by dietary hempseed inclusion.

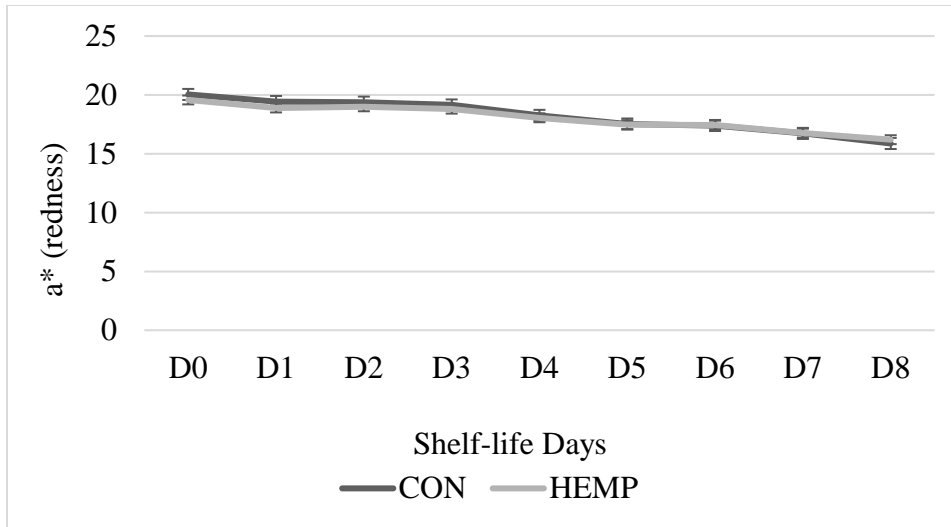


Trt \* Withdrawal Day P Value = 0.3247

Figure 2.1. Eight-day shelf-life expression of L\* values (lightness) for ribeye steaks collected from crossbred heifers had consumed late finishing rations containing supplemental hempseed (HEMP) versus a control (CON) ration without<sup>2</sup>

<sup>†</sup>D# = number of days on simulated retail display

<sup>2</sup>Control (CON) = 20% corn silage, 55% corn grain, 20% DDGS, Hempseed cake (HEMP) = CON diet with replacement of 20% DDGS with 20% hempseed cake.

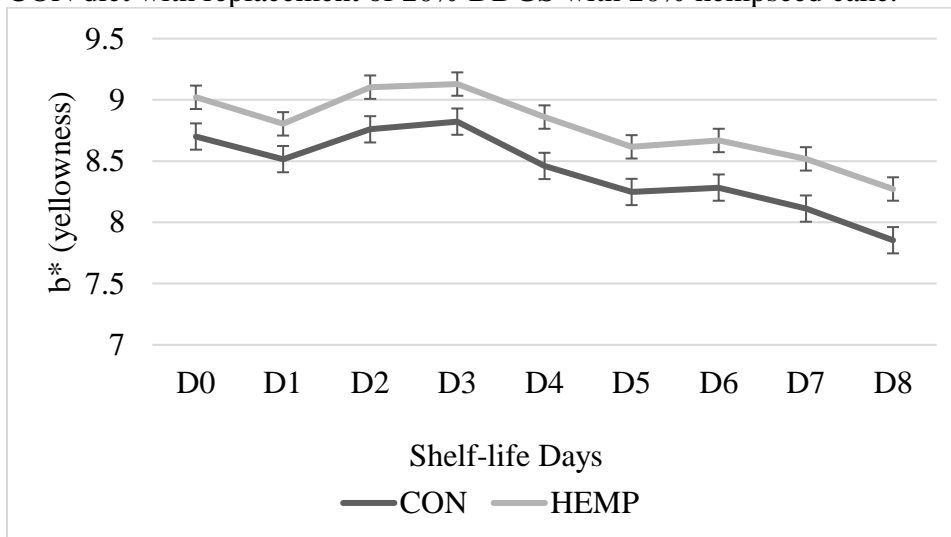


Trt × Withdrawal Day P Value = 0.097

Figure 2.2. Eight-day shelf-life expression of a\* values (lightness) for ribeye steaks collected from crossbred heifers had consumed late finishing rations containing supplemental hempseed (HEMP) versus a control (CON) ration without<sup>2</sup>

<sup>†</sup>D# = number of days on simulated retail display

<sup>2</sup>Control (CON) = 20% corn silage, 55% corn grain, 20% DDGS, Hempseed cake (HEMP) = CON diet with replacement of 20% DDGS with 20% hempseed cake.

