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Evaluation of Condensed Algal Residue Solubles as an Ingredient in Cattle Finishing Diets and Its Effects on Digestibility and Fatty Acid Flow and a Comparison of Single and Dual Implant Strategies in Finishing Heifers

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EVALUATION OF CONDENSED ALGAL RESIDUE SOLUBLES AS AN INGREDIENT IN CATTLE FINISHING DIETS AND ITS EFFECTS ON DIGESTIBILITY AND FATTY ACID FLOW AND A COMPARISON OF SINGLE AND DUAL IMPLANT STRATEGIES IN FINISHING HEIFERS

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Two experiments, a performance study (Exp. 1) and digestion study (Exp. 2) were conducted to evaluate a novel liquid feed, condensed algal residue solubles (CARS), in feedlot diets. In Exp. 1 and Exp. 2, steers were fed three inclusions of CARS at 0%, 2.5%, 5%. There were quadratic responses for greater carcass adjusted final BW, ADG, G:F, HCW, 12th rib fat, and yield grade. There was a linear increase in marbling score and a linear decrease in DMI and LM area as CARS increased. In Exp. 2, CARS did not affect intake or digestibility. Fatty acid flow at the duodenum was unaffected as CARS increased in the diet, though the fatty acid profile changed, with a linear increase of unsaturated and omega-3 fatty acids. Including CARS up to 2.5% of the diet DM improved feed efficiency. The CARS can effectively be included in feedlot finishing diets without affecting digestibility while influencing duodenal fatty acid flow composition.

Experiment 3 compared the effects of a single implant strategy utilizing a partially coated, long release heifer implant to a three non-coated implant strategy over an average of 215 d. Treatments included Revalor-XH or Revalor-IH, followed by Revalor-H followed by Revalor-

200. There were no significant differences between treatments for live or carcass adjusted final BW, DMI, ADG, or G:F. There were no significant differences between treatments for HCW, 12th rib fat, dressing percent, or calculated yield grade. Heifers that received Revalor-XH, when fed 215 d, perform similarly to heifers on a 3-implant Revalor-IH/H/200 program.

Key words: beef cattle diets, algae, novel feed, fatty acid flow, heifer implant, delayed release

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Dedication

“If any of you lack wisdom, let him ask of God, that giveth to all men liberally, and upbraideth not; and it shall be given him”

-James 1:5

“ … all that he requires of you is to keep his commandments; and he has promised you that if ye would keep his commandments ye should prosper in the land; and he never doth vary from that which he hath said…”

- Mosiah 2:22

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Introduction

Marine algae are being harvested as a source of omega-3 and omega-6 fatty acids to supplement humans, pets and aquaculture. Recent developments to improve the fermentation process of algae has allowed for higher quality supplements, and has helped to reduce the impact on wild caught fish, which has been a traditional source of omega-3 and omega-6 fatty acids (Adarme-Vega et al., 2014). Both the products and byproducts of microalgae cultivation and harvest are being explored as a feed ingredient for dairy and beef cattle. Algae can be used as a protein or lipid supplement, though the inclusion of algae is often limited by the high ash content, generally being high in sodium. However, the literature supports that algae can be successfully incorporated into dairy cattle diets without detrimental effects (Franklin et al. 1999; Stamey et al; 2012).

In 2018, production of marine microalgae for omega-3 supplementation for pets and aquaculture began near Blair, NE. Residue from the production process, known as condensed algae residue solubles (CARS; Veramaris, The Netherlands;) has been granted generally recognized as safe (GRAS) status after a trial by Norman et al. (2018). The authors concluded that CARS had no adverse effect on cattle with improved performance when fed up to 5.0% of diet DM. While live performance was measured the data was not intended to test performance, requiring only a 90 d minimum exposure required by for GRAS status. In a follow up experiment, the nutrient digestibility of CARS in finishing diets was evaluated to determine an optimal inclusion level (Norman, 2019). Norman (2019) concluded that CARS included up to 5% of the diet DM had little effect on DM and OM intake and digestibility.

