8-2021

The Role of Fatty Acids in Ruminant Diets and Novel Feed Ingredients High in Omega– 3 Fatty Acids Fed in Feedlot Diets

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THE ROLE OF FATTY ACIDS IN RUMINANT DIETS AND NOVEL FEED INGREDIENTS HIGH IN OMEGA– 3 FATTY ACIDS FED IN FEEDLOT DIETS.

By

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A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor

Andrea K. Watson

Lincoln, Nebraska

August 2021

THE ROLE OF FATTY ACIDS IN RUMINANT DIETS AND NOVEL FEED INGREDIENTS HIGH IN OMEGA– 3 FATTY ACIDS FED IN FEEDLOT DIETS.

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University of Nebraska, 2021

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A finishing study evaluated the performance, carcass characteristics and fatty acid profiles of steers fed four inclusions of a novel feedstuff Green Grass. Green Grass is a product comprised of sesame meal, giant kelp, cassava, and sorghum containing high concentrations of polyunsaturated fatty acids. No differences were observed in initial BW, final BW, BW gain, HCW, LM area, 12^{th} rib fat depth, calculated YG, or liver abscess %. Dry matter intake linearly increased as Green Grass inclusion increased in the diet. Steers fed Green Grass had lower G:F than control cattle, and steers fed 30% Green Grass had a lower marbling score. A linear increase in alpha linolenic acid was observed in steak samples, resulting in an increase of 304% comparing steers fed 30% Green Grass to control cattle. Linear increases of trans-unsaturated fatty acids, and omega-3 fatty acids were also observed in steak samples from steers fed increasing inclusion of Green Grass. Increasing Green Grass inclusion in the diet from 0 to 30% linearly improved omega-3 fatty acid concentration in beef.

A safety trial was completed by feeding a novel algal biomass to cattle. Cattle were individually fed 1 of 4 inclusions of Condensed Algal Residue Solubles (CARS). Increasing CARS inclusion in the diet quadratically increased DMI and ADG and linearly increased G:F. Net energy calculations demonstrated a linear increase in NE_m and NE_g as

CARS inclusion increased. Out of 27 organs measured, 6 had differences due to treatment in absolute weight and weight as a % of BW. Out of 21 blood chemistry measures, 8 were impacted by treatment.

A digestibility study was conducted evaluating CARS fed in finishing cattle diets. Increasing CARS inclusion in the diet resulted in a linear decrease in dry matter and organic matter intake, with no effect on dry matter and organic matter digestibility. Replacing up to 10% dry rolled corn with CARS in diets with or without wet distillers grains had little effect on digestibility of finishing beef cattle diets.

Keywords: omega 3, alpha linolenic acid, fatty acid, cattle, algae, byproduct, fat

Acknowledgments

I would like to thank my advisor Andrea Watson as well as Galen Erickson, Jim MacDonald, Chris Calkins, and Rick Stock for giving me the opportunity to pursue this degree at the University of Nebraska. I am very grateful for everything you all have taught me.

Thank you to my fellow graduate students who assisted with my research, it would not have been possible without your help.

I would also like to thank my parents, Dave and Susan Norman, my siblings, and my wife Samantha Norman for their support.

Mitch Norman

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CHAPTER I. REVIEW OF THE LITERATURE

INTRODUCTION

Fats are composed of triglycerides, which contain 3 fatty acid chains attached by an ester bond to each of the 3 carbons in the glycerol back bone. The fatty acid chains are composed of various lengths of carbon to carbon molecules bonded by covalent bonds. Hydrogen ions are bonded to the carbon in the other two orbitals. Saturated fatty acids (SFA) are fatty acids with no double bonds, making the fatty acid completely saturated with hydrogens, while unsaturated fatty acids contain at least one double bond, relieving the carbons contained in the double bond of one of two hydrogens.

Ruminant diets vary due to production scenario, diets may vary from 100% forage in grazing scenarios to as low as 5% forage in finishing diets. Inclusion of concentrates, by-products and roughages in ruminant diets can be highly variable. Due to this variation, nutrient compositions between two diets may be very different, however, inclusion of dietary fat in typical ruminant diets as a percent dry matter (DM) is usually less than 5% DM (Palmquist et al., 2005). Even at inclusion less than 5% of the diet, fats play a large role in metabolism, allowing absorption of essential fat-soluble vitamins. Lipids make up many phospholipid membranes throughout the cells in the animal. Fats provide many essential fatty acids to the animal, can improve palatability of feeds, and alleviate some heat stress due to slowing heat of fermentation (NASEM 2016). Fats are primarily supplemented to cattle to increase the energy density of the diet, or to manipulate fermentation in the rumen due to anti-microbial effects of the unsaturated fatty acids (Lourenco et al., 2010), and more recently alter fatty acid profiles of the meat.

Fats are digested differently in the ruminant animal compared to non-ruminants. The fatty acid profile contained in the animal's diet will contribute to the different profiles found in the meat or milk (Hebeisen et al. 1993). However, due to microbial functions that change these fatty acids in the rumen, the amount of unsaturated fatty acids consumed will be different than what is represented in the meat and milk. The objective of this review is to determine what fats are, how fats are digested and absorbed, and impacts of altering fatty acid profiles in diets and the effect it has on the meat.

Lipolysis

The lipid fraction of forages in cattle diets or grazing scenarios are composed almost entirely of triglycerides, glycolipids, and phospholipids. Lipolysis occurs rapidly after ingestion in the reticulum (Jenkins et al., 2008). Lipolysis is the breakdown of fats by the enzymatic function of hydrolysis to break down the ester bonds connecting the fatty acid chains and the glycerol backbone (Bauman et al., 1999). *Abaerobcibrio lipolytica* has been heavily studied in its active role in lipolysis (Lourenco et al., 2010). In concentrate diets where triacylglycerol composes most of the lipid portion of the diet, *A. lipolytica* lipolytic role has been shown to hydrolyze the ester linkages which must occur prior to biohydrogentation. In forage diets containing mostly phospho- and glucolipids *Butyribivio* species were dominant in hydrolyzing those fatty acids (Lourenco et al., 2010). As the fatty acids leave the rumen and enter the duodenum, lipolysis is considered complete. Within these classes of lipids, the major fatty acids making up the profile are the unsaturated fatty acids linolenic and linoleic acid (Bauman et al., 1999). Unsaturated fatty acids affect the permeability of microbial cell membranes and reducing gram negative microbes ability on fermentation of fiber (Nagaraja et al., 1997) therefore,

as fatty acids go through lipolysis, microbes convert unsaturated fatty acids into more saturated fatty acids in a process called biohydrogenation.

Biohydrogenation

The idea of biohydrogenation in the rumen dates back to the 1930's as described by Banks and Hilditch (1931). They found higher concentrations of stearic acid to palmitic acid found in fats associated within the animal tissue compared to what was found in diets and suggested that palmitic acid is destroyed or that stearic acid is synthesized from unsaturated fats. It is now understood that the latter of the two hypotheses occur in the rumen.

In cattle diets where grass forage makes up the largest proportion of the diet unsaturated fatty acids such as α–linolenic acid (cis-9, cis-12,cis-15, 18:3) and linoleic acid (cis-9, cis-12-18:2) are typically found in large concentrations of the lipid profile. In contrast, the concentrations of these unsaturated fatty acids are found in much lower amounts in the fat contained in meat and milk (Jenkins et al., 2008).

Currently the scientific community cannot name all species of bacteria involved in biohydrogenation, especially because the whole rumen microbiome is still unknown. Several models (Dijkstra model, Ribeiro Model, Moate model, Harvatine and Allen Model), have been developed depicting different pathways of 18:3 or 18:2 to 18:0 (Dijkstra et al. 2000; Ribeiro et al., 2007; Moate et al., 2014; Harvatine and Allen., 2006). Biohydrogenation occurs in strictly anaerobic environments, as most of the rumen bacteria are anaerobic. Biohydrogenation from 18:2 to 18:1 to 18:0 is a multi-species process. Concentrations of 18:2 shifting to 18:1 occurred with pure *Butyrivibrio fibrosolvins* strains, but 18:0 was not generated with this strain alone. Complete

biohydrogenation can be demonstrated with two different species of rumen bacteria as done in vitro by Kemp and Lander (1984).

One bacterium is responsible for converting α –linoleic acid to trans-octadec-11-enoic acid which would be the end product for that bacteria. The second species then uses trans-octadec-11-enoic acid as a substrate to convert it to stearic acid. Kemp and Lander (1984) describe species capable of doing the first reaction Group A bacterium, and Group B bacterium capable of doing the second reaction to stearic acid. Kemp and Lander (1984), determined that F2/6 *Ruminoccus albus* and S2 *Butyrivibrio sp.* were involved in the first reaction. These Group A bacteria used substrates of (18:1; *cis9, cis11, trans, trans 11*) (18:2; *cis 9, cis 12*) (18:3; *cis 9, cis 12, cis 15*). Major products from these reactions were 18:1; *trans 11* (+ *trans 10*), 18:1; *trans 11* (+ *trans 10* and 18:2; *trans 11, cis 15*).

Group B bacteria, *Fucosillus babrahamensis, Fusocillus sp*., and *R8/5 gram-negative rod* were demonstrated in Kemp and Lander (1984) to use the products from group A bacteria as substrates to form the end products stearic acid, and 18:1 cis 15, 18:1 trans 15, and cis/trans 13, 14, and 16 with these three species.

The biohydrogenation process begins with the isomeration of linoleic acid (*cis-9, cis-12*) to CLA (*trans-11-octadecenoate*) (Hughes et al.,1982). The isomerization reaction is unique because it requires no cofactor, as well as it occurs in the middle of a long fatty acid chain and distant from any activating functional groups (Bauman et al., 2000). Linoleate isomerase is the enzyme responsible for the first isomerization step in biohydrogenation, which is located on the cell membrane of the bacterial cell. It has been

shown that as pH in the rumen drops below 6, inhibition of the isomerization of *cis*-C18:2 to CLA occurs (Troegeler-Meynadier et al., 2003)

B. fibrosolvins has been shown to contain *Butyrivibrio fibrosolvens cis-9, trans-11-octadecadienoate reductase* enzyme. This reaction occurs in a way such that, linoleic acid 18:2 is converted to monoenoic acid 18:1 but monoenoic acids are not converted to stearic acid 18:0 until monoenoic acid levels exceed that of dienoic acids. The kinetics of this reaction show that the reduction to *trans-11* C18:1 is the rate limiting step (Bauman et al., 2000).

The idea that biohydrogenation occurs in a multi-bacterial system was seen previously in a study conducted by Polan et al. (1964), with mixed bacteria including *B. fibrosolvens*, *Peptostreptococcus elsdenii,* and unnamed *Selenomonas* needed for 18:0 to be generated. But when only one bacteria was used no stearic acid was produced. Thus far, *Butyrivibrio fibrosolvens* are known to be the most abundant and active participating bacteria in this process. Polan et al. (1964), identified that age of the bacteria, environment, and substrate all contribute to level of biohydrogenation that occurs.

The incomplete biohydrogenized 18:2 to 18:0 intermediates such as the *cis-9, trans-11* CLA have potential anti-carcinogenic effects, as well the *trans*-10, *cis*-12 which is believed to have benefits attributed to anti-obesity (Bauman et al., 1999). Increasing these intermediates escaping the rumen to be absorbed in the small intestine and distributed to peripheral tissues, will give humans the opportunity to increase their CLA consumption. AbuGhazaleh and Jacobson (2007), showed combining a docosahexaenoic acid (DHA) source, such as fish oil or algae, and linoleic acid source under high ruminal pH (6.7) conditions increases ruminal vaccenic acid production and cis-9, trans 11 CLA

levels. This allows humans to achieve the health benefits believed to be associated with these CLA's (Loor et al., 2004). Increasing the concentration of CLA's in the meat by allowing polyunsaturated fatty acids (PUFA's) to escape the rumen will also lower the concentration of saturated fatty acids (SFA's) deposited in the meat. Consuming large amounts of SFA's is understood to be linked to coronary heart disease in humans (Lourenco et al., 2010). Although it is believed protozoa and fungi in the rumen have little if any contribution to biohydrogenation, the role of protozoa is still important. Protozoa in the rumen engulf bacteria increasing the concentrations of fatty acids within the protozoa. Because of this role, it is believed that up to 40% of the *cis* 9, *trans* 11-CLA and 30-36% of the *trans*10, cis9-CLA isomer reaching the duodenum is due to protozoa (Yáñez-Ruiz et al., 2006).

Rumen Protected Fat

A few methods have been used to decrease biohydrogenation in the rumen and increase the amount of PUFA flowing to the small intestine. These methods and technology have had success although the results are variable. The dairy industry has used protected fats to alter desired FA absorption in the small intestines. One method is by encapsulation of PUFA in formaldehyde-treated protein. This method seems effective with reporting of 75-90% of fat was protected from rumen biohydrogenation and readily digested in the small intestine (Gulati et al., 2005). This method reduces the amount of biohydrogenation and minimizes the detrimental effects when supplementing fat on fiber digestion (Zinn et al., 2000). Calcium salts is another known method which requires post ruminal degradation for release and absorption of the constituent unsaturated fatty acid. Fatty acids are released from calcium salts primarily by acidic dissociation in the

abomasum. This method has had mixed results as in many studies either the calcium salt dissociated in the rumen or biohydrogenation still occurred (Jenkins, 1993). The last method is to increase the amount of the desired PUFA in the diet. Increasing the amount PUFA in the diet will increase the amount of PUFA absorbed, up to the point where it begins to affect fiber digestion in the rumen (Jenkins et al., 2007).

Lipid digestion and absorption in the small intestine

In non-ruminants, little digestion of lipids occurs before the small intestine, however in ruminants, lipolysis is considered complete prior to digesta entering the small intestine. Very little absorption of non-soluble fatty acids takes place in the rumen, reticulum, omasum and abomasum. The fatty portions of digesta entering the small intestine are composed of non-esterified fatty acids (70%) and small amounts of phospholipids (10 to 20%; Bauchart, 1992). These portions form micelles which are then able to associate with the unstirred water layer involved with the small intestine epithelial tissue. The enterocyte absorbs the micelle and within the endoplasmic reticulum, free fatty acids, fat-soluble vitamins, phospholipids, triacylglycerol, cholesterol esters, and protein form lipoproteins. The lipoproteins are transported out of the enterocyte and into the lymphatic system where they slowly enter the blood.

Studies presented by Bauchart (1993) and Laplaud et al., (1990), by using intestinal lymph duct cannulated dairy cows and calves suggest that in conventional dairy diets 15 to 20% of fatty acids are absorbed in the upper jejunum, and 55 to 65% of fatty acids are absorbed in the middle and lower jejunum. However, in diets containing protected lipids, or in finishing diets where high concentrate diets are fed and more

unsaturated lipids reach the small intestine (NASEM, 2016) it is more similar to non-ruminants where pancreatic lipase, emulsification with bile salts and enzymes break down the triacylglycerol into fatty acids and 2-monoacylglycerols, which play a role in the formation of micelles. In this case Bauchart (1993) suggests that fatty acid absorption does not take place until mid and lower jejunum. These micelles and packaged lipoproteins transport lipid to peripheral tissues. Lipoproteins are categorized by size, density, and apolipoprotein affiliation and include chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoproteins (HDL; D. Bauchart, 1993). In non-ruminants where rumen biohydrogenation does not occur, changing the FA profile of the feed has more of a direct change of FA concentrations in the animal products such as meat and eggs. Alterations of increased PUFA and fat-soluble vitamins from feed sources such as flax seed, fish oil and bioengineered algae in laying hen's diet, showed an in improvement in healthier fat profiles found in the yokes of these eggs (Scheideler et al., 1997). Nutrient composition comparisons of regular USDA large eggs compared to Omega eggs showed similar Calories, protein, carbohydrate, and total fat between the standard eggs compared to the Omega egg. Differences were seen in the shift of saturated fat at 1.5 grams in the Omega Egg as compared to 2.2 g in the Standard Egg. The PUFA concentrations of the Omega Egg were higher at 1.35 g compared to 0.90 g found in the standard egg. Concentrations of C18:3 were over 6 times higher in the Omega egg at 250 mg compared to the standard egg at 40 mg.

Fat in grazing diets

Supplementing fat to grazing cattle is usually not a common strategy for producers as high fat diets can hinder fiber fermentation. In all forage species, 18:3, 18:2 and 16:0 fatty acids make up 51%, 18%, and 19% on average of the total fatty acids in the forages, respectively (Boufaïed et al., 2003). Maturity of the plant has an effect on the fatty acid profile concentration and amount. Boufaïed et al (2003) reported a linear decrease in concentrations of C16:0, C16:1, C18:2, C18:3 and total fatty acid amount over four growth stages (stem elongation, early heading, late heading, and early flowering) in timothy grass ($P < 0.05$). Noci et al (2014), demonstrated a trend of improved performance with heifers supplemented sunflower oil (SO), or linseed oil (LO), while grazing pasture. The control group cattle were allowed access to 8.8 kg of DM per animal daily, while the SO and LO groups were supplemented 1.6 kg daily of concentrate which contained the test oil as well as a daily pasture allowance of 7 kg of DM per animal, which compensated for the energy supplied from the concentrates. A major difference in these oils are the concentrations of C18:1, C18:2, C18:3, MUFAs, and PUFAs. SO was higher in concentrations C18:1, C18:2 and MUFAs, while LO was higher in concentrations C18:3 and PUFAs. Heifers fed SO showed 519 kg preslaughter weights and LO showed 507 kg compared to 495 kg of the control. Daily gain was numerically improved in both SO at 1.12 kg and 1.09 kg with LO, compared to 1.00 kg of the control; however, it was not statistically significant ($P \geq 0.05$). Muscle samples were obtained from the seventh rib of the LM muscle. They detected no difference by supplementation type for total fatty acid content in the muscle, at 2,571 mg/100 g of muscle. An increase in the concentration of PUFA's were observed for both SO at 34.04

g/ 100 g of fatty acids and LO at 32.16 g/ 100 g of fatty acids which were not statistically different between treatments but both were statistically different from the control at 30.52 g/ 100 g of total fatty acids ($P \leq 0.001$). An increase of CLA c9, t11 was observed for SO treatments at 0.71 g/ 100 g of total fatty acids compared to LO at 0.51 g/ 100 g of total fatty acids, and 0.32 g of total fatty acids for the control ($P \leq 0.001$). Concentrations of n-3 PUFA were greatest for the Control and LO treatment at 12.12 and 11.38 g/ 100 g total fatty acid respectively, statistically different from the SO treatment at 8.99 g/ 100 g total fatty acids ($P \leq 0.001$).