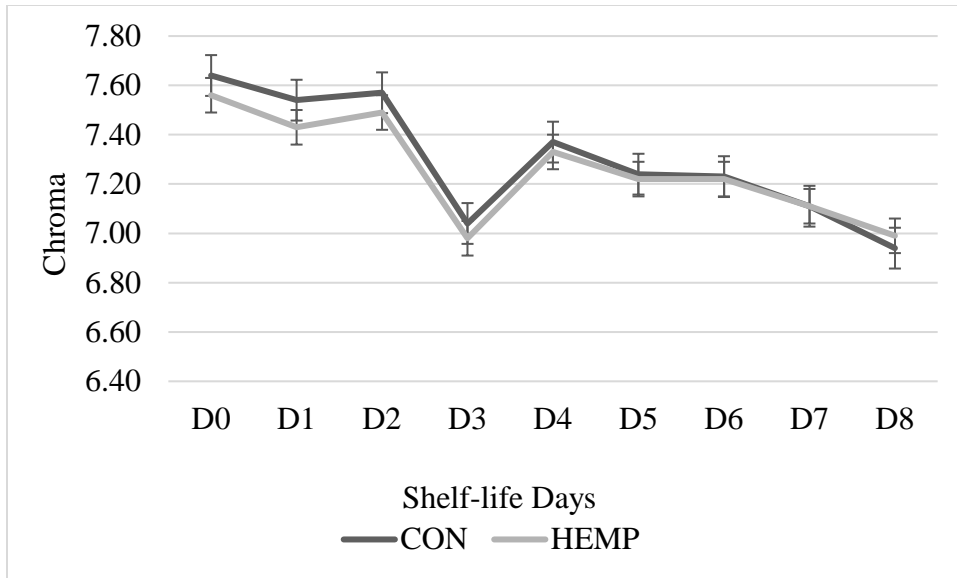


Trt × Withdrawal Day P Value = 0.1729

Figure 2.3. Eight-day shelf-life expression of b\* values (lightness) for ribeye steaks collected from crossbred heifers had consumed late finishing rations containing supplemental hempseed (HEMP) versus a control (CON) ration without<sup>2</sup>

<sup>†</sup>D# = number of days on simulated retail display

<sup>2</sup>Control (CON) = 20% corn silage, 55% corn grain, 20% DDGS, Hempseed cake (HEMP) = CON diet with replacement of 20% DDGS with 20% hempseed cake.

