

Influence of hempseed cake inclusion on growth performance, carcass characteristics, feeding behavior and blood parameters in finishing heifers

Thomas M. Winders^{*}, Eric M. Serum[†], David J. Smith[†], Bryan W. Neville[‡], Golam K. Mia^{*}, Samat Amat[§], Carl R. Dahlen^{*}, Kendall C. Swanson^{*1}

^{*}Department of Animal Sciences, North Dakota State University, Fargo, ND 58108-6050, USA

[†]USDA ARS-ETSARC – Fargo, ND, 58102, USA

[‡]Carrington Research Extension Center, Carrington, ND 58421-0219, USA

[§]Department of Microbiological Sciences, North Dakota State University, Fargo, ND 58108-6050, USA

¹Corresponding author: kendall.swanson@ndsu.edu

Lay Summary

This experiment evaluated the effects of dietary inclusion of hempseed cake in comparison to dried corn distillers grains plus solubles on growth performance, feeding behavior, carcass characteristics, and blood parameters in finishing heifers. Angus-crossbred heifers were assigned randomly to one of two treatments (20% hempseed cake; HEMP; or 20% dried corn distillers grains plus solubles; DDGS; dry matter basis) and were fed for 111 days until slaughter. Heifers receiving the DDGS treatment had greater final body weights, average daily gain, gain efficiency, dietary concentration of net energy for maintenance and gain, and carcass weight than heifers fed the HEMP treatment. All other carcass characteristics as well as feeding behavior were not influenced by treatment. Plasma urea nitrogen was greater in heifers fed the HEMP diet compared to the DDGS diet, while glucose was not influenced by treatment. Several plasma amino acid concentrations were influenced by treatment. Although inclusion of hempseed cake decreased growth performance, it could be a viable alternative feed source for cattle.

Teaser Text

Hempseed cake has potential for use as an alternative feedstuff for cattle finishing diets.

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**) $\pm$ 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 ($P \leq 0.05$) by 2.3%, 7.7%, 7.7%, and 2.6% respectively 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.

Key words: animal performance, finishing cattle, hempseed cake, nutrition, plasma metabolites

Accepted Manuscript

Abbreviations

AA, amino acids

ADF, acid detergent fiber

ADG, average daily gain

BW, body weight

CP, crude protein

DM, dry matter

DMI, dry matter intake

EAA, essential amino acids

G:F, gain:feed

HCW, hot carcass weight

LM, longissimus muscle

N, nitrogen

NDF, neutral detergent fiber

NEAA, non-essential amino acids

NE_m, net energy for maintenance

NE_g, net energy for gain

OM, organic matter

PUN, plasma urea nitrogen

THC, delta-9 tetrahydrocannabinol

Introduction

Industrial hemp has been produced for thousands of years, largely for its fiber, but production decreased in favor of cheaper alternatives (Fike, 2016). The Marihuana Tax Act of 1937 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 with the goal to reinstate industrial hemp production. 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% [dry matter (**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 its relatively high CP and ADF concentration, hempseed cake could be a useful feed ingredient for ruminants because of the potential as a dietary protein and energy source. 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 (Table 1). 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). Hessle et al. (2008) reported that feeding hempseed cake does not negatively influence dry matter intake (**DMI**) but performance metrics such as average daily gain (**ADG**) and gain:feed (G:F) are decreased when fed to growing bulls at 20% of diet DM. In the same manuscript, these authors reported that feeding hempseed cake to finishing steers at 9% of diet DM did not influence DMI, ADG, G:F or final BW. Karlsson and Martinsson (2011) fed hempseed cake to ewe lambs at 22% of diet DM and observed a decrease in ADF, G:F and final BW while not influencing DMI. 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). Furthermore, the most common inclusion rate for corn distillers grains is between 10-30% of diet DM when used as the primary byproduct (Samuelson et al., 2016). Feeding hempseed cake in comparison to corn distillers grains at a common inclusion rate in finishing cattle diets has not been evaluated. 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.

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.

Animals, experimental design, and dietary treatments

An 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**) $\pm$ SE = 494 $\pm$ 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; Table 1). The dried corn distillers grains plus solubles was sourced from Dakota Spirit Midwest Ag Energy (Spiritwood, ND, USA) and the hempseed cake was sourced from Healthy Oilseeds, LLC (Carrington, ND, USA). 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). 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 meet or exceed ruminally degradable protein intake and metabolizable protein requirements (NASEM, 2016). 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 according to USDA standards. 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.

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 (Hokofarm 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 of 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). Dry matter intake and 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.

Feed sample analyses

Feed samples were collected weekly for DM analysis and dried at 60 °C 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 (assayed with heat stable amylase and sodium sulphite) were quantified as described by Van Soest et al. (1991). Crude protein concentration was calculated as $6.25 \times \text{N}$. Samples were also analyzed for starch concentration (Herrera-Saldana and Huber, 1989). Cannabinoid concentrations in hempseed cake were measured using electrospray ionization rapid screening using similar methodology as those described by Chakrabarty et al. (2021). Total concentrations of 10 cannabinoids (including cannabidiol and THC) were 13 mg/kg of hempseed cake (DM-basis). Heifers fed the HEMP diet had an average 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.

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 ($3,000 \times g$ at 4 °C) and

stored at -20 °C 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 C₁₈ 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).