To prevent negative associative effects, supplementing fat to increase dietary energy should not exceed 4% of DMI to avoid hindering performance. Fat supplementation should not exceed 2% of DMI if decreased forage intake is not desired (Hess et al., 2008). Several theories have been proposed to try to explain the effect of fat on fiber fermentation in the rumen. Two of the more popular theories include where lipids attach to fiber and prevent microbial attachment to occur, and another is the cytotoxic effect of fatty acids especially unsaturated fatty acids where the FA interfere with bacterial cell membrane function (Jenkins, 2003). Both theories suggest that fiber fermenting bacteria in the rumen are affected by the increased fat in the diet and can be seen in a shift in the A: P ratio.

Grazing and grass silage studies show effects of increased ALA and PUFA concentrations and total omega-3 fatty acids, compared to diets with greater inclusion of grains (Nuernberg et al, 2005; Nuernberg et al, 2008, Warren et al., 2008, Realini et al, 2004). Steers finished on concentrate diets having greater weight, conformation, degree

of finishing, fat depth and ribeye area compared to cattle pasture fed cattle (Realini et al, 2004). This is consistent with Nuernberg et al. (2005) reporting a decrease in percent of total intramuscular fat in grass fed diets compared to concentrate based diets fed to German Simmental and German Holstein bulls.However, Warren et al. (2005) reported an increase of fatness on Aberdeen Angus × Holstein-Friesian and Holstein-Friesian steers harvested at 6, 14, 19 or 24 months of age fed grass silage compared to concentrate. Warren et al. (2005) is unique because in the attempt to feed concentrate diets and grass silage to a similar finishing point, and to do so they limited intake on the concentrate diet. The concentrations of fatty acids as well as the amount of total fat deposited in the meat must both be considered when considering health benefits from altering fatty acid content of meat. Grass fed beef generally has higher concentrations of desired fatty acids however lower amounts of total fat compared to conventional grain fed finished cattle.

Fat in finishing diets

Feeding supplemental fats in the form of tallow in finishing diets to increase the dietary energy content of the diet, is a common practice in the cattle feeding industry. Increasing supplemental fats up to 8% of the diet can improve weight gain, feed efficiency, and marbling score (Zinn, 1989). Feeding tallow at 4% of the diet, in corn based diets to steers and large-frame calves has been reported to improve efficiency, with greater improvements with large-framed calves (Krehbiel et al., 1995). However, differences in performance are reported on the type of fat supplementation. Vander Pol et al. (2009) reported depressed G:F when supplementing 5% corn oil in finishing steer diets; however, cattle fed up to 40% WDGS in that study had improved performance.

Many finishing diets today contain by-products of the ethanol industry. Distillers grains were first fed as a protein source, however, due the low price of distillers compared to corn it has typically been also fed to provide energy. In the process of making ethanol most of the starch is removed leaving a by-product that contains roughly three times the amount of fat, fiber, and protein compared to corn (Larson et al., 1993). Feeding high concentrate diets decreases biohydrogenation of linoleic and linolenic fatty acids (Loor et al., 2004). The amount of FA that escape biohydrogenation can greatly depend on growth conditions for the microbial population in the rumen, which can in turn affect lipolysis and then biohydrogenation (Jenkins, 2003). Fat contained in WDGS is useful in the ruminant diet because it is hypothesized that not all the unsaturated fatty acids go through complete biohydrogenation in the rumen allowing for absorption of unsaturated fats and CLA in the small intestine (Vander Pol et al., 2009) and the animal depositing these healthy fats in meat and muscle. Increasing the concentration of fat in a diet up to the break point where it inhibits fiber fermentation will increase the energy density of the diet, improving performance in finishing diets.

Larson et al. (1993) demonstrated in a 121-day yearling finishing trial (replicated over 2 years and pooled) that when wet distiller's grains plus thin stillage inclusion was increased in the diet, cattle became more efficient. The diets contained 0, 5.2, 12.6 and 40% inclusion of wet distiller's byproduct (WDB) as a percent of diet DM. Fat intake, measured as nutrient intake in kg/d, also increased as WDB inclusion increased, 0.41 kg/d, 0.48 kg/d, 0.55 kg/d, and 0.71 kg/d, respectively. Average dry matter intake deceased linearly ($P < 0.01$) as WDB inclusion increased. There was no significant change in ADG ($P = 0.07$) due to treatment, but numerical changes in ADG of 1.65 kg/d,

1.71 kg/d, 1.76 kg/d, 1.76 kg/d for the 0, 5.2, 12.6 and 40% inclusion of WDB, respectively. This resulted in gain:feed (G:F) increasing linearly with dietary inclusion of WDB (0.144, 0.152, 0.160, 0.188 for the 0, 5.2, 12.6, and 40% inclusions of WDB, respectively; $P < 0.01$). Larson et al. (1993) was then able to duplicate the trends shown in the 121-day yearling trial, on a 186-day calf-fed trial (replicated over 2 years and pooled). Improvements in performance have been reported at up to 50% inclusion of WDGS resulting in a feeding value range of 178 to 121% the value of corn (Watson et al., 2014). Similarly, improvements in performance were reported for MDGS at inclusion up to 50% of the diet resulting in a feeding value range for MDGS at 125 to 111% the value of corn (Watson et al., 2014)

Because protein, fat, and fiber concentrations are all increased in distiller's by-products, the results of Larson et al. (1993) and Watson et al. (2014) could also be contributed to increased dietary protein. Mammals are able to utilize protein as energy after deamination--the removal of the amino group of the amino acid. The remaining carbon skeleton is converted into energy through the TCA cycle. Ruminants are also able to recycle nitrogen from deaminated amino groups of amino acids and allow microbes to use the N again to form more bacterial crude protein (Marini et al., 2004). Lodge et al. (1997) fed diets based on wet corn gluten feed (WCGF) with added fat (tallow) and protein to obtain similar CP and energy to the distillers by-products plus solubles like those in the study by Larson et al. (1993). The results suggest that the fats and protein both play a role in the increased efficiency. An important outcome of Lodge et al. (1997) shows that inclusion level of lipids up to 6% of diet DM did not result in negative performance, and although fiber digestion was not measured the authors suggest that

fiber digestion was not inhibited, and did have the added benefit of energy density of fats which may have enhanced performance. Conroy et al. (2016) observed that feeding corn bran at 16.4% of diet DM with 20% corn gluten meal and 3.6% corn germ in growing diets steer diets containing 50% grass hay resulted in similar performance as MDGS at 40% inclusion. Both diets outperformed the control with no fat supplementation, suggesting the dietary lipid did not compromise fiber digestion.

Vander Pol et al. (2009) designed studies to determine if corn oil or tallow could mimic the effects of the fat within wet distiller's grains plus solubles (WDGS) when diets were formulated to be isocaloric. The experiments showed that cattle did not perform differently ($P > 0.10$) with the fats from WDGS. However, cattle on the WDGS diets did have different fatty acids absorbed from the small intestine. Increased absorption of CLAs in the small intestine was observed in WDGS diets compared to corn oil diets. More saturated fatty acids were found to be absorbed by the small intestine in corn oil diets and less in WDGS diets. Specifically, for C18:3 there was not a significant difference between WDGS (0.29 g/100g of fatty acid) and the control diet (0.23 g/100 g of fatty acid) in dudodenal fat content, but a decrease was observed for corn oil diets at 0.19 g/100g of fatty acids. This suggests that less extent of biohydrogenation occurs in the rumen for fats associated with WDGS compared to corn oil, or that fats in WDGS contain more unsaturated fats in general and more CLAs pass the rumen.

Koger et al. (2010), used two hundred forty Angus crossbred steers fed differing quantities of wet and dry distillers in a finishing diet containing dry rolled corn, soybean meal, and alfalfa hay. The dry rolled corn, soybean meal and alfalfa hay was used as the control diet. Treatment diets were 20% DDGS, 40% DDGS, 20% WDGS and 40%

WDGS on DM basis, displacing 10.5% soybean meal and dry rolled corn in the control diet. They reported no difference in HCW, LM area, KPH or marbling score. USDA yield grade increased from 2.94 for control to 3.25, 3.27, 3.24 and 3.16 for 20% DDGS, 20% WDGS, 40% DDGS and 40% WDGS respectively ($P \geq 0.17$). Concentrations of C18:0 and C18:1*t*, and C18:2 cis 9, cis 12, as well as total PUFAs found in LM were increased in diets containing DGS. They reported no change in the CLA cis-9, trans-11 isomer of CLA. They also reported no differences detected in color evaluation in ground beef patties during retail display ($P > 0.10$), however they did report an increase in TBARS, a measurement of lipid oxidation, with ground beef from steers fed 40% DGS ($P < 0.05$) compared to ground beef of steers fed the control diet. Interestingly Koger et al. (2010), stated that even with greater proportion of PUFA which would be more susceptible to oxidative rancidity, there was little to no effect on meat quality, retail display life of ground beef or fatty acid profile of LM.

Sudbeck et al. (2014) utilized 229 crossbred heifers in a 2×2×2 factorial, where treatments were low or high winter supplementation of 0.91(LW) or 2.30 kg (HW; DM basis) of modified distillers grains (DGS) while grazing corn stalk residue. Summer supplementation of 0.60% of heifer BW of dried DGS (DM basis) or no supplementation while grazing summer range, and finishing diets fed 40% wet corn gluten feed (Sweetbran; CGF) or 40% MDGS with the remainder of the diet consisting of 50% high moisture corn, 5% wheat straw and 5% supplement. Contradicting Koger et al. (2010), Sudbeck et al. (2014) observed a 15% increase of discoloration in the HW group compared to the LW group ($P < 0.01$) for the main effect of winter backgrounding. They observed greater numerical discoloration averages ($P < 0.09$) for steak samples collected

from WDGS finishing diets compared to CGF finishing diets. Similarly to Koger et al. (2010), Concentrations of C18:2, PUFA were higher ($P < 0.01$) in DGS finishing diets compared to CGF diets. Both studies would support the hypothesis that less extent of biohydrogenation occurs with fats associated with distillers grains and more unsaturated fatty acids reach the small intestine for absorption.

Lipid oxidation resulting in rancid flavors, and decreased shelf life is associated with high PUFA's found in meat from cattle fed high inclusions of DGS (Rober et al., 2005, Gill et al., 2008). The degree of lipid oxidation depends on several factors, one being the amount of polyunsaturated fatty acids (Calkins and Hodgen, 2007). Feeding supplemental vitamin E to feedlot cattle beef has been shown to increase tissue alpha-tocopherol, slowing metmyoglobin formation, resulting in less oxidation, and increasing shelf life and product display by 24 to 48 h (Smith et al., 1996). Field studies documented that supplementing diets of feedlot cattle with 500–1000 IU per head per day of vitamin E for 90–100 days prior to harvest was efficacious for beef marketed in both domestic and export trades (Smith et al., 1996).

Algae as feed in cattle diets

Large scale production of heterotrophic microalgae for production of oils, both in the biofuel and food industry is currently underway. Similar to the ethanol industry, byproducts from the algae industry have a great opportunity to be used in animal feeds. With advancement in technologies to extract the lipid from algae more efficiently, it is becoming more common. Ruminant animals have a unique ability to convert what would be waste in these industries to a useful feedstuff. As a novel feed, many variations of algal byproducts are becoming available but differ in nutrient composition.

In a 55 d growing trial, microalgae meal (ALG) was used in combination with soyhulls as an ingredient in growing steers diets (Van Emon et al., 2015). The ALG contained 57% microalgae and 43% soyhulls on a DM basis. ALG displaced WCGF in the grower diets at 15%, 30% and 45% of the diet DM. The remainder of the diet contained 15% brome grass hay, and 2.63% supplement with distillers as a carrier for the supplement. Van Emon et al. (2015), reported no difference in final BW or ADG from steers fed the control diet compared to the ALG diets. They observed a linear increase in DMI as ALG inclusion in the diet increased, resulting in a linear decrease in G:F. This decrease in efficiency agrees with Stokes et al. (2015), who reported a linear decrease in DM and OM digestibility as ALG increased from 0% to 30% inclusion, in lamb diets. Van Emon et al. (2015) reported that steers readily consumed the algae meal and Stokes et al. (2016), fed the same algae meal (ALG) as Van Emon et al. (2015), in a finishing trial. The finishing diets were corn based and treatments included 14% ALG, 28% ALG, or 42% ALG displacing dry rolled corn on a DM basis. Stokes et al. (2016) reported no difference in final BW or HCW. Similar to the growing diet, Stokes et al. (2016) observed cattle that were fed ALG had increased DMI and a linear decrease in G:F. Feeding ALG had no effect on LM area or quality grade. Algae contain unique fatty acid profiles compared to other plant derived feedstuffs. Interestingly, Stokes et al. (2016) reported no change in SFA, MUFA, PUFA, PUFA:SFA ratio, n-6:n-3 ratio or % lipid, and a tendency for a linear decrease in n-3 fatty acids as ALG increased inclusion in the diet. They concluded that ALG can substitute for corn in feedlot diets with minimal effect on ADG and carcass traits.

Post-extraction algal residue (PEAR) a byproduct of the biofuels industry was fed to cattle in finishing diets (Morrill et al., 2017). The nutrient composition of PEAR is approximately 12% ash, 33.8% CP. It is primarily fed as a protein substitute and to capture value from by-products of the biofuel industry, similar to the idea of distillers grains from the ethanol industry. Post-extraction algal residue was fed in a diet containing 42.3% DRC, 18% ground milo, 13.5% cottonseed hulls 10% grass hay, 6.7% molasses, 5.4% cottonseed meal, and the remainder vitamin and supplements. The PEAR was hand mixed into the diets at 1 kg of OM/d. They observed an increase in DMI, and a decrease in digestion of NDF in PEAR diets. They reported no difference in USDA yield or hot carcass weight between treatments. Steers fed PEAR had higher concentrations 14:0, 14:1n-5, 16:0, 16:1n-7,18:1, which suggest that much of the FA in PEAR went through biohydrogenation in the rumen (Morrill et al. 2017b).

Literature Review Summary

The importance of fats in beef cattle diets are often over-looked due to their low inclusion. Fats are generally added to diets to increase the energy of the diet. It has been demonstrated that adding fats up to 6% of the diet in finishing cattle can improve efficiency (Vander Pol et al., 2009; Larson et al.,1997), without negative effects on carcass performance due to reducing fiber digestion. In grazing or high forage diets, supplementing fat to increase dietary energy should not exceed 4% of DMI to avoid hindering fiber digestion and should not exceed 2% of DMI if the goal is to not substitute forage intake with supplemental fat intake (Hess et al., 2008).

Biohydrogenation in the rumen impacts the fatty acid profiles of meat and milk. A better understanding of this process in the future will be useful in the attempt increase

CLA's found in these food products. Cattle on high forage diets have more PUFAs reach the small intestine allowing more unsaturated fatty acids, CLAs and omega-3 fatty acids to be absorbed. Dietary fat sources that differ in high forage diets compared to common concentrate diets give different amounts of saturated or unsaturated fatty acids available for absorption in the small intestine.

Recommendations for consumption and reporting of values of concentrations of PUFA in human foods is all over the board. The *Dietary Guidelines for Americans 2005* report states, "Evidence suggests consuming approximately two servings of fish per week (approximately 227 g; 8 ounces total) may reduce the risk of mortality from coronary heart disease and that consuming EPA and DHA may reduce the risk of mortality from cardiovascular disease in people who have already experienced a cardiac event" (USDA, 2005). Multiple benefits come with consuming EPA, DHA, DPA and ALA with cardioprotective effects of n-3 fatty acids (Djousse et al., 2001) and benefits with attention deficit hyperactivity disorder, neuroprotection and autoimmune diseases (Stark et al., 2008). Human sources of these PUFAs in different human foods vary dramatically in concentrations. In pork samples of longissimus muscle ALA concentration can vary from 0.09 mg/g of fresh sample in a "normal" diet compared to 0.32 mg/g in pigs fed a PUFA enhanced diet (Morel et al., 2006). Walnuts have especially high ALA concentrations, with 11.58 % of the total fatty acid comprised of ALA and an oil content of 50.8 % (Maguire et al., 2004). This equates to 5.88 g ALA/100g of walnuts, or 58.8 mg/g. Further research is needed as most of the recommendations are based on epidemiology. With that said, cardioprotective benefits have been associated with intakes of ALA as low as 0.58 g/d, and potentially greater benefits are achievable with intakes up

to 2.81 g ALA/d. (Gebauer et al., 2006). Benefits are clearly present with increased ALA, however further research and summarization of research is greatly needed in this field.