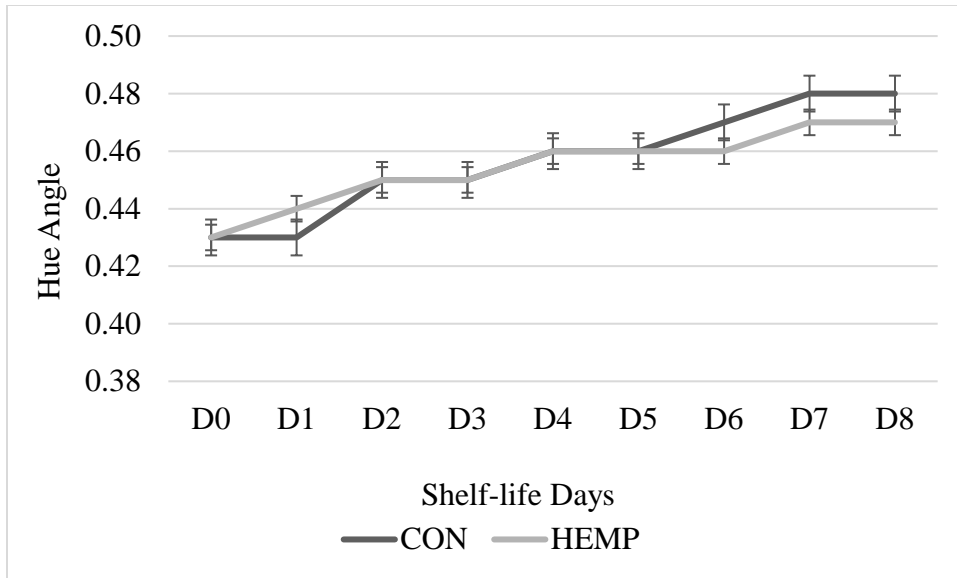


Trt \* Withdrawal Day P Value = 0.3889

Figure 2.4. Eight-day shelf-life expression of color saturation (chroma) for ribeye steaks collected from crossbred heifers had consumed late finishing rations containing supplemental hempseed (HEMP) versus a control (CON) ration without<sup>2</sup>

<sup>†</sup>D# = number of days on simulated retail display

<sup>2</sup>Control (CON) = 20% corn silage, 55% corn grain, 20% DDGS, Hempseed cake (HEMP) = CON diet with replacement of 20% DDGS with 20% hempseed cake.



Trt × Withdrawal Day P Value = 0.5476

Figure 2.5. Eight-day shelf-life expression of true redness (hue angle) for ribeye steaks collected from crossbred heifers had consumed late finishing rations containing supplemental hempseed (HEMP) versus a control (CON) ration without<sup>2</sup>

<sup>†</sup>D# = number of days on simulated retail display

<sup>2</sup>Control (CON) = 20% corn silage, 55% corn grain, 20% DDGS, Hempseed cake (HEMP) = CON diet with replacement of 20% DDGS with 20% hempseed cake.

#### 2.4.4. Proximate Analysis

Treatment had no significant effect on crude protein, moisture, crude fat, crude fiber, and ash content of beef *Longissimus dorsi* (Table 2.6). However, treatment × withdrawal day had a significant impact on moisture, crude fat, and ash. The significance of treatment × withdrawal day on moisture can be explained by the higher percentage of fat displacing the water content, or moisture, in the samples. Forage fed cattle have demonstrated to contain a lower fat content and have leaner carcasses (Nogoy et al., 2022). However, the HEMP ration for the present study had an increased amount of fiber and therefore, it was anticipated that the crude fat would be lower in the HEMP treatment group. There was, however, no noticeable differences in FD or MS between the CON and HEMP carcasses. With that being said, it was observed that, crude fat had significance of treatment × withdrawal day on d0 and d1 with lower crude fat concentration

(Table 2.6). This could be further explained by the lower amount of grain the HEMP treatment was exposed to prior to the assigned slaughter day. The explanation of significance for treatment × withdrawal day for ash (the inorganic residue remaining after the complete oxidation of organic matter), is challenging to explain, but could be the consequence of the specific slaughter days.

Table 2.6. Interactions of withdrawal days and treatment groups for proximate analysis of *Longissimus dorsi* muscle obtained from crossbred beef heifers fed CON or HEMP diets.