Dietary calculations

Rumen degradable protein (RDP) balance was modeled using NASEM (2016) empirical level of solution. Because RDP of hempseed cake was not known, only the DDGS diet was evaluated to determine RDP balance. Using BW and DMI from this experiment, the DDGS diet provided an estimated excess of 77 g/d of RDP. 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, DMI and 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 = $(0.2 \times 2.21 \text{ Mcal/kg}) + (0.8 \times x)$. The HEMP diet NE_m was set equal to 0.8×1.825 (base diet NE_m , Mcal/kg calculated in previous step) + $(0.2 \times x)$, where x is equal to the NE_m of hempseed cake. Calculations for dietary NE_m and NE_g were done for individual heifers.

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 it 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 or 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 $P \leq 0.05$.

Results and Discussion

Growth performance

Heifers fed the DDGS diet had greater ($P \leq 0.05$) final BW, ADG, and G:F than heifers fed the HEMP diet (Table 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 whereby DMI was not influenced in sheep fed hempseed meal at 20% of the diet. However, Hesse et al. (2008) reported an increase in DMI when hempseed cake is included at 20% of the diet DM in comparison to a mixture of soybean meal and rolled barley in calf diets. Furthermore, when hempseed cake was included in dairy rations up to 31% of diet DM (displaced a compound pellet comprised largely of barley) DMI increased (Karlsson et al., 2010).

The observed decrease in ADG and G:F in the present experiment is similar to what Hesse (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 hempseed contain more oil than hempseed cake (28.4% vs 7.4% oil) 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 a 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 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. Although caution should be used when comparing estimates between studies, our estimated NE_m and NE_g concentrations of hempseed cake 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). 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 the most common industry recommendation of averages of 1.50 Mcal/kg NE_g (Samuelson et al., 2016), 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. The reduced predicted net energy of hempseed cake relative to DDGS indicate that finishing cattle performance could 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 value of hempseed cake could be more dependent on the quality of dietary energy more so than dietary protein.

Feeding behavior

No differences were observed ($P \geq 0.32$) in feeding behavior between treatments (Table 3). While the effect of hempseed cake on cattle feeding behavior has not been reported elsewhere, the lack of effect may not be 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 and concentration (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.

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 ($P \geq 0.37$). 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).

Plasma parameters

Amino acid concentrations in plasma are influenced by many factors including dietary amino acid supply, ruminal fermentation, feed digestibility, amino acid 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 across treatments ($P \geq 0.09$; Table 4). Lack of differences between treatments may indicate that feeding hempseed cake did not result in substantial 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, isoleucine, leucine, lysine, methionine, and phenylalanine because treatment did not influence concentration on day 0 but did on other days and because of variability in concentrations over time. When examining dietary treatment effects within each sampling day, plasma arginine concentration was greater ($P = 0.04$) on day 98 in heifers fed the HEMP diet than in heifers fed the DDGS diet. Plasma isoleucine concentrations were lower ($P < 0.01$) on day 7 and 56, and plasma 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 lower ($P = 0.01$) 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 contribution of lysine in hempseed cake to cattle metabolizable lysine requirements 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 ($P = 0.03$) 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 intakes, 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.

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. Plasma urea nitrogen was affected quadratically ($P = 0.04$) by day. 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. Potential site of CP digestion differences between treatments could influence PUN as well. Furthermore, both treatment diets likely provided excess MP relative to requirements which can lead to greater urea production (Jennings et al., 2018). 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 (22.6%; 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).

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. 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.

Acknowledgements

This project was partially supported by a cooperative agreement between NDSU and the USDA Agricultural Research Service. The authors would like to thank the employees of the NDSU Beef Cattle Research Complex and the Nutrition, Physiology, and Meat Science Laboratories in the Department of Animal Science at North Dakota State University (Fargo, ND) for assistance with the project.

Conflict of Interest

The authors declare that they have no known financial, non-financial, or personal interest that could influence the subject matter or materials discussed in this paper in any way.

Accepted Manuscript

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
- Chakrabarty, S., W. L. Shelver, and D. J. Smith. 2021. Electrospray ionization inlet tandem mass spectrometry: A hyphenated method for the sensitive determination of chemicals in animal tissues and body fluids. *J. Mass. Spectr.* 32: 14-20. doi:10.1021/jasms.9b00114
- 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. Proceedings of the American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics (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. Thornsby. 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

Figures Legend

Figure 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 * ($P < 0.05$).

Accepted Manuscript

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

Ingredient, % of diet DM	Treatments ¹		Byproducts ²	
	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

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

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

Performance ²	Treatments ¹		SEM	P-Value
	DDGS	Hemp		
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
NEm, Mcal/kg feed	1.92	1.82	0.04	0.02
NEg, Mcal/kg	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

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

²Performance measures analyzed over the 111-day feeding period.

³Marbling score: 400 = Slight⁰⁰, 450 = Slight⁵⁰, 500 = Small, etc.

⁴Yield 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, cm²; USDA, 2016).

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

Item	Treatments ¹		SEM	P-Value
	DDGS	Hemp		
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

¹Treatments consisted of 20% DDGS or 20% Hemp (DM basis) in a finishing ration.

²Visit defined as each time the Insentec system detected a heifer at the bunk.

³Meal defined as eating periods combined if break between was not longer than 7 minutes.

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

Item, μ M	Treatments ¹								SEM	Trt	P-Value ³	
	DDGS ²				Hemp ²						Day	TxD
	Day				Day							
	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
----------	-------	-------	-------	-------	-------	-------	-------	-------	----	------	---	------

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

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

³Standard 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.

Figure 1