Future research should also focus on ways to increase the amount of CLA reaching the small intestine of cattle. The increased absorption of unsaturated fatty acids in the small intestine should lead to an increase in the amount of omega-3 and CLAs deposited in milk and meat. Unsaturated fats tend to oxidize faster, decreasing shelf life of meat. Whether or not to include more Vitamin E in the diet and how it affects the rate of oxidation in these healthier red meat products is also important. Using newly developed feeds and feed co-products such as condensed algal residue solubles (CARS) and Green Grass may provide sources of omega 3 unsaturated fatty acids that can provide healthier fat profiles in beef. Thus, the objective of this thesis is to evaluate Green Grass as an ingredient in a beef finishing ration and to determine its effect on fatty acid profile in the meat. Another objective is to determine the nutrient digestibility of CARS, and finally to evaluate the safety and efficacy of CARS in a beef cattle diet.

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CHAPTER II. EVALUATION OF GREEN GRASS AS A FEED INGREDIENT IN BEEF FINISHING RATIONS AND IMPACT ON CATTLE PERFORMANCE, CARCASS CHARACTERISTICS, AND FATTY ACID PROFILES IN MEAT

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ABSTRACT

A finishing study utilizing 240 crossbred steers (initial BW=340 ± 24 kg) evaluated the performance, carcass characteristics and fatty acid profiles of finishing steers fed four inclusions (0, 10, 20, 30% DM basis) of Green Grass, displacing dry rolled corn in the finishing diet. Steers were blocked by initial BW into light, medium and heavy BW, stratified by day 0 BW, and assigned randomly to pen. Due to an uneven distribution of initial BW and to maintain less than a 40 kg range in body weight by bock, replication 1 (40 hd) was assigned to block 1, replications 2, 3, and 4 (120 hd) were assigned to block 2, and replications 5 and 6 (80 hd) were assigned to block 3. Pens were assigned randomly to 1 of 4 treatments with 10 steers/pen and 6 pens/treatment. Steers were harvested over 3 days (d 190, d 199, d 203). There were no differences in initial BW, final BW, BW gain, HCW, LM area, 12^{th} rib fat depth, calculated YG, or liver abscess % ($P \geq 0.09$). Dry matter intake linearly increased ($P = 0.04$) as Green Grass inclusion increased in the diet. Steers fed Green Grass had lower G:F ($P = 0.01$) than control cattle, and steers fed 30% Green Grass had a lower marbling score ($P = 0.05$). Steak samples were collected from the 6th rib and analyzed for fatty acid profile. A linear increase in alpha linolenic acid ($P < 0.01$) was observed in steak samples, resulting in 4-fold increase when comparing steers fed 30% Green Grass to control cattle. Linear increases of trans-unsaturated fatty acids, and omega-3 fatty acids ($P \leq 0.01$) were also observed in steak samples from steers fed increasing inclusion of Green Grass. Including up to 20% inclusion of Green Grass on a DM basis in finishing steer diets decreased efficiency but had little other effect on performance or carcass characteristics. Increasing Green Grass

inclusion in the diet from 0 to 30% linearly improved omega-3 fatty acid concentration in beef.

Keywords: alpha linolenic acid, cattle, fatty acid, finishing, omega 3

INTRODUCTION

With human health studies showing benefits from consuming omega-3 fatty acids and conjugated linoleic acid (CLAs; Belury, 1995), there is interest in increasing the amount of omega-3 fatty acids in beef, which typically have small amounts of polyunsaturated fatty acids (PUFAs) and omega-3 fatty acids (Jenkins et al., 2008; Scollan et al., 2001). Ribeye steaks from steers fed a typical Midwest corn based diet with distillers grains had 5.24% polyunsaturated fatty acid (PUFA), and 0.20% alpha linolenic acid (ALA; de Mello et al., 2018). In comparison, walnuts contain 69% PUFA, and 11.58 % of total fatty acid composition is comprised of ALA (Maguire et al., 2004). Through a process called biohydrogenation, ruminant microbes convert dietary unsaturated fatty acids into mono-unsaturated fatty acids, or completely saturated fatty acids (Banks and Hilditch., 1931). Therefore, even in diets containing PUFAs, many of these fatty acids are converted to more saturated fatty acids or intermediates thereof. Alpha-Linolenic acid is the most abundant fatty acid by concentration found in forages across most species, comprising around 50% of the total fatty acids found in forages (Boufaïed et al., 2003). A Korean feed product called Green Grass (GG, Sunseo Omega 3; Chungcheong Duk-Do, South Korea), a feed comprised of sesame meal, giant kelp, cassava, and sorghum has a high concentration of PUFA, specifically ALA. There has been interest in using this product in livestock diets, primarily to alter the fatty acid profile of the animal products (meat, milk, eggs). Research was conducted to determine if feeding Green Grass, which is high in omega-3 fatty acids, would alter the fatty acid profile of beef or impact finishing cattle performance or carcass characteristics.

MATERIALS AND METHODS

The following experiment was conducted at the Panhandle Research and Extension Center (PHREC; Scottsbluff, NE), and the University of Nebraska Animal Science Complex (Lincoln, NE). Animal handling and space for the experiment were in accordance to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). All Procedures outlined as part of this study were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee approval # 1420. Because Green Grass is not currently approved by the FDA to be fed to cattle entering the human food chain in the U.S, documentation was acquired from the USDA for custom slaughter and to export the beef to South Korea.

Experimental Design

A 203-d finishing trial was conducted using 240 crossbred steers (initial BW = 340 ± 23.6 kg). At receiving, 12 days before the initiation of the trial, steers were penned in groups of 10 and fed a common receiving diet of 45% corn silage, 35% alfalfa hay, 15% WDGS, and 5% supplement on a DM basis. On d -10, steers were given electronic and panel tag ID ear tags to be individually identified and vaccinated for the prevention of bovine viral diarrhea virus Type I and II, infectious bovine rhinotracheitis, parainfluenza, bovine respiratory syncytial virus, *Mannhemia haemolytica*, and *Pasteurella multocia* (Bovi-Shield Gold 5, Zoetis Inc., Kalamazoo, MI). Additionally, steers were orally drenched for parasite control (Safe-Guard, Merck Animal Health, Desoto, KS). Steers were limit fed the common diet at 2% of BW for 5 days and weighed for 2 consecutive days at the beginning of the trial to minimize variation in gut fill and establish initial BW (Watson et al., 2013), with the average of those 2 days used to

establish initial BW (Stock et al., 1983). Steers were blocked by initial BW into light, medium and heavy BW, stratified by day 0 BW, and assigned randomly to pen. Due to an uneven distribution of initial BW, replication 1 (40 hd) was assigned to block 1, replications 2, 3, and 4 (120 hd) were assigned to block 2, and replications 5 and 6 (80 hd) were assigned to block 3, given the target range across blocks. Pens were assigned randomly to 1 of 4 treatments with 10 steers/pen and 6 pens/treatment.

Treatments consisted of Green Grass product fed at 0, 10, 20, or 30% of diet DM, displacing dry-rolled corn (DRC) in the diet (Table 2.1). The remaining diet consisted of 15% wet distillers grains plus solubles (WDGS), 20% corn silage, and 6% liquid supplement on a DM basis. Two supplements were used. Supplement in the control diet supplied extra rumen degradable protein in the form of urea. Supplements were formulated to provide 33 mg / kg monensin (Rumensin, Elanco Animal Health, Greenfield, IN) and 9.7 mg/ kg tylosin (Tylan, Elanco Animal Health) on a DM basis. Cattle were stepped-up to their assigned diets over the course of 24 d starting on d 1 with 5 steps. As step-up diets progressed, alfalfa hay and corn silage were displaced by the ratio of dry rolled corn and Green Grass product in each of the treatment diets. Each step did not exceed over a 10% DM displacement of roughage by concentrate. Cattle were fed *ad libitum* and feed bunks were evaluated daily at approximately 0530 h for feed refusals, so that trace amounts of feed were left in the bunk at the time of feeding. Feed was delivered once daily starting at 0800 h with a truck mounted mixer and delivery unit (Roto-Mix, Dodge City, KS). For all feed refusals, bunks were scooped, weighed, and subsampled, then dried in a 60 ºC forced air oven for 48 hours to calculate a dry matter

intake (DMI) per pen (AOAC,1999, method 4.1.03). Green Grass product was sampled weekly and composited into lot samples (3 lots over the feeding period). Ingredient samples (dry rolled corn, WDGS, corn silage) were collected weekly and composited into monthly samples and subsequently analyzed for DM, crude protein (CP, AOAC, 2006, method 930.03), total fatty acids (TFA; O'Fallon et al., 2007), acid detergent fiber (ADF; ANKOM Technology Method 14), macro- and micro- minerals, and fatty acid profiles (Dairy One Co-Op Inc., Ithaca, NY). Nutrient composition, and fatty acid profiles of the diet were then calculated by prorating days fed of each sample to get the weighted average.

On day 35, cattle were implanted with 200 mg trenbolone acetate and 20 mg estradiol (Revalor 200, Merck Animal Health), and revaccinated to aid in the prevention of bovine viral diarrhea virus Type I and II, infectious bovine rhinotracheitis, parainfluenza$_3$, bovine respiratory syncytial virus, *Mannhemia haemolytica*, and *Pasteurella multocia* (Express 5, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO), and poured for parasite control (StandGuard, Elanco Animal Health). Cattle were harvested at a commercial packing plant (J F O'Neil Packing Co., Omaha, NE) over 3 harvest days (day 190, 199, 203). Due to the uneven distribution of initial body weight, replications 1 and 2 (80 hd) were harvested on d 190, replications 3 and 4 on d 199, and replications 5 and 6 on d 203. Hot carcass weight (HCW) and liver abscess occurrence were recorded on day of harvest. Ribeye area, marbling score, and 12th rib back fat were recorded after a 48-h chill. Final BW, average daily gain (ADG), and feed efficiency (G:F) were calculated from HCW corrected to a common 63% dressing percentage. Yield

grade (YG) was calculated using the USDA YG equation, (USDA, 1997) where YG = 2.5 + (0.98425 x 12^{th} rib fat, cm) + (0.2 x 2.5 [KPH]) + 0.0038 x HCW, kg) – (0.32 x LM area, cm^2). Steak samples (239 steak samples) were collected from all but one animal, which was missed on the production line, by cutting an approximate 3.8 cm wide steak from the 6th rib. Steak samples were transported on ice to the University of Nebraska meat lab for fatty acid analysis. Upon arrival at the Loeffel Meat Laboratory, the 2 d aged beef steaks, cut at the 6th rib, were trimmed of all muscles except the *longissimus thoracis* muscle. Steaks were vacuum packaged (MULTIVAC 500, Multivac, Inc., Kansas City, MO) in Prime Source Vacuum pouches (3 mil STD barrier, Prime Sources, St. Louis, MO) and kept frozen (-80 °C) until ready to powder for future analyses. All steaks subsequently used for laboratory analyses were diced into small portions before freezing in liquid nitrogen and powdering in a metal blender cup (Model 51BL32, Waring Commercial, Torrington, CT) from February 4 to 8, 2019 and kept frozen (-80°C) for following analyses.

Extraction of the total lipids was completed with the chloroform-methanol procedure described by Folch et al. (1957). Following the extraction process, lipids were reduced to fatty acid methylated esters according to Morrison and Smith (1964), and Metcalfe et al. (1966). One gram of powdered sample was homogenized with 5 mL of 2:1 chloroform: methanol and kept at room temperature (23°C) for 1 hr. Afterwards, the homogenate was filtered through Whatman #2 paper into a screw cap tube and brought to a final volume of 10 mL with the 2:1 chloroform: methanol solution. Samples were vortexed for 5 s with 2 mL 0.74% KCl solution and centrifuged for 5 minutes at 1,000 x

g, and the top layer was aspirated off. After aspirating, the samples were placed in a heating block at 60°C and continually flushed with nitrogen until dried. Once dry, 1 mL of 0.5 NaOH in methanol was added, vortexed for 5 s, and heated for 10 min at 100°C. Subsequently, one mL of 14% boron trifluoride in methanol was added, vortexed for 5 s, and the samples were heated again at 100°C for 5 min. Following the heating period, 2 mL of saturate salt solution and 2 mL of hexane were added to the samples and vortexed (5 s). Samples were then centrifuged at 1,000 x g for 5 min and the hexane layer (top layer) was removed to be analyzed using gas chromatography (TRACE 1310 Gas Chromatography; ThermoFisher Scientific, Waltham, MA). Separation of the fatty acids was done using a capillary column (Chrompack CP-Sil 88- 0.25 mm x 100 m; Inlet temp: 260°C, Oven: 140°C hold for 5 min, increase at 4°C/min to 240°C and hold for 15 min. FID temp: 280°C. Injected at 30:1 ratio) and identified based on their retention times compared to known commercial standards (NU- Check Prep, Inc., Elysiam, MN; # GLC-68D, GLC-79, GLC-87, GLC-455, and GLC-458). Determination of fatty acids percentage was done with the peak areas in the chromatograph and values were converted to mg/100 g tissue:

Fatty acid mg/100 g tissue = (% of fatty acid peak area * fat content of samples) * 1000

Fat, moisture, and ash (%) were determined from powdered-raw meat samples. Moisture and ash (%) were measured in duplicate using a Thermogravimetric Analyzer (Model 604- 100-400, LECO Corporation, St. Joseph, MI). Total fat (%) was done in triplicate through the Soxhlet procedure of ether extraction after taking the difference of

the already quantified moisture percentage (AOAC, 1990; Appendix VI) with protein calculated by overall difference.

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) as a randomized block design. Pen was used as the experimental unit while treatment and kill block nested within BW block were included in the model as fixed effects. Orthogonal contrasts were used to test significance for linear, quadratic and cubic responses due to Green grass inclusion. Treatment differences were considered significant when $P \leq 0.05$. A tendency was declared when $P > 0.05$ and $P \leq 0.10$.

Over the course of the feeding period, 4 steers were removed due to death, health or lameness issues including 2 steers on 20 Green Grass and 2 steers on 30 Green Grass. These animals were removed from the statistical analysis by removal from those pen averages. Logistical difficulties resulted in a shortage of Green Grass product to feed at the end of the feeding period. On d 150 to 176, Green Grass 10, 20, and 30 diets were dropped to 7.5%, 15%, and 22.5% Green Grass inclusion, respectively. On d 177 to 187, Green Grass 10, 20, and 30% diets were dropped to 5%, 7.5%, and 15% Green Grass inclusion, respectively. On d 188 through the remainder or the trial, Green Grass 10 and 20 were switched to the control diet, while Green Grass 30 was dropped to 7.5% Green Grass inclusion. On day 189 through the remainder of the trial, Green Grass 30% was switched to the control diet.

RESULTS AND DISCUSSION

Performance and Carcass Characteristics

There were no differences in initial BW, final BW, HCW, ADG, calculated YG, % liver abscesses, or LM area ($P \geq 0.11$) across all treatments (Table 2.2). Our results

would agree with Scollan et al. (2001), who reported no significant differences in ADG or cold carcass weight for steers fed a barley and sugar beet pulp diet with differing sources of lipid. A linear increase (P = 0.04) in DMI was observed for steers fed increasing inclusions of Green Grass. Steers fed Green Grass in this experiment consumed on average 0.3 to 0.4 kg/d more than 0% Green Grass. A cubic response was observed (P = 0.02), but was generally quadratic (P = 0.07) for G:F as Green Grass inclusion increased. As inclusion of Green Grass increased from 0 to 30% of the diet, G:F decreased from 0.147 to 0.137. Steers fed Green Grass had similar efficiencies of 0.139, 0.142, and 0.137 for 10, 20, and 30% Green Grass, respectively (P = 0.07). Steers fed 30% Green Grass had a lower marbling score of 430 (small 30) compared with steers fed 0, 10, or 20% Green Grass which had marbling scores averaging 470 (small 70; Quadratic P = 0.03). Steers fed Green Grass had greater intakes and equivalent ADG resulting in poorer feed efficiency. This resulted in a calculated NEm of 1.83 , 1.76, 1.77, and 1.77 Mcal/kg from 0 % Green Grass to 30 % Green Grass respectively. The calculated NEg of the diets were 1.19, 1.13, 1.14 and 1.14 Mcal/kg from 0% Green Grass to 30% Green Grass. The cattle's performance results indicate Green Grass has a similar, but lower energy value relative to corn, with the treatment diets resulting in feeding values of 99.48 % (10 Green Grass), 99.79 % (20 Green Grass), 99.86 % (30 Green Grass) compared to the control diet . Cattle were able to increase DMI with Green Grass diets and eat to a similar energy end point, resulting in no effect on ADG and HCW. Interestingly, G:F decreased compared to the control, but was relatively constant for 10, 20, or 30% inclusion. It is unclear whether altering the Green Grass inclusions from day

150 to 203 impacted performance, but some impacts were expected for Green Grass replacing energy dense corn during the finishing period.