Proximate Analysis	Trt	Withdrawal Days				P Values	
		0	1	4	8	Trt	Trt x Withdrawal Day
Crude Protein	CON	15.11 ± 2.16	20.67 ± 2.16	19.79 ± 2.16	18.77 ± 2.16	0.42	0.94
	HEMP	16.24 ± 2.16	23.37 ± 2.16	19.72 ± 2.16	20.01 ± 2.16		
Moisture	CON	16.17 ± 1.74 <sup>a</sup>	12.1 ± 1.74 <sup>a</sup>	13.63 ± 1.74 <sup>a</sup>	16.19 ± 1.74 <sup>a</sup>	0.47	0.05
	HEMP	21.65 ± 1.74 <sup>b</sup>	9.44 ± 1.74 <sup>ac</sup>	14.93 ± 1.74 <sup>a</sup>	11.73 ± 1.74 <sup>a</sup>		
Crude Fat	CON	63.61 ± 1.64 <sup>a</sup>	62.41 ± 1.64 <sup>a</sup>	63.82 ± 1.64 <sup>a</sup>	61.05 ± 1.64 <sup>a</sup>	0.95	0.04
	HEMP	58.84 ± 1.64 <sup>b</sup>	65.14 ± 1.64 <sup>bc</sup>	60.07 ± 1.64 <sup>a</sup>	63.44 ± 1.64 <sup>a</sup>		
Crude Fiber	CON	0.015 ± 0.017	0.008 ± 0.017	0.065 ± 0.017	0.018 ± 0.017	0.53	0.66
	HEMP	0.02 ± 0.017	0.003 ± 0.017	0.033 ± 0.017	0.02 ± 0.017		
Ash	CON	0.895 ± 0.037 <sup>a</sup>	1.17 ± 0.037 <sup>b</sup>	1.06 ± 0.037 <sup>bc</sup>	1.03 ± 0.037 <sup>bc</sup>	0.34	0.03
	HEMP	0.803 ± 0.037 <sup>ac</sup>	1.15 ± 0.037 <sup>b</sup>	1.18 ± 0.037 <sup>b</sup>	1.13 ± 0.037 <sup>b</sup>		

\*Trt represents treatment; Control (CON) = 20% corn silage, 55% corn grain, 20% DDGS, Hempseed cake (HEMP) = CON diet with replacement of 20% DDGS with 20% hempseed cake.

<sup>1</sup> Values within the same row with different letter indicate significance at ( $P < 0.05$ ).

#### 2.4.5. Fatty Acid Profile

Fatty acid concentrations will be presented according to individual fatty acids that comprise less than (Table 2.7) or greater than (Table 2.8) 1.0% of total crude fat concentration of subcutaneous fat obtained adjacent the wholesale brisket. Brisket fat from late-finishing heifers supplemented the HEMP treatment possessed significantly greater concentration of vaccenic ( $P < 0.0001$ ), linolenic ( $P < 0.0001$ ),  $\gamma$ -Linolenic ( $P < 0.0001$ ), and homoalinolenic ( $P < 0.01$ ) while arachidic ( $P < 0.04$ ) and behenoic ( $P < 0.04$ ) differed for the interaction term of treatment × withdrawal day (Table 2.7). The treatment by slaughter day interaction observed for arachidic



and behenoic acid are likely due to random chance and the small sample size of livestock participating in this experiment, or the differences in accumulation of specific fatty acids and/or the different presence desaturase activity (not measured) within tissues (Hood and Thornton, 1976; Chang et al., 1992). The difference between CON and HEMP concentration of vaccenic, linolenic,  $\gamma$ -Linolenic, and homoalinolenic acid is 85, 122.5, 30.25, and 3.25 mg per 100 gram serving of brisket fat, respectively. However, the average consumer of beef brisket will not consume 100 grams of pure brisket fat; therefore, these differences will likely not impart a physiological impact on the humans consuming them.

Vaccenic acid has been identified to have an assortment of health benefits that include increasing insulin sensitivity and anti-inflammatory properties within intestines (Singh et al., 2021.) Plus, with the increased concentrations of vaccenic acid within beef fat, there is an opportunity to label retail beef cuts as healthier for consumers due to the increased amount of trans fat (Singh et al., 2021). A potential explanation for the increased vaccenic, linolenic,  $\gamma$ -Linolenic, and homoalinolenic acid in the brisket fat is due to decreased rumen pH which reduced *Butyrivibrio fibrisolvens*, an anaerobic rumen bacterium responsible for the microbial biohydrogenation of linoleic acid and  $\alpha$ -linolenic acid (Bessa et al., 2000). These small differences could still impart volatile flavor components after heat treatment (cooking) of the beef brisket, but there is not any current literature that indicates vaccenic and/or homoalinolenic acid impart off flavors in beef. In fact, Arshad et al. (2018) concluded that meat flavor is most profoundly impacted by fat and low molecular weight water soluble compounds. Linoleic and oleic acid have been found in higher concentration in beef from grain-fed diets versus grass-fed diets (Vasta et al., 2006). Resulting in linolenic acid compounds to be present in increased concentration in grass-fed animals (Larick et al., 1987). A taste panel analysis of CON versus

HEMP treatments was not concluded in the present study and is necessary to determine if these small differences impact the acceptability of beef flavor.