Implanting heifers improves growth performance as well as hot carcass weight compared to heifers that received no implant (Adams et al. 1990; Cleale et al. 2013). Heifers at the same age tend to have increased fat deposition compared to steers, thus more aggressive implant strategies to increase the potency and exposure to trenbolone acetate (TBA) and estradiol (E2), are typically used in feedlot finishing heifers. However, when comparing implant strategies among heifers, the literature suggests that the total amount of TBA and E2 or estradiol benzoate (EB) given throughout the feeding period does not always result in greater performance (Folmer et al. 2009; Hilscher et al. 2016). Single dose heifer implants with delayed payouts of TBA and E2 have been shown to perform similar to other heifer implant strategies which use single or multiple non-coated implants over the feeding period, though results can vary depending on the days on feed (Crawford et al. 2018; Schumacher et al. 2019; Smith and Johnson, 2020).

CHAPTER I. Review of the Literature

Lipids

Lipids in the Beef Industry

Relative to carbohydrates and protein, lipids are energy dense and can be relatively inexpensive (NASEM, 2016). From a management perspective, lipids can also help hold mixed rations together and improve or limit intake based on the interactions with microbial populations (Lourenço et al., 2010). Once in the body, energy is easily stored as fat in the peripheral tissues of the animal which can then be released and used when needed. Lipids have many functions, for example fat is necessary for the utilization of fat-soluble vitamins, regulation of body temperature, and can effect reproductive health (Zinn and Jorquera, 2007). These energy stores also contribute to the taste and tenderness of meat (Furnols and Guerrero, 2014) and may provide health benefits to human consumers (Sinclair et al. 2006).

As a brief explanation, lipid supplements differ by the way they contribute to the biological processes of the animal. For example, soybean oil, which contains a higher concentration of poly-unsaturated fatty acids (PUFA) compared to corn oil may inhibit the growth of cellulolytic microbes (Pantoja et al., 1994; Zinn and Jorquera, 2007). Evidence suggest that high concentrations of free fatty acids (FFA) can inhibit ruminal biohydrogenation activity, and increases intestinal digestion of UFAs (Zinn and Jorquera, 2007).

Lipid in the Rumen

Fatty acids in dietary fat come in many different forms: free fatty acids, long or short chained fatty acids, mono, di, or tri-acylglycerides (fatty acid attached to a glycerol backbone),

or saturated (SFA), unsaturated (UFA), and poly-unsaturated fatty acids (PUFA). In forage diets, fatty acids are primarily PUFA (albeit lower in concentration) and in finishing diets UFAs tend to dominate the lipid profile. Fat can be supplemented in liquid form such as with tallow, corn biproduct solubles, or vegetable oil, or may also come as a solid such as from calcium salts and distillers grains (de Mello et al., 2018; Zinn and Jorquera, 2007). In ruminants, lingual lipase from the serous and mandibular glands immediately begin breaking down triglycerides by hydrolyzing at the sn-3 position of the glycerol backbone which begins to free the fatty acids attached (Hamosh, 1986). Rumen microbes also secrete their own lipase to hydrolyze the lipid, breaking down the mono, di, or triacylglycerides into FFA and glycerol. This process leaves about 75% of the total lipid as FFA with a few mono and diacylglycerides (Jenkins et al., 2008). Research suggests these lipases come from many bacterial species such as *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens* bacteria species (Henderson, 1968; Lourenço et al., 2010; Rattray and Craig, 2007).

Biohydrogenation and other Microbial Activities

To the microbes living in the rumen, dietary UFA are toxic, acting as a detergent which negatively affects the cell wall, with PUFA being especially toxic (Pantoja et al., 1994). This toxic effect is greatest on fibrolytic bacteria, mainly responsible for forage digestion, thus limiting digestion when cattle are fed high fiber diets (Pantoja et al., 1994). In order to deal with both UFA and PUFA, some bacterial species such as *butyrivibrio fibrisolvens* have adapted a process known as biohydrogenation (Lourenço et al., 2010). Biohydrogenation replaces the double bonds with a hydrogen, thus saturating the unsaturated fatty acid (Mosley et al., 2002). This process is completed through isomerization and hydrogenation, producing stearic (C18:0)

and palmitic (C16:0) acids. Biohydrogenation, therefore, is responsible for the high concentration of SFA entering the small intestine (Bauman et al., 2003).