Fatty Acid Profile Analysis

As inclusion of Green Grass increased in the diet, a linear decrease ($P \leq 0.02$) was observed for C12:0, C14: 1, C15:0, C16:1, C17:0, C17:1, C18:1, C20:3ω6, and total ω6 (omega-6) in mg/100 g of lean tissue (Table 2.3). A linear increase ($P \leq 0.01$) was observed for concentrations of C18:1T, C18:2T, C18:2, C18ω3, C20:5ω3, and C22:5 in mg/100 g of lean tissue as Green Grass product inclusion in the diet increased. This agrees with Scollan et al. (2001) who reported an increase of C18:3ω3 and C18:1T in cattle fed diets supplemented with whole linseed as well as linseed + fish oil. Nuernberg et al. (2005) and Warren et al. (2008) also reported similar results with cattle fed grass silage and grass-fed diets compared to concentrate diets. The decrease in many of the saturated fatty acids (SFA's) and increase in conjugated linoleic acid (CLA) and ω3 may be due to fatty acid content of the diet but also suggest that less biohydrogenation occurred in the rumen as Green Grass inclusion increased. A quadratic effect ($P = 0.06$) was observed for mono-unsaturated fatty acid (MUFA) concentrations with an increase as Green Grass increased in the diet from 0 to 20% inclusion, then a decrease with 30 Green Grass. The concentration of C18:3ω3 and total ω3 (omega-3) fatty acids linearly increased ($P \leq 0.01$) in mg/100 g of lean tissue, as Green Grass inclusion increased in the diet. Comparing 30 Green Grass to control there was a 3-fold increase for C18:3ω3 and for total ω3 (omega-3) fatty acids. Poly-unsaturated fatty acids (PUFA), and trans-unsaturated fatty acid (Trans) concentrations also linearly increased ($P \leq 0.01$) in mg/100

g of lean tissue as Green Grass inclusion increased in the diet. Concentrations of total ω6, and the ratio of ω6:ω3 linearly decreased ($P \leq 0.01$) as Green Grass inclusion increased in the diet which agrees with Scollan et al. (2001). de Mello et al., (2018) observed increased C18:3ω3, PUFA, n- 6, n-3 and n-6: n-3 when feeding increased inclusions of WDGS finishing diets. Wet DGS is a different product than Green Grass and is made up of differing fatty acid concentrations but this illustrates that altering fatty acid profiles of the diet will change fatty acid concentrations deposited in the meat. Increased PUFA deposited in the meat of animals fed distillers grains has increased oxidative rancidity, decreasing shelf life, color stability, and producing off flavors. A follow up study should be done to determine the effects of oxidative rancidity from steers fed Green Grass, and if feeding vitamin E can provide protection from unsaturated fatty acid oxidation (Burken et al., 2012).

A quadratic response ($P = 0.04$) was observed for total fat % from the proximate analysis, with 10 and 20 Green Grass having greater % fat within lean steak samples (11.41% and 11.51 %) compared to 0 and 30 Green Grass (10.96% and 10.43%; Table 2.4.). The percent of moisture in steak samples from the proximate analysis also had a quadratic response ($P = 0.02$), with 0 and 30 Green Grass having greater moisture (68.13% and 68.75%), compared to 10 and 20 Green Grass (67.76% and 67.71%).The increase in concentration of PUFA, total ω3, and C18:3ω3 support the hypothesis that increasing the amount of dietary omega-3 fatty acids from feeding Green Grass positively influences fatty acids deposited in the meat, with dramatic increases in ω3 (omega-3) fatty acids.

IMPLICATIONS

Steers fed Green Grass had greater intakes and equivalent ADG compared to control cattle resulting in a 5% decrease in feed efficiency. Other cattle performance parameters and carcass characteristics were not affected as Green Grass inclusion in the diet increased up to 30% of diet DM. Steers fed 30% Green Grass had lower marbling scores; however, they had greater concentrations of PUFA, total ω3, and C18:3ω3. Displacing corn with up to 30% of diet DM with Green Grass product did not affect gain, and improved the PUFA, total ω3, and C18:3ω3 concentrations in steak samples. Further research is needed to determine the energy content and digestibility of Green Grass, and the significance of the change in ω3 fatty acid concentrations in the steaks on human health.

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Table 2.1 Diet Composition (DM basis) for finishing steers fed 4 inclusions of Green Grass product

	Treatment[1] % Inclusion			
Ingredient	0	10	20	30
Dry-rolled corn	59	49	39	29
Wet Distillers Grains plus Solubles	15	15	15	15
Green Grass[1]	0	10	20	30
Corn Silage	20	20	20	20
Supplement[2]	6	6	6	6
CP, %	46.0	7.0	7.0	7.0
Ca, %	5.7	5.2	5.2	5.2
P, %	0.05	0.09	0.09	0.09
Salt, %	3.1	3.1	3.1	3.1
K, %	2.6	3.2	3.2	3.2
Vitamin A, IU	10,820	10,820	10,820	10,820
Dietary Nutrient Composition[3], %				
DM	54.3	54.3	54.3	54.3
CP, % DM	14.0	14.0	16.3	18.7
Acid detergent fiber, % DM	10.3	12.5	14.7	16.9
Ca, % DM	0.40	0.47	0.56	0.65
P, % DM	0.45	0.51	0.58	0.64
Mg, % DM	0.14	0.17	0.20	0.23
K, % DM	0.81	0.91	0.96	1.02
Na, % DM	0.03	0.04	0.06	0.07
S, % DM	0.17	0.21	0.26	0.30
Fe, PPM	65.8	157.2	248.5	339.9
Zinc, PPM	26.8	32.0	37.8	43.6
Cu, PPM	2.9	6.1	9.2	12.4
Manganese, PPM	15.2	22.6	30.1	37.5
Dietary Fatty Acid Profile[3], % of Total Fatty Acid				
C12:0	0.05	0.06	0.06	0.06
C14:0	0.01	0.01	0.01	0.01
C16:0	13.69	13.51	13.33	13.15
C16:1	0.13	0.17	0.21	0.25
C18:0	1.63	1.93	2.24	2.54
C18:1	21.80	22.00	22.20	22.40
C18:2	52.71	49.05	45.38	41.71
C18:3	2.95	5.51	8.08	10.64
C20:0	0.30	0.32	0.34	0.36
C20:1	0.26	0.29	0.32	0.35

C20:5	0.05	0.08	0.12	0.15
C22:0	0.09	0.10	0.11	0.12
C22:6	0.00	0.04	0.07	0.11
C24:0	0.10	0.11	0.12	0.13
Other	6.24	6.83	7.43	8.02

[1]Differences in dietary treatment were due to Green Grass (Sunseo Omega 3, Chungcheongbuk-do, Korea) inclusion (0 ,10, 20, 30 % of diet DM)

[2]Supplements were formulated to provide 33 mg/kg Monensin (Rumensin-90; Elanco Animal Health, DM Basis), 9.7 mg/kg Tylosin (Tylan; Elanco Animal Health, DM Basis), ≥10,820 IU Vitamin A, supplement in diet 0 provided rumen degradable protein in the form of urea (13.6% of supplement)

[3]Nutrient Compositions and fatty acid profiles were calculated from ingredient sample composition

Table 2.2 Effect of increasing inclusion of Green Grass on cattle performance and carcass characteristics

	Treatment[1]					Contrast		
Item,	0	10	20	30	SEM	L[2]	Q[3]	C[4]
Carcass adjusted Performance								
Initial BW, kg	340	340	342	341	0.50	0.91	0.20	0.09
Final BW, kg	683	674	684	673	4.63	0.16	0.98	0.11
DMI, kg/d	11.9[a]	12.2[ab]	12.3[b]	12.2[b]	0.13	0.04	0.16	0.78
ADG, kg	1.75	1.70	1.75	1.70	0.022	0.14	0.89	0.13
Gain:Feed	0.147[a]	0.139[b]	0.142[b]	0.137[b]	0.0011	< 0.01	0.07	0.02
Carcass characteristics								
HCW, kg	430	425	431	424	2.90	0.16	0.96	0.11
LM area, cm^2	80.7	78.1	80.0	80.0	0.903	0.85	0.16	0.21
Fat depth, cm	1.85[ab]	1.78[a]	1.98[b]	1.78[a]	0.064	0.88	0.33	0.02
Calculated YG[5]	4.45	4.44	4.62	4.30	0.091	0.43	0.12	0.12
Liver abscess, %	8.97	8.97	12.74	10.89	4.075	0.58	0.83	0.60
Marbling[6]	470[a]	470[a]	480[a]	430[b]	9.75	0.05	0.03	0.35

[1] Differences in dietary treatments were due to Green Grass inclusion (0, 10, 20, or 30% of diet DM).
[2] L= *P*-value for the linear response to Green Grass inclusion
[3] Q= *P*-value for the quadratic response to Green Grass inclusion
[4] C= *P*-value for the cubic response to Green Grass inclusion
[5] Calc. YG (calculated yield grade), Calculated as 2.5 + (2.5 x 12^{th} rib fat, cm) + (0.2 x 2.5 (KPH, %)) + (0.0038 x HCW, kg) – (0.32 x REA, cm^2)
[6] 400 = $Small^0$, 500 = $Modest^0$
[ab] Means in a row with different superscripts differ ($P < 0.05$).

Table 2.3 Fatty acid profile of steak samples collected at the 6th rib from steers fed increasing inclusion of Green Grass product in mg/100g of lean tissue (DM basis)

	Treatment[1]					Contrast		
Fatty acid	0	10	20	30	SEM	L	Q	C
C10:0	9.30	7.77	5.93	5.66	1.222	0.03	0.62	0.74
C12:0	5.22^{a}	3.89^{ab}	2.87^{b}	1.80^{b}	0.786	< 0.01	0.87	0.93
C14:0	342	361	343	328	11.8	0.28	0.16	0.46
C14:1	103^{a}	106^{a}	89.8^{b}	89.1^{b}	4.15	< 0.01	0.64	0.08
C15:0	43.91^{ab}	47.51^{a}	40.57^{b}	37.24^{b}	2.345	0.02	0.16	0.20
C15:1	139	162	156	140	8.4	0.95	0.03	0.06
C16:0	2796	2892	2915	2680	83.2	0.39	0.63	0.63
C16:1T	25.90	30.95	23.36	35.78	6.165	0.43	0.56	0.25
C16:1	374^{a}	348^{a}	345^{a}	299^{b}	11.7	< 0.01	0.39	0.22
C17:0	117^{a}	127^{a}	113^{ab}	98.7^{b}	5.667	< 0.01	0.05	0.40
C17:1	141^{ab}	155^{b}	127^{ab}	116^{a}	9.7	< 0.02	0.20	0.18
C18:0	1525	1631	1647	1494	61.2	0.79	0.05	0.77
C18:1T	302^{a}	392^{b}	425^{b}	414^{b}	20.4	< 0.01	0.02	0.88
C18:1	4099^{a}	4059^{a}	4130^{a}	3555^{b}	139.2	0.02	0.07	0.24
C18:1V	185	181	203	182	9.8	0.74	0.40	0.14
C18:2T	47.00^{a}	48.25^{a}	52.04^{a}	62.80^{b}	3.349	< 0.01	0.17	0.77
C19:0	13.57^{a}	23.71^{a}	31.90^{b}	24.30^{ab}	3.638	0.02	0.03	0.41
C18:2	355^{a}	449^{b}	484^{bc}	508^{c}	14.5	< 0.01	0.03	0.48
C18:3ω6	10.53^{a}	4.14^{b}	4.57^{b}	3.63^{b}	2.042	0.04	0.20	0.38
C18:3ω3[2]	21.71^{a}	53.04^{b}	68.29^{c}	87.77^{d}	3.819	<0.01	0.14	0.25
C20:0	11.78	17.47	12.08	3.75	5.943	0.28	0.25	0.76

C20:1	47.46	50.80	49.02	51.53	3.980	0.57	0.92	0.60
C20:2	35.35[a]	41.74[a]	23.27[b]	9.29[c]	4.371	< 0.01	0.03	0.15
C20:3 ω6	26.27[a]	24.05[ab]	21.63[bc]	19.71[c]	1.209	< 0.01	0.90	0.90
C20:3 ω3	1.73	1.47	1.65	2.19	1.325	0.79	0.77	0.99
C20:4ω3	0.0	0.0	0.0	0.0	-	-	-	-
C20:4ω6	72.88[a]	79.21[a]	68.84[ab]	61.07[b]	3.125	< 0.01	0.04	0.19
C20:5ω3	0.0[a]	1.87[b]	1.99[b]	7.12[c]	0.511	< 0.01	< 0.01	< 0.01
C22:0	1.47	1.95	1.13	0.00	0.659	0.09	0.24	0.74
C22:1	10.79	3.96	0.00	3.31	2.970	0.06	0.11	0.74
C22:2	0.00	0.00	0.26	0.00	0.124	0.64	0.30	0.17
C22:4	5.59[a]	5.36[a]	3.43[ab]	0.0[b]	1.200	< 0.01	0.20	0.97
C22:5	9.33[a]	18.46[b]	20.48[bc]	24.15[c]	1.511	< 0.01	0.09	0.21
C22:6	0.30	1.14	4.22	5.09	1.410	0.01	0.99	0.49
C23:0	0.99	0.55	0.00	1.68	0.691	0.63	0.14	0.46
C24:1	17.49[a]	6.56[b]	2.06[c]	2.39[c]	1.244	< 0.01	< 0.01	0.78
TOTAL	10,894	11,335	11,417	10,352	336.7	0.32	0.04	0.61
Other	64.00	75.02	90.91	79.21	8.993	0.14	0.22	0.43
SFA[3]	4854	5105	5102	4659	155	0.41	0.04	0.79
UFA[4]	6040	6230	6315	5693	186	0.27	0.04	0.48
SFA:UFA	87.88	93.49	93.19	85.23	2.987	0.54	0.04	0.90
MUFA[5]	5440	5483	5544	4891	175.5	0.06	0.06	0.36
PUFA[6]	600[a]	747[b]	772[b]	803[c]	22.1	< 0.01	0.02	0.21
Trans[7]	376[a]	470[b]	496[b]	510[b]	25.0	< 0.01	0.13	0.62
ω6[8]	112[a]	110[a]	97.2[ab]	86.4[b]	5.09	< 0.01	0.36	0.54
ω3[9]	24.19[a]	56.99[b]	73.01[c]	97.30[d]	4.320	< 0.01	0.34	0.22
ω6:ω3	5.64[a]	2.28[b]	1.55[b]	0.93[b]	0.552	< 0.01	0.02	0.32

[1]Differences in dietary treatment were due to Green Grass (Sunseo Omega 3, Chungcheongbuk-do, Korea) inclusion (0 ,10, 20, 30% of diet DM)
[2]C18:3ω3= Alpha linolenic acid [3]SFA = saturated fatty acids, [4]UFA=unsaturated fatty acids, [5]MUFA = monounsaturated fatty acids, [6]PUFA = polyunsaturated fatty acids, [7] Trans= Trans-unsaturated fatty acids, [8]ω6= total omega 6 fatty acids, [9]ω3=total omeg-3 fatty acids
[abcd] Means in a row with different superscripts differ ($P < 0.05$).

Table 2.4 Proximate analysis of lean steak samples from steers fed increasing inclusion of Green Grass product

	Treatment[1]					Contrast		
Item	0	10	20	30	SEM	L[2]	Q[3]	C[4]
Fat, %	10.96[ab]	11.41[ab]	11.51[a]	10.43[b]	0.340	0.34	0.04	0.60
Protein, %	19.83	19.75	19.75	19.82	0.115	0.96	0.49	0.98
Ash, %	1.07[a]	1.01[c]	1.03[bc]	1.05[ab]	0.014	0.68	< 0.01	0.16
Moisture, %	68.13[ab]	67.76[b]	67.71[b]	68.75[a]	0.260	0.20	0.02	0.57

[1]Differences in dietary treatment were due to Green Grass inclusion (0 ,10, 20, 30% of diet DM)
[2] L= *P*-value for the linear response to Green Grass inclusion
[3] Q= *P*-value for the quadratic response to Green Grass inclusion
[4] C= *P*-value for the cubic response to Green Grass inclusion
[ab]Means in a row with different superscripts differ ($P < 0.05$).

Chapter III. Evaluation of the safety of an algal biomass as an ingredient for finishing cattle

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ABSTRACT

A study was conducted evaluating the safety of feeding a novel algal biomass to cattle. Crossbred cattle (20 steers and 20 heifers, 255 kg initial BW, SD = 14) were individually fed 4 inclusions of Condensed Algal Residue Solubles (**CARS**; 0, 2.5, 5, 7.5 % of diet DM) displacing corn in the finishing diet for a minimum of 97 days. Increasing CARS inclusion in the diet quadratically increased DMI and ADG ($P \leq 0.01$) and linearly increased G:F ($P < 0.01$). Net energy calculations demonstrated a linear increase in NE_m and NE_g as CARS inclusion increased. Out of 27 organs measured, 6 had differences due to treatment in absolute weight and weight as a % of BW. Weight of the liver, pancreas, jejunum, and heart linearly increased ($P \leq 0.05$) while weight of the thyroid and gall bladder quadratically increased ($P \leq 0.04$) as CARS inclusion in the diet increased. However, organ weights were all within expected ranges and histopathology analysis of organs revealed no differences due to treatment ($P \geq 0.24$). Hemoglobin and hematocrit concentrations quadratically decreased ($P = 0.05$) and red blood cell distribution width linearly increased ($P = 0.02$) as CARS inclusion increased, no other differences were observed for hematology measures ($P \geq 0.11$). Out of 21 blood chemistry measures, 8 were impacted by treatment ($P \leq 0.02$). Inclusion of up to 7.5% of diet DM as CARS had no adverse effect on cattle and improved performance when fed up to 5.0% of the diet DM.