Of the fatty acids present in beef brisket fat at a concentration greater than 1.0%, linoleic acid was the only one significantly lower ( $P = 0.002$ ) in HEMP versus CON (Table 2.8). Subcutaneous fat obtained from CON possessed 502.5 mg more linoleic acid per 100-gram sample than fat from HEMP heifers. Despite the high concentration of linoleic acid in the dietary hempseed supplement, the linoleic content of adipose tissue from HEMP fed heifers did not differ from the CON. Even so, Rugamba (2013) reported 0.071 grams of linoleic acid per 100 grams sample of pure subcutaneous fat. However, it should be noted the subcutaneous fat collected by Rugamba was collected from an unreported number of carcasses. While the USDA nutrient database (FoodData Central, accessed 1 July 2022) reports that uncooked (raw) USDA choice grade beef brisket will contain 0.18 grams of linoleic acid per 100 grams sample of brisket. Unfortunately, this cannot be used as a direct comparison, as our study collected pure subcutaneous fat for analysis, while the USDA nutrient database reports for the whole beef brisket. That being said, it does serve as a means to compare the amount of linoleic acid in an average cut of beef.

The USDA nutrient database (FoodData Central, accessed 1 July 2022) indicates the average amount of grams of myristic in 100g of whole beef brisket is 0.22g. However, the amount of myristic in the subcutaneous fat of brisket averaged 2.87g for the control diet fed heifers and 2.93g for the hempseed cake diet fed heifers. Due to the increased concentration of myristic acid in the present study, the potential for a negative (metallic and/or grassy aroma) beef fat aroma has been reported by Melton et al. (1982).

The average amount of palmitic acid found in whole beef brisket (100g) is 1.63g, but 24.22g of was found in the control heifers, and 24.69g in hempseed heifer. The drastically increased amount of palmitic acid could be explained by the increased concentration of omega-3 and omega-6 in the present study's hempseed ration influenced the fuel metabolism (suppressing the hepatic lipogenesis, decreasing the hepatic triacylglycerol output, enhancing ketogenesis, and inducing fatty acid oxidation, accompanied by a decrease in body fat deposition). Additionally, with the digestive efficiency of polyunsaturated fatty acids, there is a high degree of biohydrogenation that increases the probability of saturated fatty acids (palmitic acid) to be identified in tissues (Clarke, 2000; Petit et al., 2002).

The average amount of stearic acid found in whole beef brisket per 100g is 0.73g. The average amount of stearic found in the CON treatment group was 25.19g and 24.53g in the HEMP treatment group. Stearic acid is the primary saturated fatty acid found in meat. This is due to the biohydrogenation process in the rumen manipulating feedstuffs to stearic acid (Hodgen, 2006). Leizer et al. (2000) revealed an average 17.4% less stearic acid on w/w basis in the fatty acid profile than the w/w basis of stearic acid revealed in Table 2.8. This significantly higher concentration of stearic acid identified in brisket subcutaneous fat may be due to heavier heifers utilized in the present study, or due to the biohydrogenation process in the rumen with the utilization enzymes and bacteria in the intestines. With that being said, stearic acid has not been proven to have any significant impact on cholesterol concentrations in humans (Yu et al., 1995; Williamson et al., 2005). This is vital to understand due to the controversy assumption that consumption of red meat negatively impacts serum cholesterol concentrations in humans even though red meat cholesterol content is similar to other meats; beef 73, pork 79, lamb 85, chicken 76, and turkey 83 mg/100g (Wheeler et al., 1987; Daley et al., 2010).

The average amount of palmitoleic acid in whole beef brisket (100g) is 0.35g, but 1.33g were identified in the control diet group and 1.35g in the hempseed diet group. The differences in the average amount of grams found in this study verse the USDA nutrient database (FoodData Central, accessed 1 July 2022) could be attributed to the type of sample taken (subcutaneous fat of brisket verse whole beef brisket) and the much larger beef heifers utilized in this study.