While biohydrogenation is an effective way to neutralize most of the toxic effects from UFA and PUFA, the process of biohydrogenation is often incomplete and results in partially biohydrogenated PUFA, as *trans*-monoene intermediates, as byproducts that flow out of the rumen (Mosley et al. 2002; Jenkins et al., 2008). In their research to examine the biohydrogenation pathway of oleic acid (C18:1 cis-9) to stearic acid, Mosley et al. (2002) reported that there were several isomers of oleic acid formed as *trans*-monoenes along with stearic acid. These products, mainly as C18:1 *trans*-6, *trans*-7 as well as all *trans*- 9-16 positions, signified that the process of biohydrogenation was incomplete. Shortland et al. (1957), while working to understand biohydrogenation effects of the ruminal microbes, also confirmed the conversion of oleic, linoleic (C18:2 *cis*-9,12) or linolenic acid (C18:3 *cis*-9,12,15) to stearic acid with *trans* isomers. However, they noted that biohydrogenation effects on oleic acid to stearic acid were least efficient, with only 59.0% of the original oleic acid remaining un-hydrogenated, while of the linoleic and linolenic acids, 18.9 and 0.7%, respectively, were not altered. Of the portions that were not biohydrogenated, the *trans*-monoene formation was 17.2% in oleic acid, with 47.9 and 67.3% for Linoleic and Linolenic, respectively.

These partially biohydrogenated linoleic and linolenic fatty acids are also known as conjugated linoleic acids (CLA) and are known to have certain health benefits in human consumption (Duckett et al., 2002). The CLA's are also available for absorption in the small intestine (Bauman et al., 2003; Zinn and Jorquera, 2007). Common CLAs include C18:2 *cis*-9,*trans*-11, C18:2 *cis*-9,*cis*-11, and C18:1 *trans*-11, along with other intermediates (Noble et al., 1974). Factors that can influence rumen biohydrogenation activity include ruminal pH, physical

form of the lipid, the fatty acid profile of the feed, level of supplementation, and rate of passage (Zinn and Jorquera, 2007). Factors may also include metabolic hydrogen acceptors in the rumen such as CO_2, or fumarate (Polan et al. 1964).

Lipids Post Rumen

Once in the small intestine, the process of digestion is similar for both ruminants and non-ruminants, with one major difference: ruminants can digest saturated fatty acids more effectively than non-ruminants because of a lower duodenal pH along with greater bile secretions (Zinn and Jorquera, 2007). Bile emulsification begins in the duodenum and helps to separate the lipids from the feed particles, while pancreatic lipase hydrolyze the lipid that were not hydrolyzed previously (NASEM, 2016). Pancreatic phospholipase works on phospholipids to create lysophospholipids that are necessary for both incorporating and solubilizing fatty acids into micelles (NASEM, 2016). Bile salts, lecithin, and pancreatic juices work together to form the micelles (Bauman et al., 2003) and can proceed to the jejunum for absorption.

Free fatty acids, along with lysolecithins and cholesterols are absorbed across the plasma membrane of the enterocyte and are re-esterified (re-attached to a glycerol) into di and triglycerides in order to be incorporated (Lock et al., 2006) into a chylomicron. Chylomicrons, also known as lipoproteins, are the transporter molecule and are made up of triacylglycerols, cholesterol esters, phospholipids along with proteins and fat soluble vitamins. The chylomicron is then transported in the lymph to the blood where it distributes the lipid contents into tissues throughout the body before reaching the liver (Bauchart, 1992). Lipoprotein lipase (LPL) found on the surface of endothelium cells of the capillaries extracts the triacylglycerols to be deposited into the cell (Bauchart, 1992). Once absorbed in the adipose cell, fatty acids and diacylglycerides

are rejoined and stored as triacylglycerol until needed (Bauchart, 1992). Conjugated linoleic acids, after passing through the rumen, are stored in the meat or in mammary glands in concentrations ranging from 1.2 to 10.0 mg/g of lipid (Schmid et al., 2006). Availability of CLAs to be stored depends mostly on the outflow from the rumen, while within peripheral tissues, desaturation effects can increase the tissue's CLA content of the fatty acid profile (Duckett et al. 2002).