Keywords: algae, cattle, coproduct, feed safety, novel feedstuffs

INTRODUCTION

With increasing interest in production of algae derived omega-3 fatty acids for both human food and animal feeds, coproducts from the algae industry could result in an alternative feed ingredient for cattle. Algal biomass is a potential source of protein, fiber and fat, which could contribute essential nutrients in cattle diets. A condensed algal residue solubles (**CARS**; Veraferm, Veramaris, Delft, Netherlands), is being commercially produced from heterotrophic algae as a result of producing omega-3 fatty acids for use in the animal feed industry, primarily aquaculture and pet foods. The CARS is produced by condensing the residue from algal fermentation of dextrose after the oil has been extracted from the algal cells without organic solvents and has a syrupy consistency.

Marine algae, commonly photoautotrophic, have been utilized in animal diets for many years, and utilize photosynthesis to harness simple inorganic substances as energy and nutrients (Lum et al., 2013). Heterotrophic algae, grown using complex organic substances for feedstuffs, may result in improved yields and growth efficiency, and thus improve the economics of utilizing algae as a livestock feedstuff (Ogbonna et al., 1997; Bryant et al., 2012). Van Emon et al. (2015), fed a heterotrophic microalgae meal (57% microalgae, 43% soyhulls) to growing cattle. They observed greater DMI, a tendency for ADG to increase, and decreased G:F as algae meal increased from 0 to 45% of diet DM, replacing wet corn gluten feed. A similar algae meal (43% partially deoiled heterotrophic microalgae and 57% soyhulls) was fed to finishing cattle (Stokes et al., 2016) replacing

corn at 0 to 42% of diet DM. They reported no change in HCW and a linear decrease in calculated dietary NEg content as inclusion increased in the diet. These results suggest the algae product is a suitable cattle feed when mixed with soyhulls. Little research has been conducted on algae as a feed ingredient and no research has been conducted feeding CARS to cattle; therefore, the objectives of this study were to evaluate the safety of CARS as a feed ingredient in cattle diets and the performance response to increasing inclusion in the diet.

MATERIALS AND METHODS

The following experiment was conducted at the Eastern Nebraska Research and Extension Center (ENREC; near Mead, NE), University of Nebraska Animal Science Complex (Lincoln, NE) and the University of Nebraska Veterinary Diagnostic Center (UNL VDC; Lincoln, NE). Animal handling and space for the experiment were in accordance to the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). All procedures outlined as part of this study were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee (protocol number 1517). Because CARS is not currently approved by the FDA to be fed to cattle entering the human food chain, all cattle were incinerated at completion of the experiment, following intensive sampling of tissues.

Experimental Design

A trial was conducted using forty crossbred cattle (20 steers and 20 heifers, 255 kg initial BW, SD = 14). At receiving, all cattle were vaccinated with a Mannheimia haemolytica, bovine rhinotracheitis virus, bovine viral diarrhea (type 1 and 2),

parainfluenza-3, and bovine respiratory syncytial virus combination vaccine (Bovi-shield One Shot, Zoetis, Florham Park, NJ), bacterin toxoid against seven clostridial diseases and Haemophilus somnus (Ultrabac-7, Zoetis), an intranasal vaccine containing bovine rhinotracheitis, parainfluenza-3, and bovine respiratory syncytial virus (Inforce 3, Zoetis), dewormed with 1% w/v doramectin (10 mg/mL, Dectomax, Zoetis), and received 10 mL of gamma-cyhalothrin pour-on (StandGuard, Elanco, Greenfield IN). Cattle were tagged with one 4-digit panel tag, a metal clip with the same four-digit identification, and electronic ID. All cattle were individually fed using the Calan gate system (American Calan Inc., Northwood, NH) within two pens separating steers and heifers. The calves underwent a 3-week training period to acclimate to the Calan gate system prior to trial initiation. Each animal had approximately 46 linear cm of bunk space. Daily observations of each individual animal were recorded after feeding by trained animal care staff at the research facility; daily observation forms were kept on record.

Five days prior to the initiation of the trial, cattle were limit fed at 2% of BW on a common diet of 50% Sweet Bran (Cargill corn milling, Blair, NE) and 50% alfalfa hay (Watson et al., 2013). Cattle were weighed on 3 consecutive days prior to feeding to reduce error from gut fill, and the average was used as initial BW. Day 1 and 2 weights were averaged, and cattle were blocked by initial BW strata into 10 blocks where blocks 1, 3, 5, 7, and 9 represented the heaviest to lightest steers and blocks 2, 4, 6, 8, and 10 represented the heaviest to lightest heifers with each treatment being represented in each block. On the third day of weighing, cattle were additionally ear tagged with the corresponding bunk ID number.

Four dietary treatments were assigned randomly to animal within block. Diets consisted of increasing inclusion of CARS (0, 2.5, 5, and 7.5% of diet DM; Table 3.1) displacing dry rolled corn in the diet (70.0, 67.5, 65.0, and 62.5%). All diets contained 15% wet distillers grains, 10% grass hay, and 5% supplement (DM basis). Because of the high Na content of CARS (Table 3.2; 8.5% of DM), 2 supplements were formulated, one for the 0% CARS and another for the 7.5% CARS treatment. Both supplements were blended together for use in the 2.5% and 5% CARS diets. Supplements were formulated to limit dietary Na to 1% of diet DM. Supplements included limestone, urea, trace mineral premix, vitamin ADE premix, tallow, Rumensin (330 mg/animal daily; Elanco Animal Health), and Tylan (90 mg/animal daily; Elanco Animal Health) with fine ground corn as the carrier. Cattle were fed ad-libitum once daily (0700 h).

Feed refusals were collected weekly, weighed and then dried in a 60º C forced air oven for 48 hours to calculate accurate DMI per individual. Approximately 400 g of each total mixed ration and individual ingredients (CARS, dry rolled corn, wet distillers grains, grass hay and supplement) were sampled weekly. Samples were composited into 3-week periods (6 composites of each of 4 diets and each ingredient) and subsequently analyzed for DM, OM, NDF, ADF, CP, macro- and micro-minerals (Ward Laboratories, Inc., Kearney, NE) and docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA; Eurofins Scientific, Des Moines, IA; Table 3.3). The DHA and EPA levels in diets were used to confirm dosage of CARS as CARS was the only source of DHA and EPA in the diets. Net energy calculations were calculated by the quadratic solution used by Vasconcelos and Galyean (2008).

Blood and Urine Analysis

Interim BW, urine, blood and Veterinary observations were obtained on days 0, 33, 61, 90 and harvest day. On each collection day, cattle were processed through a chute, weighed, and visually appraised by a veterinarian for normal behavior and general health. Cattle were then dosed with furosemide (2 mL/ 45 kg BW, Lasix, Validus Pharmaceuticals LLC, Parsippany, NJ), a diuretic, to stimulate urination. A 50-mL conical tube was used to capture a urine sample. Urine was chilled during collection and samples were immediately transported to the UNL VDC (Lincoln, NE) for urinalysis including protein, pH, ketone bodies, bilirubin, urobilinogen glucose (Chemstrip 2 GP, 2 LN, 9, 10 with SG, Roche Diagnostics, Indianapolis, IN) and microscopic examination. Samples of blood were collected by jugular venipuncture with 2 Vacuette Tube 6 mL K2E K2EDTA (Greiner Bio-One GmbH, Monroe, NC) and 2 Corvac Integrated Serum Separator Tubes (Covidien, Mansfield, MA) per animal. Blood samples were chilled following collection and immediately transported to the UNL Ruminant Nutrition laboratory (Lincoln, NE). At the laboratory, blood serum tube samples were placed in a 4°C refrigerator for 1 hour before centrifugation at 1250 × g for 10 min at 4°C. Blood and blood serum samples were sent to Iowa State University Veterinary Pathology Laboratory (Ames, IA) overnight for common hematology and blood chemistry. Hematology included white blood cell count (WBC), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), mean platelet volume (MPV), platelet count, and neutrophil, lymphocyte, monocyte,

eosinophil, basophil, plasma protein, fibrinogen, hematocrit and hemoglobin concentrations. Blood chemistry measures included Na, K, Cl, Ca, P, Mg, blood urea N (BUN), creatinine, glucose, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), creatine kinase, total bile acids, bicarbonate, and cholesterol.

Organ Harvest

Blocks were harvested at a target BW of 454 kg (419 ± 22 kg), with blocks 1 and 2 on day 97, 3 and 4 on day 104, 5 and 6 on day 111, 7 and 8 on day 118, and 9 and 10 on day 125. On each harvest day, all cattle were individually weighed at 0630 h at ENREC prior to feeding. The 8 cattle to be slaughtered that day had blood samples from the jugular vein taken while in the chute and were then held in a sort pen. Remaining cattle were also weighed and then returned to their pen. Veterinary observations were recorded on all animals. The 8 sorted animals were then transported to the University of Nebraska Animal Science complex (Lincoln, NE) where they were held in two 3.6 × 6 m pens (steers separate from heifers) and had access to water. Cattle were trailered from the Animal Science complex to the UNL VDC in groups of 2 for harvest. Slaughter order was assigned randomly within block to avoid bias of timing of euthanasia. Steers were harvested before heifers. The cattle were injected with pentobarbital sodium (390 mg/mL, 1 mL /45 kg BW, Fatal-Plus, Vortech Pharmaceuticals, Dearborn, MI) to euthanize the animal and exsanguinated.

A pathologist, blinded to treatment, supervised the necropsy and recorded gross findings. Feet were removed at the knee and the hock. The head was removed at the atlas and the hide was skinned away from the thoracic cavity. Urine collection was done post mortem by needle and syringe directly from the bladder. After evisceration, the rest of the hide was removed. Organs were isolated, removed, washed, weighed, and then sampled in duplicate (approximately 10 g per sample). Organs and tissues evaluated included: brain, spinal cord (2 segments), spleen, lung, pancreas, skeletal muscle, rumen reticulum, omasum, abomasum, duodenum, jejunum, cecum, colon, kidneys, urinary bladder, pituitary, thyroid, adrenal, liver, gall bladder, heart, mesenteric lymph node, skin, prostate, eye, bone and marrow, marrow smear, ileum, and thymus. For heifers, the ovaries, mammary gland, and uterus were also evaluated. After full tissue collection and necropsy, the cattle were incinerated at the UNL VDC.

Due to mechanical failures with the rail and hoist system on the first harvest day, block 2 heifers (4 animals) were held overnight at the University of Nebraska Animal Science complex. The heifers were individually penned and allowed access to water and their assigned treatment diet (same amount as the previous day). Overnight the rail and hoist system was fixed, and the heifers were harvested the next day following the same procedures. The remaining harvest dates proceeded as planned with 8 animals harvested per day.

Preliminary murine experiments

Prior to the cattle feeding study, the safety of this novel feed ingredient was evaluated using a bacterial reverse mutation assay (Ames test) and an *in vivo*

micronucleus test in mouse immature erythrocytes as well as repeated-dose toxicity studies rats. All studies were conducted by Eurofins Product Safety Laboratories (Dayton, NJ) in accordance with the GLP Regulations issued by the U.S. FDA (Title 21 of the CFR, Part 58; effective 1987) and followed the Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals and Food Ingredients, Section 4, Parts 471, 474, and 408.

In the Ames test (Ames et al., 1973), CARS was investigated for its potential to induce gene mutations according to the plate incorporation test using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and tester strain *Escherichia coli* WP2 uvrA. In two independent experiments several concentrations up to 5000 µg/plate of the test item were used. Each experiment was conducted with and without metabolic activation. No toxic effects of the test item were noted in any of the five tester strains. No biologically relevant increases in revertant colony numbers were observed following treatment with CARS at any concentration level, neither in the presence nor absence of metabolic activation in both experiments indicating lack of mutagenic potential of CARS.

The safety of CARS was also evaluated in a 14-day dietary toxicity study in rats followed by a sub chronic 90-day dietary study in Sprague–Dawley rats. In the 90-day study (OECD Test Guideline 474), the test material was added to the basal diet at dietary levels of 0.5% (5000 ppm), 1.5% (15,000 ppm) and 5.0% (50,000 ppm). Each experimental group consisted of 10 animals per sex. The stability, homogeneity and concentration of CARS in the diet were confirmed by analysis based on Docosahexaenoic Acid (DHA) content in the diet (Eurofins Central Analytical Laboratories, Metairie, LA).

There were no changes in BW, BW gain, feed consumption or feed efficiency in male and female rats attributable to the administration of test substance. There were no test substance-related changes in hematology, coagulation, clinical chemistry and urinalysis parameters. There were no CARS-related macroscopic or organ weight changes. Test substance related microscopic findings consisted of pancreatic acinar cell hyperplasia observed in High Dose (50,000 ppm) males (found in 3 out of 10 animals).

Therefore, under the conditions of the study and based on the toxicological endpoints evaluated, the No-Adverse-Effect Level for administration of CARS in the rodent diet was determined to be 1.5% of the diet (15,000 ppm), equivalent to an overall average CARS intake of 1071 mg/kg BW daily for male and female rats. These preliminary experiments were completed prior to the current cattle feeding trial and suggested no toxic effects of CARS.

Statistical Analyses

Performance data (BW, ADG, DMI, G:F, HCW, NEm, NEg, and organ weights) were analyzed using the mixed procedure of SAS (SAS Inc., Cary, NC) as a randomized complete block design with treatment, gender, and treatment by gender interactions as fixed effects, BW block as a random effect and individual animal as the experimental unit. Interactions were removed from the model if not significant. Orthogonal contrasts were used to test significance for linear, quadratic and cubic responses due to CARS inclusion. Blood and urine data were analyzed as repeated measures with an optimized covariate structure selected based on the lowest Akaike information criterion score suggesting the best model fit (Littell et al., 1998). For a few variables measured in the

urine (epithelial cells, amorphous crystals, triple phosphate crystals, WBC, blood, protein, anisocytosis, acanthocyte, and echinocyte) qualitative data were collected and then transposed to numerals for analysis (0 = none, 1 = few, 2 = moderate, 3 = many). Probabilities less than or equal to 0.05 were considered significant, less than or equal to 0.10 were declared tendencies.

RESULTS AND DISCUSSION

Cattle Performance

There were no interactions between sex and treatment ($P \geq 0.25$) for performance data. Sex was significant for all variables ($P \leq 0.04$) with steers having greater DMI, initial BW, ADG, HCW and final BW, compared to heifers. There were no differences in initial BW between CARS treatments ($P \geq 0.27$). There was a quadratic response ($P = 0.01$; Table 3.4) observed for DMI with cattle fed 2.5% CARS having the greatest DMI of 8.98 kg/d. There was a quadratic ($P < 0.01$) response for ADG with cattle fed 2.5% and 5% CARS having the greatest numerical values of 1.40 and 1.37 kg, respectively. Live final BW responded quadratically ($P < 0.01$) and was the greatest for cattle fed 2.5% and 5% CARS, 428 and 427 kg, respectively. The cattle fed 7.5% CARS had the lowest DMI and ADG ($P \leq 0.01$); however, this treatment elicited a greater G:F of 0.166, linearly ($P < 0.01$) increasing with increased algae inclusion in the diet. Both NEm and NEg linearly increased ($P < 0.01$) with increasing inclusion of CARS.

The CARS evaluated in this trial differs from other algal based feedstuffs fed to cattle and evaluated in previous research (Franklin et al., 1999; Drewery et al., 2014; Van Emon et al., 2015; Costa et al., 2016; Stokes et al., 2016). The nutrient profile is unique

due to both the initial algae feedstock and the processing methods of CARS production. Much of the previous research has also fed the algal residue in combination with other feeds, such as soyhulls (Van Emon et al., 2015; Stokes et al., 2016) or to growing cattle (Drewery et al., 2014; Van Emon et al., 2015; Costa et al., 2016). In a trial with finishing cattle, a meal consisting of 43% partially deoiled microalgal residue and 57% soyhulls replaced up to 42% of the dietary dry rolled corn (Stokes et al., 2016). Authors reported no differences in final BW or ADG, but a linear decrease in G:F as the algal meal replaced corn in the diet. This resulted in a linear decrease in both dietary ME and NEg as algal meal inclusion increased. Results from the current trial suggest feeding algal residue up to 7.5% of dietary DM linearly increased G:F and dietary NEg. This would be a similar algae inclusion as the lowest inclusion of algae meal (14% diet DM) in the Stokes et al. (2016) trial. Algal residues are somewhat variable depending on the species grown and the manufacturing process used for production. The CARS product evaluated in the current trial appears to be a suitable replacement for corn in finishing diets, up to 7.5% of diet DM, and improved ADG and G:F up to 5% inclusion of the diet DM.

Organ Weights

Organ weights were analyzed as absolute organ weight as well as organ weight as a percent of shrunk BW (SBW, final BW shrunk 4% to account for gut fill). Table 3.5 reports organ weights with the sex × treatment interaction removed from the statistical model for all organs. There were no significant differences ($P \geq 0.16$) among treatments for organ weight of spleen, lungs, rumen, reticulum, omasum, ileum, cecum, kidneys, pituitary, adrenal, eye, thymus, uterus, ovaries, prostate, and seminal vesicle.