No treatment or treatment by withdrawal interactions were observed for any fatty acids (Table 2.8) except for palmitoleic ( $P = 0.04$ ) and linoleic ( $P = 0.002$ ). The high linoleic acid composition of hempseed cake was not transferred to the muscles of the cattle that consumed it.

Table 2.7. Fatty acids concentrations lower than 1.0% for total crude fat of subcutaneous fat of brisket from crossbred feeder heifers fed a standard finishing diet (CON) or a finishing diet containing hempseed cake (HEMP) withdrawn from diet 0, 1, 4, or 8 days prior to harvest.

Fatty Acids	Trt	Withdrawal Days				P Values	
		0	1	4	8	Trt	Trt x Withdrawal Day
Myristoleic (%)	CON	0.25 ± 0.03	0.23 ± 0.03	0.16 ± 0.03	0.31 ± 0.03	0.22	0.13
	HEMP	0.28 ± 0.03	0.29 ± 0.03	0.26 ± 0.03	0.24 ± 0.03		
Pentadecanoic (%)	CON	0.49 ± 0.03	0.52 ± 0.03	0.47 ± 0.03	0.46 ± 0.03	0.86	0.86
	HEMP	0.47 ± 0.03	0.51 ± 0.03	0.48 ± 0.03	0.49 ± 0.03		
Methyl pentadecanoate (%)	CON	0.01 ± 0.0016	0.01 ± 0.0016	0.01 ± 0.0016	0.005 ± 0.0016	1	0.09
	HEMP	0.0075 ± 0.0016	0.01 ± 0.0016	0.0075 ± 0.0016	0.01 ± 0.0016		
Decanoic (%)	CON	0.53 ± 0.05	0.50 ± 0.05	0.51 ± 0.05	0.51 ± 0.05	0.62	0.87
	HEMP	0.46 ± 0.05	0.52 ± 0.05	0.49 ± 0.05	0.50 ± 0.05		
Vaccenic (%)	CON	0.51 ± 0.02 <sup>a</sup>	0.46 ± 0.02 <sup>a</sup>	0.48 ± 0.02 <sup>a</sup>	0.50 ± 0.02 <sup>a</sup>	<0.0001	0.14
	HEMP	0.55 ± 0.02 <sup>a</sup>	0.59 ± 0.02 <sup>b</sup>	0.58 ± 0.02 <sup>b</sup>	0.57 ± 0.02 <sup>b</sup>		
Linoelaidic (%)	CON	0.07 ± 0.013	0.05 ± 0.013	0.04 ± 0.013	0.045 ± 0.013	0.08	0.77
	HEMP	0.075 ± 0.013	0.055 ± 0.013	0.062 ± 0.013	0.075 ± 0.013		
Linolenic (%)	CON	0.23 ± 0.03 <sup>a</sup>	0.21 ± 0.03 <sup>a</sup>	0.21 ± 0.03 <sup>a</sup>	0.20 ± 0.03 <sup>a</sup>	<0.0001	0.79
	HEMP	0.31 ± 0.03 <sup>a</sup>	0.35 ± 0.03 <sup>b</sup>	0.33 ± 0.03 <sup>b</sup>	0.35 ± 0.03 <sup>b</sup>		
gLinolenic (%)	CON	0.023 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>a</sup>	0.02 ± 0.01 <sup>a</sup>	0.015 ± 0.01 <sup>a</sup>	<0.0001	0.42
	HEMP	0.047 ± 0.01 <sup>b</sup>	0.062 ± 0.01 <sup>b</sup>	0.045 ± 0.01 <sup>b</sup>	0.055 ± 0.01 <sup>b</sup>		
Arachidic (%)	CON	0.23 ± 0.01 <sup>a</sup>	0.23 ± 0.01 <sup>a</sup>	0.21 ± 0.01 <sup>a</sup>	0.19 ± 0.01 <sup>b</sup>	0.48	0.04