Increasing CLA Content in Food Products

While in meat or mammary cells, CLA can also be converted into gamma-linoleic acid by way of delta-6-desaturase, an enzyme which adds double bonds back to the lipid molecule. Gamma-linoleic acid can then be used to make other essential FA in the body, though this mechanism is not fully understood (Horrobin and Manku 1983). When evaluating CLA supplementation to increase tissue CLA content, Corl et al. (2003) fed both a control and a modified butter high in vaccenic acid (CLA 18:1 *trans*-11) and CLA *cis*-9,*trans*-11 to rats. They observed that the effects of both CLA *cis*-9,*trans*-11 and CLA *trans*-11 on the mammary fat pad were correlated to a subsequent decrease in tumor formation in the mammary gland itself. They concluded that CLA *trans*-11 and CLA *cis*-9,*trans*-11 enriched food products may be a viable way to deliver the anti-carcinogenic benefits for public consumption. The CLA *trans*-10,*cis*-12 has also been associated with anti-obesity properties (Hargrave et al., 2002). Hargrave et al. (2002) reported the effects of CLA *trans*-10,*cis*-12 in mice could produce the effects of adipocyte apoptosis to remove the adipose cells when fed in the purified form.

The process of increasing CLA in meat has been the focus of many feeding trials. Schmid et al. (2006), in a review of CLA content in meat noted that, among other factors such as animal

genetics and management strategies, diet appeared to be the most important factor determining CLA concentrations in meat. They observed that steers on pasture had greater CLA content of fat within the *longissimus* and *semitendinosus* muscle compared to steers on a concentrate diet, 13.1mg/g compared to 2.0 mg/g fatty acid methyl ester (FAME). This was attributed to the higher PUFA concentration in forage. French et al. (2000) suggested that forage diets affect the ratio of PUFA:SFA in the *longissimus* muscle, as concentrates in the diet decreased, in g/100g FAME. Schmid et al. (2006) went on to suggest that grass silage may also increase the CLA concentration in intramuscular tissue, though not as much as grass alone. They speculated that this is due to fewer sugars and soluble fibers available after ensiling which then changed the rumen microbial profile. Accounting for the fat content of the meat (as a % of meat intake) is key in determining if changes in the fatty acid profile (as a % of the fat) will affect human consumption of those fatty acids (g per day).

Duckett et al. (2002) studied the effects of feeding high-oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. They observed that the internal mechanisms in adipose and mammary tissue can also alter CLA, specifically in reference to converting CLA *trans*-11 to CLA *cis*-9,*trans*-11 through the enzyme delta-9 desaturase. Santora et al. (2000) reported that when feeding CLA *trans*-11, the only known precursor for CLA *cis*-9,*trans*-11 (Field et al., 2009), to rodents there was an increase of CLA *cis*-9,*trans*-11 within adipose tissue. Similar results were also reported by Griinari et al., (2000) when CLA *trans*-11, was infused into the abomasum of dairy cows within milk fat. There was a similar increase of CLA *cis*-9,*trans*-11, though these processes are not well understood.

Omega-3 Fatty Acids

During biohydrogenation, PUFAs can be incompletely saturated to become a variety of CLA's which are stored in the tissues (Duckett et al., 2002). The long chain Omega-3 (n-3) PUFA: eicosapentaenoic acid (EPA; C20:5, n-3) and Docosahexaenoic acid (DHA; C22:6, n-3), are widely used in human, pet, aquaculture, and livestock supplements and treatments. These fatty acids are associated with enhancing the quality and duration of life and being involved in both development and normal function of the brain (Kidd, 2007; Swanson et al., 2012). Much of the benefit to dietary n-3 PUFA can be traced to the central nervous system which utilizes long chain n-3 and n-6 as well as in visual functions (Dyall et al., 2008). In ruminants, there are reproductive and carcass quality benefits when supplemented long chain n-3 FA (Sinclair et al. 2006). As a result, much research has been done to understand how dietary lipid is deposited in the ruminant and what concentration of the lipid is stored as n-3 FA or CLA.