Pancreas weight linearly increased ($P = 0.02$) as CARS inclusion increased in the diet; however, this could be attributed to the difficulty of distinguishing pancreas and fat connected to the pancreas. There was a quadratic response observed for brain weight ($P = 0.04$); cattle fed 5% CARS had the greatest brain weight of 387 g, which was not different from cattle fed 0 and 2.5% CARS ($P \geq 0.10$) but was greater than cattle fed 7.5% CARS at 356 g ($P = 0.01$). Liver weight linearly increased ($P < 0.01$) as CARS inclusion increased in the diet. Thyroid weight had a quadratic response ($P = 0.02$), with cattle fed 2.5% CARS having the greatest weight of 31.8 g, statistically different from cattle fed 0% CARS ($P < 0.01$), but not different from cattle fed 5% and 7.5% CARS ($P \geq 0.11$). There was a quadratic ($P = 0.04$) response for abomasum weight with cattle fed 0% CARS having the lightest weight of 1.25 kg and cattle fed 5% CARS having the greatest weight of 1.41 kg. Similarly, there was a quadratic response ($P = 0.03$) for duodenum weight with cattle fed 0% CARS having the lightest weight of 273 g and cattle fed 5% CARS having the greatest weight of 326 g. The difference in duodenum weight between treatments could be attributed to variation in discretion of where the duodenum ends and the jejunum begins. There was a cubic response observed for urinary bladder weight with cattle fed 2.5% CARS having the greatest weight of 116 g, and cattle fed 5% CARS having the smallest weight of 96.4 g. Differences in urinary bladder weight were small, and the cubic response suggests differences were due to variation and error in measurement, not biological differences due to treatment.

There was a tendency for a sex × treatment interaction ($P = 0.08$; Table 3.6) for jejunum weight with steers fed 7.5% CARS having the greatest weight of 6.33 kg and

heifers fed 5% CARS having the greatest weight of 5.69 kg. There was a sex × treatment interaction ($P = 0.02$) for gall bladder weight, with a quadratic ($P < 0.01$) response. Steers fed 2.5% CARS had the greatest weight of 81.6 g while heifers fed 5% CARS had the greatest weight of 107 g. The heart also had a sex × treatment interaction ($P = 0.04$) with steers fed 7.5% CARS having the greatest heart weight (2.21 kg) and heifers on the 5% CARS treatment having the greatest heart weight (2.07 kg). The colon also had a sex × treatment interaction ($P = 0.02$) with steers fed 7.5% CARS having the greatest colon weight (4.38 kg) and heifers fed 2.5% CARS having the greatest colon weight (4.93 kg).

Organ Weight as % of SBW

There were no significant differences ($P \geq 0.07$) among treatments in organ weight as a % of SBW for spleen, lungs, rumen, reticulum, omasum, abomasum, duodenum, ileum, cecum, kidneys, urinary bladder, brain, pituitary, adrenal, thymus, prostate, seminal vesicles, uterus, ovaries, and colon. A difference in liver weight as % of SBW was observed, with a quadradic response ($P < 0.01$); cattle fed 7.5 % CARS had the greatest weight (2.05 kg). The thyroid also had a quadratic response ($P = 0.04$), but differences due to treatment were small, varying from 0.006 to 0.008% of SBW. The weight of both the pancreas and eye linearly ($P \leq 0.01$) increased as CARS inclusion increased in the diet.

The jejunum had a sex × treatment interaction (Table 3.6, $P = 0.04$), and linearly ($P < 0.01$) increased as CARS increased in the diet. There was a sex × treatment interaction ($P = 0.04$) in colon weight with steers fed 2.5% and 5% CARS having the smallest colon and an increase in colon weight for heifers fed 2.5% and 5% CARS ($P \leq$

0.04). There was a tendency (P = 0.07) for colon weight as a % of SBW to be greater in heifers than steers. There was a sex × treatment interaction (P= 0.01) in gall bladder weight as a % of SBW, with steers fed 2.5% CARS having the greatest gall bladder weight and heifers fed 5% CARS having the greatest gall bladder weight. There was a sex × treatment interaction (P = 0.03*)* for heart weight as a % of SBW. Heart weight linearly increased (P = 0.01) from 0.444% to 0.554% of SBW in steers and from 0.454% to 0.515% in heifers as inclusion of CARS increased in the diet.

Absolute organ weights and organ weights as a % of SBW are similar to values published in the literature (Hersom et al., 2004; McCurdy et al., 2010). Differences due to CARS inclusion were relatively minor and likely due to nutrient load. Differences in liver, pancreas, and gall bladder weights between treatments were the most pronounced. These organs function in nutrient digestion and excess nutrient excretion. With increasing inclusion of CARS, some minerals, primarily Na, were increased in the diet and would have been processed by the liver.

Hematology

Both hemoglobin and hematocrit concentrations quadratically decreased (P = 0.05) with increasing inclusion of CARS (Table 3.7). For both measures, minimum concentrations were observed for cattle fed 2.5% CARS. Red blood cell distribution width (RDW) linearly increased (P = 0.02) from 20.9 to 22.0% with increasing inclusion of CARS. There was a linear tendency (P = 0.09) for monocyte concentrations to increase as CARS inclusion in the diet increased, but all treatments fell within the expected

laboratory reference range. There was no difference due to sex ($P = 0.80$) and no treatment × sex interaction ($P = 0.48$) for monocyte concentrations.

Sex was not significant ($P \geq 0.16$), and there were no treatment × sex interactions ($P \geq 0.42$) for WBC, RBC, hemoglobin, hematocrit, MCHC, RDW, platelet count, MPV, and lymphocyte, eosinophil, basophil, and fibrinogen concentrations (data not shown). Sex was significant ($P = 0.02$) for MCV, with heifers having an average volume of 40.8 fl, and steers having an average volume of 38.6 fl, but no treatment × sex interaction ($P = 0.38$) was observed. Sex was significant ($P = 0.02$) for neutrophil concentrations, with heifers having greater concentration of neutrophils at 3.57×10^3/ul and steers having a concentration at 2.84×10^3/ul, but there were no differences between treatments ($P = 0.18$). There was a difference due to sex ($P = 0.02$) in the concentration of plasma protein with heifers having a concentration of 8.36 g/dL and steers having a concentration of 8.09 g/dL, and there was tendency for a treatment × sex interaction ($P = 0.08$), but no differences among treatments ($P = 0.11$).

Laboratory reference intervals of hematology variables measured in cattle are shown in Table 3.7 as expected ranges (Veterinary Pathology, 2011). Nearly all variables were well within the prescribed expected range. The RDW was greater than expected, averaging 21.4% for all treatments with 8.0 to 15% considered the expected range. Fibrinogen concentrations were slightly elevated above the laboratory reference range for cattle fed 0% and 2.5% CARS at 516 and 582 mg/dL, respectively. The maximum upper limit of the laboratory reference range is 500 mg/dL. The MCV value for cattle fed 2.5% CARS was slightly lower than expected at 38.9 fl with the lower end of the expected

range at 40.0 fl. The MPV of cattle fed 7.5 % CARS was greater than expected at 8.27 fl and the upper end of the expected range at 8.0 fl. Plasma protein concentrations of all treatments were greater than expected, averaging 8.22 g/dL and the upper end of the expected range at 7.7 g/dL. These expected ranges may have been established using different animal populations that may not be representative of normal feedlot animals on a finishing diet. Daily cattle observations and visual health observations all suggested cattle were healthy and showed no adverse effects to any dietary treatment.

Blood Chemistry

There were no differences due to sex ($P \geq 0.11$), no treatment × sex interactions ($P \geq 0.29$) and no differences among treatments ($P \geq 0.10$) observed for blood Na, blood K, blood P, blood Ca, BUN, blood glucose, total bile acids, and AST concentrations. There was a tendency for a linear decrease (Table 3.8; $P = 0.06$) in ALT concentration as CARS inclusion increased. There were no treatment × sex interactions ($P = 0.46$) and no differences due to sex ($P = 0.47$) for ALT concentration. There was a linear decrease ($P \leq 0.01$) in blood Cl concentration as CARS increased in the diet and a difference due to sex ($P \leq 0.01$), with heifers having a concentration of 101 mEq/L and steers having a concentration of 100 mEq/L. There were no treatment × sex interactions ($P = 0.45$) for Cl concentration and blood Cl concentrations were within the expected ranges for cattle. There was a linear increase ($P < 0.01$) in blood bicarbonate concentration as CARS increased in the diet and a difference due to sex ($P = 0.03$), with heifers having a lower concentration than steers, 27.7 and 28.5 mEq/L respectively. There were no treatment × sex interactions ($P = 0.55$) for blood bicarbonate concentration and measured values were

within the expected ranges for cattle. There was a cubic response ($P = 0.03$) for blood Mg with cattle fed 5% CARS having the highest blood Mg concentration of 2.07 mg/dL. There was no difference due to sex ($P = 0.11$), and no treatment × sex interaction ($P = 0.50$) for blood Mg concentration. Stokes et al. (2016) reported no differences due to algal meal inclusion in the diet on plasma Mg levels; values they reported are similar to the current trial averaging 2.36 mg/dL. There was a tendency for a cubic response ($P = 0.09$) for blood albumin concentrations with cattle fed 5% CARS having the greatest concentrations of 3.27 g/dL; all treatments were within the expected range for cattle. Blood creatinine concentration linearly increased ($P < 0.01$) from 1.07 to 1.16 mg/dL as CARS inclusion increased in the diet. There was a tendency for a treatment × sex interaction ($P = 0.09$) in total protein concentration; however, there were no differences among treatments ($P \geq 0.10$) and measured values were within the expected range for cattle. Sex was not significant ($P = 0.50$), for blood creatine kinase concentrations; however, there was a tendency for a treatment × sex interaction ($P = 0.10$), and a quadratic decrease ($P = 0.02$) was observed with cattle fed 7.5% CARS having the greatest concentration of 217 IU/L. The creatine kinase concentration for all treatments was within the expected range for cattle. Alkaline phosphatase concentrations decreased linearly ($P < 0.01$) from 65.4 to 43.7 IU/L as CARS inclusion increased in the diet, but were within the expected range for cattle. There was a tendency for a difference due to sex ($P = 0.08$) for GGT, and a quadratic ($P < 0.01$) response was observed with cattle fed 0 and 7.5% CARS having the greatest concentrations of 46.8 and 45.1 IU/L respectively. Total bilirubin concentration had a cubic response ($P < 0.01$) with cattle fed 5% CARS

having the greatest concentration at 0.366 mg/dL. Sex was significant ($P = 0.04$) for total bilirubin with heifers having a greater concentration at 0.351 mg/dL and steers at 0.323 mg/dL. All treatments had higher concentrations than would be expected for cattle, with the upper limit being 0.18 mg/dL. There was a tendency ($P = 0.08$) for steers and heifers to be different in total bile acids; steers had a concentration of 38.8 umol/L and heifers 29.4 umol/L, but no differences among treatments ($P \geq 0.10$). There was a tendency for a treatment × sex interaction ($P = 0.09$) in cholesterol, but no differences due to sex ($P = 0.70$). Cholesterol had a tendency to linearly increase ($P = 0.07$) as CARS inclusion in the diet increased. There was a difference due to sex ($P = 0.02$) for LDH levels; heifers had LDH levels of 4390 IU/L and steers had levels of 4120 IU/L. There was a quadratic ($P = 0.04$) response observed for LDH with cattle fed 7.5% CARS having the greatest concentration of LDH at 4494 IU/L, which is above the upper limit of the expected range, 410 IU/L. Feedlot cattle have a large metabolic activity due to the high energy diets they are fed. This can lead to greater hepatocellular swelling and leakage, which is a primary source of LDH. Also, younger animals generally have greater levels of LDH. The expected range was developed from a mix of cattle, likely cows on forage based diets as cattle on all treatments had elevated LDH concentrations relative to the expected range.

Laboratory reference intervals for blood chemistry variables measured in cattle are shown in Table 8 as expected ranges. Nearly all variables were well within the prescribed expected range. However, these expected ranges may have been established using different animal populations that may not be representative of normal feedlot animals on a finishing diet. Total bilirubin was greater than expected, averaging 0.338

mg/dL while the upper end of the expected range is 0.18 mg/dL. Blood concentrations of Ca and P were also greater than expected, averaging 10.3 and 8.17 mg/dL, while upper expected limits are 10.1 and 7.9 mg/dL. Blood Mg concentration averaged 2.00 mg/dL, less than the expected value of 2.10 mg/dL. Daily cattle observations and visual health observations all suggested cattle were healthy and showed no adverse effects of any treatment.

Urine Analysis

Sex did not impact pH ($P = 0.45$) or specific gravity ($P = 0.95$) of urine. Urine pH did not have a treatment × sex interaction ($P = 0.21$) but there was a quadratic response (Table 3.9; $P < 0.01$) as CARS increased in the diet with cattle fed 5% CARS having the greatest pH (8.70). There were no differences among treatments ($P \geq 0.96$) for specific gravity.

There were no differences among treatments and no treatment × sex interactions ($P \geq 0.17$) for epithelial cells, amorphous crystals, WBC, protein or blood measured in the urinalysis. Epithelial cell count was quantified as few (1-10 cells/field) in all treatments. Amorphous crystals, WBC, protein and blood were all quantified as none for all treatments. Triple phosphate crystals had a numerical difference of none for cattle fed 0% CARS and few (1-10 crystals/field) for cattle fed 2.5, 5, or 7.5 % CARS, but no statistical difference between treatments ($P = 0.10$).

Histopathology

Two treatments (cattle fed 0% CARS and 7.5% CARS) were compared for all histopathology analysis. The pathologist was blinded to treatments and slides from tissues

of cattle fed 0 and 7.5% CARS were evaluated as either 0 = normal, or 1 = abnormal. There were no significant differences due to treatment ($P \geq 0.24$) for: brain (5 slides evaluated), spinal cord (2), eye, spleen, left cranial lung, right caudal lung, pancreas, longissimus (skeletal muscle), brisket (skeletal muscle), rumen (3), reticulum, omasum, abomasum, duodenum, jejunum (3), cecum, ileum, thymus, colon (2), right kidney, left kidney, urinary bladder, pituitary, thyroid, adrenal, left liver, right liver, gall bladder, left side of heart, right side of heart, mesenteric lymph node (2), prostate, ovary (2), skin, hoof c band, hoof wall, hoof sole, and bone marrow. It was assumed that with no differences between the 2 extreme inclusions of CARS (0 vs 7.5%), the intermediate treatments were also not affected. Histology results from 0 and 7.5% CARS suggest that there were no differences in tissue health of the cattle whether CARS was included in the diet or not.

IMPLICATIONS

The feedstuff CARS demonstrated to be a safe and efficacious feed ingredient in cattle diets. Feeding CARS to finishing cattle improved G:F as inclusion in the diet increased up to 7.5% of diet DM. Cattle HCW, ADG, and DMI all increased quadratically and were maximized when cattle were fed 2.5 or 5% CARS. No adverse effects of feeding CARS were observed in hematology, blood chemistry, or histopathology analyses. An increase in organ weight was observed for the liver, thyroid, gall bladder, pancreas, jejunum, and heart when CARS was fed, but no impact on health was observed and no differences in tissues were found. Further research is needed to

determine the optimal inclusion of CARS on performance and carcass traits when fed an entire feeding period, as well as potential for CARS to be used in growing cattle diets.

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Table 3.1 Composition of diets containing increasing inclusions of Condensed Algal Residue Solubles (CARS) and individually fed to steers and heifers

	Treatment			
Ingredient, % diet DM	0%	2.5%	5%	7.5%
Dry rolled corn	70.0	67.5	65.0	62.5
Wet distillers grains	15.0	15.0	15.0	15.0
Grass hay	10.0	10.0	10.0	10.0
CARS	--	2.5	5.0	7.5
Supplement[1]	5.0	5.0	5.0	5.0
Fine ground corn	2.28	2.49	2.70	3.12
Limestone	1.69	1.69	1.69	1.69
Tallow	0.125	0.125	0.125	0.125
Urea	0.54	0.405	0.27	--
Salt	0.30	0.225	0.15	--
Trace mineral premix[2]	0.05	0.05	0.05	0.05
Vitamin A-D-E premix[3]	0.015	0.015	0.015	0.015

[1] Two supplements were formulated and blended together for the 2.5% CARS and 5% CARS treatments. Supplement provided Rumensin (330 mg/animal daily; Elanco, Greenfield, IN), and Tylan (90 mg/animal daily; Elanco).

[2] Trace mineral premix contained 10% Mg, 6% Zn, 4.5% Fe, 2% Mn, 0.5% Cu, 0.3% I, and 0.05% Co.

[3] Vitamin A-D-E premix contained 1500 IU vitamin A, 3000 IU vitamin D, and 3.7 IU vitamin E per g.

Table 3.2 Nutrient composition of Condensed Algal Residue Solubles (CARS)

Item	CARS[1]
DM, %	41.7
%, DM basis	
CP	29.3
NDF	34.6
ADF	2.3
Ca	0.16
P	0.82
K	1.51
S	2.54
Na	8.52
mg/kg, DM basis	
Mg	0.33
Zn	43.87
Fe	86.33
Mn	13.5
Cu	6.00
Mo	0.69

[1]Nutreint composition of CARS was analyzed by Ward Laboratories, Inc. (Kearney, NE).