	HEMP	0.22 ± 0.01 <sup>a</sup>	0.21 ± 0.01 <sup>a</sup>	0.21 ± 0.01 <sup>a</sup>	0.24 ± 0.01 <sup>ac</sup>		
Gonodic (%)	CON	0.18 ± 0.02	0.16 ± 0.02	0.17 ± 0.02	0.15 ± 0.02	0.2	0.87
	HEMP	0.15 ± 0.02	0.14 ± 0.02	0.15 ± 0.02	0.15 ± 0.02		
Docosanoic (%)	CON	0.042 ± 0.005	0.04 ± 0.005	0.045 ± 0.005	0.035 ± 0.005	0.11	0.15
	HEMP	0.03 ± 0.005	0.042 ± 0.005	0.03 ± 0.005	0.037 ± 0.005		
Homoglinolenic (%)	CON	0.037 ± 0.007	0.037 ± 0.007	0.037 ± 0.007	0.035 ± 0.007	0.09	0.3
	HEMP	0.035 ± 0.007	0.06 ± 0.007	0.045 ± 0.007	0.04 ± 0.007		
Homoalinolenic (%)	CON	0.007 ± 0.002 <sup>a</sup>	0.005 ± 0.002 <sup>a</sup>	0.01 ± 0.002 <sup>ac</sup>	0.005 ± 0.002 <sup>a</sup>	0.01	0.41
	HEMP	0.01 ± 0.002 <sup>c</sup>	0.01 ± 0.002 <sup>c</sup>	0.01 ± 0.002 <sup>c</sup>	0.01 ± 0.002 <sup>c</sup>		
Arachidonic (%)	CON	0.013 ± 0.004	0.018 ± 0.004	0.018 ± 0.004	0.018 ± 0.004	0.83	0.92
	HEMP	0.01 ± 0.004	0.02 ± 0.004	0.018 ± 0.004	0.015 ± 0.004		
Heneicosanoic (%)	CON	0.43 ± 0.039	0.34 ± 0.039	0.37 ± 0.039	0.45 ± 0.039	0.14	0.06
	HEMP	0.4 ± 0.039	0.49 ± 0.039	0.45 ± 0.039	0.41 ± 0.039		
Behenoic (%)	CON	0.038 ± 0.004	0.035 ± 0.004	0.028 ± 0.004	0.03 ± 0.004	0.06	0.04
	HEMP	0.038 ± 0.004	0.03 ± 0.004	0.04 ± 0.004	0.043 ± 0.004		
Erucic (%)	CON	0 ± 0.002	0.003 ± 0.002	0 ± 0.002	0.003 ± 0.002	0.22	0.59
	HEMP	0 ± 0.002	0.003 ± 0.002	0.005 ± 0.002	0.005 ± 0.002		
Arachidic (%)	CON	0.008 ± 0.003 <sup>a</sup>	0.003 ± 0.003 <sup>a</sup>	0 ± 0.003 <sup>b</sup>	0.005 ± 0.003 <sup>a</sup>	<0.0001	0.13
	HEMP	0.01 ± 0.003 <sup>ac</sup>	0.015 ± 0.003 <sup>bc</sup>	0.01 ± 0.003 <sup>ac</sup>	0.013 ± 0.003 <sup>ac</sup>		
Lignoceric (%)	CON	0.02 ± 0.003	0.018 ± 0.003	0.013 ± 0.003	0.013 ± 0.003	0.33	0.41
	HEMP	0.02 ± 0.003	0.015 ± 0.003	0.018 ± 0.003	0.018 ± 0.003		

\*Trt represents treatment; Control (CON) = 20% corn silage, 55% corn grain, 20% DDGS, Hempseed cake (HEMP) = CON diet with replacement of 20% DDGS with 20% hempseed cake.

<sup>1</sup> Values within the same row with different letter indicate significance at ( $P < 0.05$ ).

Table 2.8. Fatty acids concentrations higher than 1.0% for total crude fat from subcutaneous fat of brisket from crossbred feeder heifers fed a standard finishing diet (CON) or a finishing diet containing hempseed cake (HEMP) withdrawn from diet 0, 1, 4, or 8 days prior to harvest.