Table 3.3 Dry matter composition of diets containing increasing inclusions of Condensed Algal Residue Solubles (CARS) with overall ± SD

	Treatment[1]			
Nutrient analysis, %[2]	0	2.5	5	7.5
DM	69.0 ± 3.04	67.1 ± 1.42	66.0 ± 1.69	64.6 ± 1.45
OM	65.1 ± 0.491	61.8 ± 0.854	60.3 ± 0.428	58.4 ± 0.440
CP	13.3 ± 0.705	14.4 ± 1.02	14.2 ± 0.339	14.1 ± 0.534
NDF	15.2 ± 0.686	14.0 ± 2.39	16.6 ±3.35	17.5 ± 2.78
ADF	6.9 ± 1.05	6.6 ± 0.769	8.5 ± 2.35	9.4 ± 1.97
Ca	0.550 ± 0.149	0.875 ± 0.265	0.815 ± 0.241	0.687 ± 0.105
P	0.377 ± 0.032	0.403 ± 0.045	0.408 ± 0.053	0.430 ± 0.045
K	0.635 ± 0.049	0.678 ± 0.061	0.713 ± 0.079	0.723 ± 0.067
S	0.200 ± 0.013	0.280 ± 0.024	0.348 ± 0.046	0.415 ± 0.027
Na	0.153 ± 0.023	0.385 ± 0.053	0.593 ± 0.067	0.778 ± 0.087
Mg	0.147 ± 0.019	0.163 ± 0.021	0.165 ± 0.020	0.168 ± 0.022
Zn, mg/kg	50.6 ± 7.04	59.7 ± 10.1	58.7 ± 2.84	56.1 ± 6.08
Fe, mg/kg	162.5 ± 32.6	191.5 ± 35.0	185.5 ± 12.9	201.0 ± 47.0
Mn, mg/kg	32.8 ± 4.26	38.3 ±5.32	38.5 ± 2.74	36.3 ± 2.73
Cu, mg/kg	13.9 ± 1.44	17.6 ± 7.94	17.3 ± 2.90	14.8 ± 1.06
Mo, mg/kg	0.678 ± 0.093	0.842 ± 0.141	0.863 ± 0.104	0.825 ± 0.115
DHA[3]	< 0.02	0.148 ± 0.023	0.300 ± 0.038	0.475 ± 0.061
EPA[3]	< 0.02	0.038 ± 0.008	0.077 ± 0.008	0.113 ± 0.015

[1] Differences in dietary treatments were due to CARS inclusion (0, 2.5, 5, or 7.5% of diet DM).
[2] Nutrient analysis was measured on weekly grab samples of total mixed diets, composited into six, 3 week period samples and analyzed by Ward Laboratories, Inc., Kearney, NE.
[3] DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; measured by Eurofins Scientific, Des Moines, IA

Table 3.4 Performance of steers and heifers individually fed Condensed Algal Residue Solubles (CARS) at increasing inclusions

	Treatment[1]					Contrast		
Item	0	2.5	5	7.5	SEM	Linear	Quadratic	Cubic
Initial BW, kg	255	255	258	254	1.85	0.94	0.27	0.33
Final BW, kg	417^{ab}	428^{a}	427^{a}	404^{b}	5.28	0.10	< 0.01	0.71
HCW, kg	238^{a}	243^{a}	244^{a}	226^{b}	3.97	0.05	0.01	0.50
DMI, kg/d	8.80^{a}	8.98^{a}	8.21^{b}	7.35^{c}	0.204	< 0.01	0.01	0.32
ADG, kg	1.31^{ab}	1.40^{a}	1.37^{a}	1.21^{b}	0.040	0.07	< 0.01	0.97
G:F	0.149	0.156	0.166	0.166	0.0033	< 0.01	0.26	0.34
NE_m	1.82^{a}	1.86^{a}	1.98^{b}	2.03^{b}	0.027	< 0.01	0.78	0.21
NE_g	1.19^{a}	1.22^{a}	1.33^{b}	1.37^{b}	0.024	< 0.01	0.78	0.21

[1] Differences in dietary treatments were due to CARS inclusion (0, 2.5, 5, or 7.5% of diet DM).
[abc] Within a row, means a without a common superscript differ ($P < 0.05$).

Table 3.5 Organ weight and organ weight as a percent of shrunk BW (SBW) of steers and heifers individually fed increasing inclusions of Condensed Algal Residue Solubles (CARS)

	Treatment[1]					Contrast		
Organ[2]	0	2.5	5	7.5	SEM	Linear	Quadratic	Cubic
Final SBW	417[b]	428[a]	427[a]	404[b]	5.28	0.10	< 0.01	0.71
HCW	238[a]	243[a]	244[a]	226[b]	3.97	0.05	0.01	0.50
Spleen								
kg	1.38	1.25	1.19	1.37	0.107	0.86	0.16	0.68
% SBW	0.345	0.304	0.290	0.352	0.028	0.96	0.07	0.70
Lung								
kg	2.77	2.83	2.74	2.63	0.103	0.30	0.42	0.79
% SBW	0.693	0.688	0.670	0.679	0.030	0.61	0.79	0.74
Pancreas								
g	364[ab]	375[ab]	409[ab]	411[b]	16.2	0.02	0.77	0.45
% SBW	0.091[a]	0.091[ab]	0.100[ab]	0.106[b]	0.004	<0.01	0.45	0.54
Rumen								
kg	8.34	8.14	8.57	7.55	0.329	0.20	0.22	0.17
% SBW	2.08	1.98	2.09	1.94	0.064	0.33	0.72	0.12
Reticulum								
g	862	853	831	817	29.3	0.23	0.92	0.86
% SBW	0.215	0.208	0.203	0.210	0.006	0.46	0.25	0.75
Omasum								
kg	3.65	3.58	3.53	3.54	0.250	0.73	0.86	0.98
% SBW	0.911	0.871	0.861	0.911	0.066	0.98	0.50	0.92
Abomasum								
kg	1.25	1.36	1.41	1.27	0.059	0.63	0.04	0.63
% SBW	0.312	0.332	0.347	0.328	0.015	0.36	0.19	0.66
Duodenum								
g	273[a]	311[ab]	326[b]	276[ab]	19.0	0.77	0.03	0.63

% SBW	0.069	0.076	0.080	0.071	0.005	0.58	0.11	0.67
Ileum								
g	123	125	232	122	62.3	0.70	0.36	0.24
% SBW	0.031	0.031	0.055	0.032	0.015	0.67	0.41	0.25
Cecum								
g	323	328	323	364	26.5	0.33	0.50	0.65
% SBW	0.081	0.080	0.079	0.093	0.006	0.21	0.23	0.60
Kidneys								
kg	1.16	1.19	1.25	1.21	0.050	0.34	0.56	0.53
% SBW	0.291	0.288	0.307	0.312	0.013	0.16	0.76	0.52
Urinary Bladder								
g	109^{ab}	116^{a}	96.4^{b}	109^{ab}	6.87	0.51	0.67	0.05
% SBW	0.025	0.028	0.024	0.028	0.002	0.57	0.82	0.07
Liver								
kg	6.76^{a}	6.73^{a}	7.36^{b}	7.98^{c}	0.200	< 0.01	0.11	0.46
% SBW	1.69^{abc}	1.63^{b}	1.80^{c}	2.05^{d}	0.039	< 0.01	< 0.01	0.50
Brain								
g	375^{ab}	379^{ab}	387^{a}	356^{b}	7.99	0.17	0.04	0.25
% SBW	0.094	0.093	0.095	0.092	0.003	0.74	0.83	0.43
Pituitary								
g	2.84	2.82	2.76	2.73	0.095	0.37	0.94	0.87
% SBW	0.00007	0.00007	0.00007	0.00007	0.00003	0.80	0.36	0.79
Thyroid								
g	23.4^{a}	31.8^{b}	29.1^{b}	27.4^{ab}	1.95	0.28	0.02	0.16
% SBW	0.006^{a}	0.008^{b}	0.007^{ab}	0.007^{ab}	5.0×10^{-4}	0.04	0.04	0.13
Adrenal								
g	26.5	25.5	22.0	23.6	1.75	0.13	0.48	0.33
% SBW	0.007	0.006	0.005	0.006	4.0×10^{-4}	0.21	0.24	0.32
Eye[3]								
g	28.8	28.3	30.3	29.2	0.86	0.43	0.76	0.16
% SBW	0.007^{ab}	0.007^{a}	0.007^{ab}	0.008^{b}	3.0×10^{-4}	0.01	0.26	0.18

Thymus								
g	355	379	385	413	47.9	0.41	0.97	0.87
% SBW	0.088	0.092	0.093	0.107	0.012	0.27	0.70	0.78
Seminal Vesicle[4]								
g	38.6	35.3	21.5	32.6	8.09	0.28	0.28	0.19
% SBW	0.006	0.007	0.004	0.005	0.001	0.49	0.99	0.25
Prostate[4]								
g	90.0	70.8	82.9	92.5	11.05	0.70	0.22	0.51
% SBW	0.022	0.017	0.019	0.023	0.003	0.57	0.11	0.56
Ovaries[5]								
g	27.0	17.9	14.3	15.6	6.25	0.20	0.42	0.98
% SBW	0.007	0.005	0.004	0.004	0.002	0.21	0.38	0.96
Uterus[5]								
g	249	317	280	295	24.5	0.38	0.29	0.17
% SBW	0.064	0.079	0.070	0.078	0.006	0.26	0.51	0.16
Jejunum								
kg	5.08	5.36	5.38	5.86	0.240	0.04	0.70	0.50
% SBW[6]	1.27	1.31	1.32	1.51	0.0540	< 0.01	0.18	0.40
Gall Bladder								
g[7]	59.3	79.2	84.8	63.7	7.31	0.57	< 0.01	0.71
% SBW[6]	0.00147	0.00192	0.00210	0.00164	0.0018	0.41	0.02	0.65
Heart								
kg[7]	1.79	1.99	1.88	2.08	0.0805	0.05	0.97	0.01
% SBW[6]	0.449	0.485	0.462	0.535	0.019	0.01	0.34	0.08
Colon								
kg[6]	4.10	4.52	4.37	4.32	0.24	0.64	0.33	0.54
% SBW[6]	1.03	1.11	1.08	1.11	0.0629	0.42	0.73	0.53

[1] Differences in dietary treatments were due to CARS inclusion (0, 2.5, 5, or 7.5% of diet DM).

[2] All organ weights reported after separating and washing individual organs. Organ weight as a percent of SBW was calculated by washed organ weight divided by final BW with a 4% shrink.

[3] Weight from one eye is reported.

[4] Steers only
[5] Heifers only
[6] Sex×treatment interactions were significant and are reported in Table 6.
[ab] Within a row, means a without a common superscript differ ($P < 0.05$).

Table 3.6 Organ weight and organ weight as a percent of shrunk BW (SBW) of steers and heifers individually fed increasing inclusions of Condensed Algal Residue Solubles (CARS) for organs having a sex by treatment interaction[1]

	Steer				Heifer					*P*-value		
Organ[2]	0	2.5	5	7.5	0	2.5	5	7.5	SEM	TRT	Sex	TRT×Sex
Jejunum												
kg	5.37^{b}	5.31^{b}	5.06^{b}	6.33^{a}	4.78^{b}	5.42^{ab}	5.69^{ab}	5.39^{b}	0.318	0.13	0.39	0.08
% SBW	1.29^{bcd}	1.26^{bcd}	1.20^{d}	1.59^{a}	1.24^{cd}	1.35^{bcd}	1.43^{ab}	1.42^{abc}	0.007	<0.01	0.60	0.04
Colon												
kg	4.51^{ab}	4.12^{ab}	3.89^{b}	4.38^{ab}	3.69^{b}	4.93^{a}	4.86^{a}	4.26^{ab}	0.305	0.58	0.34	0.02
% SBW	1.10^{ab}	0.980^{b}	0.929^{b}	1.10^{ab}	0.956^{b}	1.23^{a}	1.22^{a}	1.12^{ab}	0.081	0.70	0.07	0.04
Gall Bladder												
g	64.2^{bc}	81.6^{ab}	62.7^{bc}	51.4^{c}	54.4^{c}	76.8^{bc}	107^{a}	75.9^{bc}	9.14	0.03	0.46	0.02
% SBW	0.015^{bc}	0.019^{abc}	0.015^{bc}	0.013^{c}	0.014^{bc}	0.019^{abc}	0.027^{a}	0.022^{ab}	0.002	0.04	<0.01	0.01
Heart												
kg	1.83^{bcd}	1.98^{abcd}	1.70^{d}	2.21^{a}	1.76^{cd}	2.01^{abc}	2.07^{ab}	1.95^{abcd}	0.103	0.05	0.79	0.04
% SBW	0.444^{cd}	0.470^{bcd}	0.404^{d}	0.554^{a}	0.454^{bcd}	0.500^{abc}	0.519^{abc}	0.515^{ab}	0.024	<0.01	0.10	0.03

[1] Differences in dietary treatments were due to CARS inclusion (0, 2.5, 5, or 7.5% of diet DM).

[2] All organ weights reported after separating and washing individual organs. Organ weight as a percent of SBW was calculated by washed organ weight divided by final BW with a 4% shrink.

[abcd] Within a row, means a without a common superscript differ ($P < 0.05$).

Table 3.7 Hematology of cattle fed increasing inclusions of Condensed Algal Residue Solubles (CARS)

	Treatment							Contrast		
Item	0	2.5	5	7.5	Expected Range[1]	Unit	SEM	Linear	Quadratic	Cubic
WBC[2]	10.2	10.3	9.3	10.7	4.0-12.0	$\times 10^3$/ul	0.494	0.79	0.23	0.13
RBC[3]	8.64	8.59	8.20	8.33	5.0-10.0	$\times 10^6$/ul	0.190	0.32	0.60	0.98
Hemoglobin	12.5	12.1	12.2	12.7	8.0-15.0	g/dL	0.300	0.54	0.05	0.94
Hematocrit	35.0	33.7	34.0	35.4	24.0-46.0	%	0.683	0.65	0.05	0.88
MCV[4]	39.5	38.9	40.3	40.1	40.0-60.0	fl	0.874	0.44	0.81	0.38
MCH[5]	13.6	13.5	13.9	13.8	11.0-17.0	pg	0.318	0.57	0.94	0.39
MCHC[6]	35.5	35.5	35.6	35.7	30.0-36.0	g/dL	0.0960	0.40	0.62	0.79
RDW[7]	20.9	21.2	21.3	22.0	8.0-15.0	%	0.331	0.02	0.53	0.61
Platelet Count	479	445	477	395	100-800	$\times 10^3$/ul	32.9	0.14	0.46	0.23
MPV[8]	7.52	7.50	7.47	8.27	5.0-8.0	fl	0.370	0.19	0.27	0.62
Neutrophil	3.17	3.43	2.86	3.37	0.6-4.0	$\times 10^3$/ul	0.309	0.99	0.69	0.18
Lymphocyte	6.26	5.80	5.65	5.73	2.5-7.5	$\times 10^3$/ul	0.327	0.24	0.41	0.98
Monocyte	0.442	0.407	0.458	0.532	0.03-0.85	$\times 10^3$/ul	0.0418	0.09	0.20	0.74
Eosinophil	0.107	0.0592	0.0760	0.0568	0.0-2.4	$\times 10^3$/ul	0.0288	0.31	0.63	0.44
Basophil	0.0357	0.0289	0.0330	0.03412	0.0-0.2	$\times 10^3$/ul	0.0080	0.98	0.62	0.70
Plasma Protein	8.19	8.14	8.35	8.21	6.9-7.7	g/dL	0.0807	0.49	0.57	0.11
Fibrinogen	516	582	458	462	100-500	mg/dL	31.0	0.19	0.53	0.89

[1]Reference intervals were established at Iowa State University's Clinical Pathology Laboratory (Veterinary Pathology, 2011).
[2]WBC – white blood cells
[3]RBC – red blood cells
[4]MCV- mean corpuscular volume
[5]MCH- mean corpuscular hemoglobin
[6]MCHC- mean corpuscular hemoglobin concentration
[7]RDW- red blood cell distribution width
[8]MPV-mean platelet volume

Table 3.8 Blood chemistry of cattle fed increasing inclusions of Condensed Algal Residue Solubles (CARS)

	Treatment							Contrast		
Item	0	2.5	5	7.5	Expected Range[1]	Unit	SEM	Linear	Quadratic	Cubic
ALT[2]	54.14	52.65	47.8	51.28	n/a	IU/L	1.55	0.06	0.12	0.09
Sodium	143.5	142.5	143.0	142.7	133-147	mEq/L	0.423	0.28	0.44	0.28
Potassium	4.73	4.71	4.80	4.69	3.7-5.3	mEq/L	0.0460	0.86	0.34	0.14
Chloride	101	101	100	99.9	94-109	mEq/L	0.289	<0.01	0.52	0.72
Bicarbonate	27.2	28.1	28.3	28.6	19.5-30.3	mEq/L	0.355	<0.01	0.40	0.64
Calcium	10.2	10.2	10.3	10.4	8.2-10.1	mg/dL	0.0940	0.25	0.80	0.67
Phosphorus	8.28	8.14	8.02	8.25	3.8-7.9	mg/dL	0.158	0.75	0.26	0.63
Magnesium	2.00	1.98	2.07	2.00	2.10-2.90	mg/dL	0.0266	0.51	0.34	0.03
BUN[3]	10.2	10.1	9.91	9.68	7-32	mg/dL	0.502	0.43	0.93	0.98
Creatinine	1.07	1.07	1.16	1.16	0.7-1.9	mg/dL	0.0240	<0.01	0.90	0.13
Glucose	82.5	84.3	84.7	86.3	40-100	mg/dL	2.26	0.25	0.97	0.78
Total Protein[4]	7.39	7.32	7.49	7.42	6.7-8.7	g/dL	0.062	0.38	0.95	0.10
Albumin	3.15	3.15	3.27	3.21	3.2-3.9	g/dL	0.0388	0.12	0.48	0.09
AST[5]	83.3	81.8	81.7	84.8	68-156	IU/L	5.04	0.85	0.65	0.93
Creatine Kinase	211	151	159	217	1-350	IU/L	23.3	0.81	0.02	0.87
ALP[6]	65.4	66.4	53.6	43.7	29-136	IU/L	4.68	<0.01	0.25	0.43
GGT[7]	46.8	35.1	38.9	45.1	1-50	IU/L	2.00	0.90	<0.01	0.16
Total Bilirubin[3]	0.340	0.305	0.366	0.342	0-0.18	mg/dL	0.0130	0.14	0.83	<0.01
Total Bile Acids	42.4	28.5	31.0	34.6	n/a	umol/L	5.21	0.38	0.10	0.51
Cholesterol	99.6	110	111	113	78-120	mg/dL	5.22	0.07	0.41	0.59
LDH[8]	4268	4092	4173	4494	280-410	IU/L	11.4	0.15	0.04	0.97

[1]Reference intervals were established at Iowa State University's Clinical Pathology Laboratory (Veterinary Pathology, 2011).
[2]ALT – alanine aminotransferase
[3] BUN – blood urea nitrogen
[4]Treatment×sex interaction was significant (interaction was removed from the statistical model if no interaction was present).
[5]AST – aspartate aminotransferase
[6]ALP – alkaline phosphatase

[7]GGT – gamma-glutamyl transpeptidase
[8]LDH – lactate dehydrogenase

Table 3.9 Urine analysis of cattle fed increasing levels of Condensed Algal Residue Solubles (CARS)

	Treatment					Contrast		
Item	0	2.5	5	7.5	SEM	Linear	Quadratic	Cubic
pH	7.96[a]	8.53[b]	8.70[b]	8.56[b]	0.114	<0.01	<0.01	0.86
Specific Gravity	0.808	0.808	0.811	0.811	0.001	0.96	0.99	0.98

[ab] Within a row, means a without a common superscript differ ($P < 0.05$)

Chapter IV. Nutrient Digestibility of Condensed Algal Residue Solubles in Beef Cattle Fishing Diets

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ABSTRACT

Condensed algal residue solubles (CARS) were evaluated in finishing cattle diets. Six treatments were evaluated (2 × 3 factorial arrangement), CARS inclusion in the diet at 0, 5, or 10% of diet dry matter with 0 or 20% wet distillers grains. The remainder of the diets consisted of 57.5 to 87.5% dry rolled corn, 7.5% sorghum silage and 5% supplement. Increasing wet distillers grains in the diet had no effect on dry matter and organic matter intake ($P \geq 0.16$), but decreased dry matter digestibility ($P < 0.01$) and organic matter digestibility ($P < 0.01$) by 4.2 and 5.5%, respectively. Increasing CARS inclusion in the diet resulted in a linear decrease ($P \leq 0.01$) in dry matter and organic matter intake, with no effect on dry matter and organic matter digestibility ($P \geq 0.29$). Replacing up to 10% dry rolled corn with CARS in diets with or without wet distillers grains had little effect on digestibility of finishing beef cattle diets.

Keywords: algae, byproduct, fat

INTRODUCTION

Feeding algae to animals is not a new idea, as algae has been used in animal diets dating back 60 years; however, until recently heterotrophic algae has not been commonly used. A condensed algal residue solubles (CARS; Veraferm, Veramaris, Delft, The Netherlands) product is being commercially produced from heterotrophic algae as a co-product from producing n-3 fatty acids for aquaculture and the pet food industry. CARS was fed in a 100 d safety study where cattle fed between 2.5 and 5% CARS had similar hot carcass weight (HCW), average daily gain (ADG), and dry matter intake (DMI), with poorer gain to feed (G:F) compared to control cattle (Norman et al., 2018). The novel co-product CARS now has expert-affirmed GRAS (generally recognized as safe) status and with limited research done on this product, the objective of this study was to evaluate the digestibility of CARS at different inclusion levels, with and without wet distillers grains plus solubles (WDGS), in finishing cattle diets.

MATERIALS AND METHODS

The following experiment was conducted at the University of Nebraska Animal Science Complex (Lincoln, NE). Animal handling and space for the experiment were in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). All procedures outlined as part of this study were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Because CARS was not approved by the FDA to be fed to cattle entering the human food chain at the time of this study, all cattle were composted at completion of the experiment.

Experimental Design

A digestibility study was conducted utilizing 6 steers in a 6 × 6 Latin square design to evaluate the effects of inclusion of condensed algal residue solubles (CARS; Table 4.1). Treatments were set up as a 2 × 3 factorial arrangement with 2 levels of wet distillers grains (0 or 20% WDGS), and 3 levels of CARS (0, 5, and 10% on a DM basis). Therefore the feeding trial consisted of six, 21 day periods, resulting in each animal receiving each of the 6 diets over the feeding trial. As a novel feed ingredient palatability was of concern, therefore WDGS was included to determine if it would help with diet conditioning or feed intake. The remainder of the diets consisted of 57.5 to 87.5% dry rolled corn, 7.5% sorghum silage and 5% supplement on a DM basis (Table 4.2). Supplement consisted of limestone, vitamin A-D-E, beef trace minerals, urea in the 0% WDGS diets, and fine ground corn as the carrier. Cattle were fed *ad libitum* with feed delivered twice daily. Steers were housed in 2.1 × 3.7 m slatted floor pens and offered ad libitum water. Each period was 21 days in length consisting of 16 d adaption and a 5 d collection period. These animals were not fistulated therefore TiO_2 was top dressed, and rumen pH measurements were not taken. On d 10-21 of each period, 5 g of TiO_2 in a 100 ml mixture of distillers solubles was top dressed on the feed at each feeding for a total of 10 g of TiO_2 dosed daily. On d 16-21, fecal grab samples were collected 4 times daily at 0700, 1100, 1500 and 1900 h. Hourly fecal samples were composited by day on an equal wet weight basis. Daily fecal samples and feed ingredient samples were then freeze dried (Virtis Freezemobile 25ES, SP industries, Warminster, PA). Feed samples and fecal samples, ground through a 1 mm screen (No. 4, Thomas Scientific, Swedesboro, NJ), and

daily fecal composites were then composited on a dry weight basis by animal within period to create a period composite from the freeze dried samples. Period fecal composite and feed ingredient composited by period were analyzed for DM, OM, and NDF. Neutral detergent fiber analysis was conducted using the procedure described by Van Soest et al. (1991) with modifications to the analysis of corn and byproducts described by Buckner et al. (2013).

Furthermore, fecal samples were analyzed for titanium dioxide concentration (Spectra MAX 250, Molecular Devices, LLC, Sunnyvale, CA; Myers et al., 2004). Concentration of TiO_2 was then used to calculate fecal DM output using the following equation: [(g TiO_2 dosed per d) / (concentration of TiO_2 in feces)] (Meyers et al., 2004). Total tract digestibility was calculated using the following equation: (Cochran and Galyean, 1994) [(kg of nutrient fed – kg of nutrient refused – kg of nutrient in feces) / (kg of nutrient fed – kg of nutrient refused)] × 100.

Digestibility data were analyzed as a Latin Square using the mixed procedure of SAS (SAS Inst., Cary, N.C.) with period, WDGS, CARS, and the interaction between WDGS and CARS as fixed effects and steer as a random effect. Treatment differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

A CARS inclusion by WDGS interaction was observed for NDF digestibility (NDFD; $P < 0.01$). Steers fed 5% CARS and 20% WDGS had a NDFD of 55.0%, which was greater than the rest of the treatment diets that ranged from 39.5 to 44.7% NDFD

(Figure 4.1). Due to the soluble nature of the NDF content in CARS, it is difficult to get accurate estimates of NDF intake and NDFD, therefore it is not likely that the interaction has much biological importance. No other interactions were observed for CARS inclusion by distillers grain level ($P \geq 0.39$).

For the main effect of CARS, a linear decrease was observed for DMI ($P = 0.01$; Table 3), with 0 and 5% CARS having similar DMI at 8.3 and 8.1 kg/d respectively, and 10% CARS having lower DMI at 7.3 kg/d. In a separate experiment, feeding algae meal and post-extracted algal residue (PEAR) to cattle increased DMI (Stokes et al., 2016, Morrill et al., 2017). A linear decrease was observed for both OM intake and NDF intake ($P \leq 0.01$) as CARS increased from 0 to 10% in the diet. Morrill et al. (2017) also observed a reduction in NDF digestibility when PEAR was included in the diet at the rate of 1 kg OM/d. The CARS feed has a high Na content (Table 4.1), which may limit intake at inclusions greater that 5% of the diet. There were no differences observed for DMD or OMD ($P \geq 0.29$) across all three levels of CARS inclusion. The only statistical difference between steers fed 0% CARS and 5% CARS was NDF digestibility suggesting that CARS has a similar feeding value as dry rolled corn at 5% inclusion. These results are supported by 2 cattle performance experiments with inclusion of CARS at 2.5% and 5% of the diet (Gibbons et al., 2021; Norman et al., 2018).

For the main effect of WDGS, no differences were observed for DMI or OMI ($P \geq 0.16$; Table 4). Steers fed 0% WDGS had greater DMD ($P < 0.01$) at 76.7% compared to 72.5 % for steers fed 20% WDGS. Similarly, steers fed 0% WDGS had greater OMD ($P < 0.01$) at 78.2%, compared to 74.6% for steers fed 20% WDGS. Steers fed 20%

WDGS had greater NDF intake at 2.0 kg/d ($P < 0.01$) compared to 1.6 kg/d for steers fed 0% WDGS.

IMPLICATIONS

Results indicate decreased DMI and OMI as CARS inclusion level increased in the diet, however, this had no effect on DMD or OMD. This would agree with performance results when cattle were fed up to 7.5% CARS (Norman et al., 2018; Gibbons et al., 2021). Replacing up to 5% corn with CARS in finishing cattle diets with wet distillers grains at 0 or 20% diet DM, appears to have little effect on DMI, DMD, OMI or OMD. Further research is needed to determine the optimal inclusion of CARS in finishing cattle diets on performance, carcass characteristics, and fatty acid profiles of beef.

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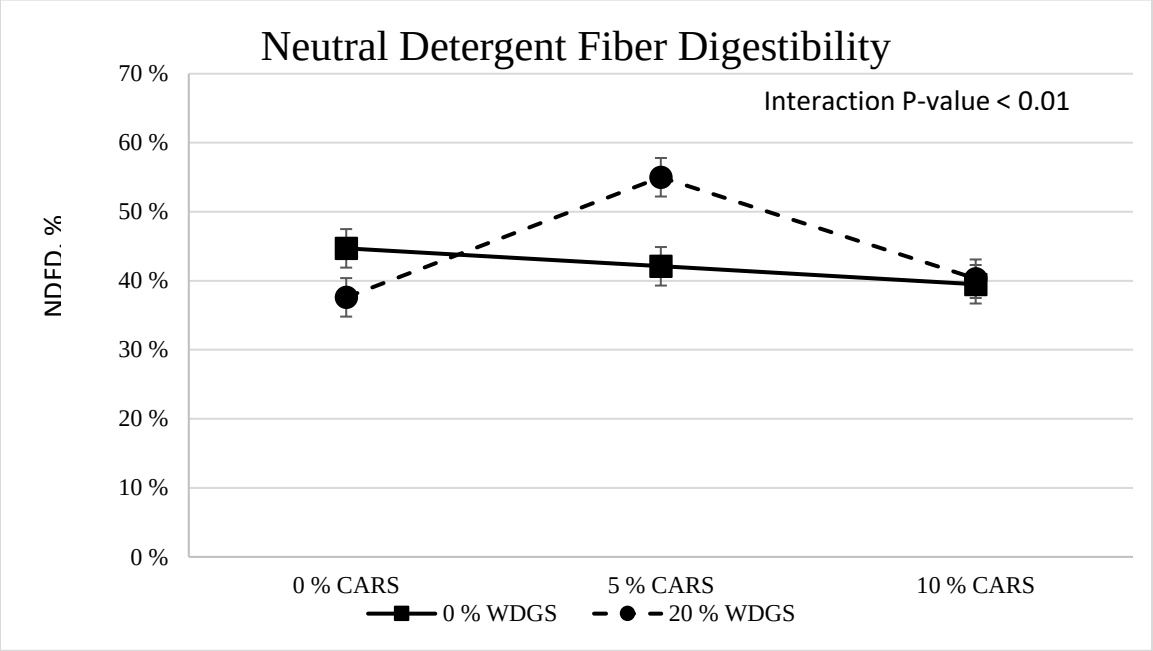

Figure 4.1 Interaction between condensed algal residue solubles (CARS) and wet distillers grains plus solubles (WDGS) inclusion in the diet on neutral detergent fiber digestibility.

Table 4.1 Nutrient composition of Condensed Algal Residue Solubles (CARS)

Item	CARS[1]
DM, %	41.7
%, DM basis	
CP	29.3
NDF	34.6
ADF	2.3
Ca	0.16
P	0.82
K	1.51
S	2.54
Na	8.52
mg/kg, DM basis	
Mg	0.33
Zn	43.87
Fe	86.33
Mn	13.5
Cu	6.00
Mo	0.69

[1]Nutreint composition of CARS was analyzed by Ward Laboratories, Inc. (Kearney, NE).

Table 4.2 Diet composition (DM basis) for finishing cattle fed 3 levels of CARS with 0 or 20% WDGS

	0 CARS[1]		5 CARS[1]		10 CARS[1]	
Item, %	0 WDGS[2]	20 WDGS[2]	0 WDGS[2]	20 WDGS[2]	0 WDGS[2]	20 WDGS[2]
WDGS	-	20	-	20	-	20
CARS	-	-	5	5	10	10
DRC	87.5	67.5	82.5	62.5	77.5	57.5
Sorghum Silage	7.5	7.5	7.5	7.5	7.5	7.5
Supplement[3]	5.0	5.0	5.0	5.0	5.0	5.0
Fine Ground Corn	1.264	2.824	1.844	3.134	2.404	3.134
Limestone	1.690	1.670	1.690	1.660	1.680	1.660
Tallow	0.125	0.125	0.125	0.125	0.125	0.125
Urea	1.540	-	1.260	-	0.710	-
Salt	0.300	0.300	-	-	-	-
Trace mineral	0.050	0.050	0.050	0.050	0.050	0.050
Rumensin	0.016	0.016	0.016	0.016	0.016	0.016
Vitamin ADE	0.015	0.015	0.015	0.015	0.015	0.015
Nutrient Composition, %						
DM	77.0	59.0	73.0	56.6	69.4	54.6
OM, % DM	98.1	97.3	96.3	95.5	94.5	93.7
CP, % DM	12.81	12.98	12.83	13.77	12.10	14.53
Fat, % DM	3.72	5.19	4.26	5.69	4.77	6.18
Na, % DM	0.15	0.18	0.68	0.71	1.33	1.36
S, % DM	0.11	0.22	0.15	0.26	0.19	0.30

[1] Treatment, % CARS, (DM basis); CARS = condensed algal residue solubles

[2] Treatment, % WDGS, (DM Basis); WDGS = wet distillers grains plus solubles

[3] Supplement targeted Rumensin at 330 mg/animal daily; (Elanco, Greenfield, IN) and Vitamin A-D-E premix contained 1500 IU vitamin A, 3000 IU vitamin D, and 3.7 IU vitamin E per g. Trace mineral premix contained 10% Mg, 6% Zn, 4.5% Fe, 2% Mn, 0.5% Cu, 0.3% I, and 0.05% Co.

Table 4.3 Main effects of condensed algal residue solubles (CARS) inclusion on digestibility of cattle finishing diets

	TREATMENT, % CARS				*P*-value		Contrast[2]	
Item	0	5	10	SEM	CARS	CARS× WDGS	Lin	Quad
Dry Matter								
Intake, kg	8.3[a]	8.1[a]	7.3[b]	0.31	0.03	0.41	0.01	0.39
Digestibility, %	75.7	74.2	73.9	1.41	0.52	0.82	0.29	0.67
Organic Matter								
Intake, kg	8.2[a]	7.7[ab]	6.8[b]	0.31	0.01	0.55	< 0.01	0.63
Digestibility, %	77.3	75.8	76.0	1.29	0.57	0.87	0.41	0.51
NDF[1]								
Intake, kg	2.0	1.9[a]	1.6[b]	0.07	< 0.01	0.39	< 0.01	0.62
Digestibility[1], %	41.1	48.6	38.9	1.97	0.01	< 0.01	0.65	< 0.01

[a-b] Values within rows with similar superscript are not different ($P > 0.05$)

[1]Neutral detergent fiber (NDF) digestibility interaction between condensed algae residue soluble (CARS) and distillers grain inclusion shown in Figure 1

[2]Lin = linear orthogonal contrast for CARS inclusion in the diet; Quad = quadratic orthogonal contrast for CARS inclusion

Table 4.4 Main effects of wet distillers grains plus solubles (WDGS) inclusion on digestibility of cattle finishing diets

	WDGS			*P*-Value	
Item	0 %	20 %	SEM	WDGS	CARS×WDGS
Dry Matter					
Intake, kg	7.7	8.2	0.27	0.16	0.41
Digestibility, %	76.7	72.5	1.25	< 0.01	0.82
Organic Matter					
Intake, kg	7.4	7.8	0.27	0.17	0.55
Digestibility, %	78.2	74.6	1.14	< 0.01	0.87
NDF[1]					
Intake, kg	1.6	2.0	0.06	< 0.01	0.39
Digestibility[1], %	42.1	44.3	1.61	0.34	< 0.01

[1]Neutral detergent fiber (NDF) digestibility interaction between condensed algae residue solubles (CARS) and distillers grain inclusion shown in Figure 1