Fatty Acids	Trt	Withdrawal Days				P Values	
		0	1	4	8	Trt	Trt x Withdrawal Day
Myristic (%)	CON	2.63 ± 0.19	3.03 ± 0.19	2.66 ± 0.19	3.14 ± 0.19	0.66	0.06
	HEMP	3.21 ± 0.19	2.99 ± 0.19	2.85 ± 0.19	2.65 ± 0.19		
Palmitic (%)	CON	23.75 ± 0.86	24.86 ± 0.86	23.30 ± 0.86	24.95 ± 0.86	0.45	0.24
	HEMP	25.68 ± 0.86	24.05 ± 0.86	24.84 ± 0.86	24.17 ± 0.86		
Palmitoleic (%)	CON	1.32 ± 0.09 <sup>a</sup>	1.27 ± 0.09 <sup>a</sup>	1.15 ± 0.09 <sup>a</sup>	1.56 ± 0.09 <sup>b</sup>	0.74	0.04
	HEMP	1.39 ± 0.09 <sup>a</sup>	1.35 ± 0.09 <sup>a</sup>	1.37 ± 0.09 <sup>a</sup>	1.27 ± 0.09 <sup>a</sup>		
Margaric (%)	CON	1.54 ± 0.09	1.59 ± 0.09	1.71 ± 0.09	1.39 ± 0.09	0.26	0.28
	HEMP	1.42 ± 0.09	1.52 ± 0.09	1.46 ± 0.09	1.51 ± 0.09		
Stearic (%)	CON	24.64 ± 1.02	25.93 ± 1.02	26.53 ± 1.02	23.67 ± 1.02	0.37	0.25
	HEMP	25.08 ± 1.02	24.13 ± 1.02	24.08 ± 1.02	24.84 ± 1.02		
Elaidic (%)	CON	4.09 ± 0.33	3.93 ± 0.33	3.94 ± 0.33	3.59 ± 0.33	0.19	0.32
	HEMP	3.96 ± 0.33	4.60 ± 0.33	3.80 ± 0.33	4.44 ± 0.33		
Oleic (%)	CON	32.94 ± 1.24	30.53 ± 1.24	31.40 ± 1.24	33.02 ± 1.24	0.66	0.43
	HEMP	30.59 ± 1.24	31.49 ± 1.24	32.52 ± 1.24	31.72 ± 1.24		
Linoleic (%)	CON	2.02 ± 0.21	2.21 ± 0.21	2.73 ± 0.21	2.03 ± 0.21	0.002	0.18
	HEMP	1.47 ± 0.21	2.01 ± 0.21	1.69 ± 0.21	1.81 ± 0.21		
Total SFA (%)	CON	17.01 ± 0.69	18.03 ± 0.69	17.49 ± 0.69	17.25 ± 0.69	0.49	0.18
	HEMP	17.99 ± 0.69	17.06 ± 0.69	17.26 ± 0.69	17.22 ± 0.69		
Total USFA (%)	CON	5.99 ± 0.33	5.77 ± 0.33	6.14 ± 0.33	5.96 ± 0.33	0.23	0.23
	HEMP	5.41 ± 0.33	5.88 ± 0.33	5.74 ± 0.33	5.78 ± 0.33		

\*Trt represents treatment; Control (CON) = 20% corn silage, 55% corn grain, 20% DDGS, Hempseed cake (HEMP) = CON diet with replacement of 20% DDGS with 20% hempseed cake.  
<sup>1</sup> Values within the same row with different letter indicate significance at ( $P < 0.05$ ).

## 2.5. Implications

Supplementation of hempseed cake phased into the diet of late-finishing, heavy feedlot heifers did not impact the economically important traits of carcass weight, USDA yield grade, marbling score, tenderness, or fresh beef color in a simulated retail display setting. These are positive findings because it provides support for the inclusion of hempseed as a replacement for DDGS in a standard finishing diet. Also, there is no evidence from the present study that

hempseed inclusion in finishing rations alters beef quality or palatability. Future work should examine longer duration feeding and subsequent impact on economically important traits associated with beef quantity and quality.

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