Increasing Omega-3 Content in Beef

In ruminant food products, a common method found to increase n-3 content in the meat has been to feed flaxseed, which is high in lipid and specifically PUFA, making up about 73% of the total lipid concentration (LaBrune et al., 2008). In flaxseed, the PUFAs are protected from rumen biohydrogenation and can be deposited intact to the small intestine (LaBrune et al., 2008; Scollan et al., 2014). In their study, LaBrune et al. (2008), fed 3 steam flaked corn (SFC) based diets and 2 dry rolled corn (DRC) diets with either no fat, with tallow, or with flaxseed in the SFC diet. The SFC/flaxseed treatment had no effect on carcass quality and was effective in maintaining performance when replacing tallow as a source of fat, and effectively increased the n-3 content in the beef. However, one of the side effects was the impact on the meat itself, which exhibited an off-flavor and off-color effect. This was also noticed by Juárez et al. (2012) and

they proposed a solution by increasing the concentration of vitamin E in the diet to allow the antioxidant property of vitamin E to counteract the effects of the increased n-3. In their trial they fed two levels of vitamin E with or without flaxseed. Vitamin E (dl-α-tocopheryl acetate) concentrations were either 451 IU/hd/d or 1051 IU/hd/d. Juárez et al. (2012) reported that the supplemented amount of vitamin E in this study was insufficient to improve the retail display scores (scored on appearance; lean color, and surface discoloration) in steers fed flaxseed. However, they reported a correlation between the n-3:vitamin E ratio, indicating that as n-3 FA in the diet increased, a reciprocal increase in vitamin E supplementation was also required to avoid the off-flavor and off-color effect on the product.

Future of Lipid Supplementation

A major challenge when altering or increasing long chain PUFA in the fatty acid profile of beef is due to the biohydrogenation and lipolysis activity in the rumen (Scollan et al., 2014). In their review analyzing the effects of nutrition on beef lipid content, Scollan et al. (2014) indicated that n-3 PUFA concentrations in beef fed forage-based diets provided 17 mg/100 of EPA and 3.3 mg/100 g of DHA in muscle. However, this estimate falls below the standards given by the European Food Safety Authority (2009) to be labelled as being “high in” or “a source of” omega 3 PUFA, a standard that requires EPA and DHA concentrations to be between 40 to 80 mg of EPA plus DHA per 100 g of product. Dunne et al. (2011) fed heifers an ad libitum grass silage diet and supplemented a pH sensitive encapsulated feed bolus containing varying levels of n-3 long chain PUFA, ranging from 0 to 275 g/kg, sourced from fish oil. The capsule allowed the bolus to pass through the rumen without digestion but was then able to be broken down in the small intestine. Protecting the long chain PUFA from rumen

biohydrogenation activity increase the EPA content of the muscle up to 52.3 mg/100 g of muscle and the DHA content to 15.4 mg/100 g of muscle, reaching the level of required EPA plus DHA. Moloney et al. (2017) also observed an increase in EPA plus DHA in the muscle, ranging from 19 to 24.1 mg/ 100 g of muscle when feeding heifers a grass based diet supplemented with rumen protected fish oil. Though there was an increase, these levels fall short of the required 40 to 80 mg/ 100g of muscle.

When observing the effects of CLA, *trans*-10,*cis*-12, an anti-obesity CLA, researchers gave a supplement of 1.8 g/d or 3.6 g/d of CLA to overweight patients and reported that either dose reduced appetite (Kamphuis et al., 2003) and decreased body fat mass (Blankson, 2000). In contrast, Chen et al. (2012) observed that it took 1.7 g/d of CLA to see a reduction in body fat composition in Chinese adults. While there is evidence for CLA's to have health benefits, the concentrations are generally minimal per serving of animal product. Estimates of CLA concentrations range from 1.2 to 10.0 mg/g of lipid in beef and 2.42 ± 0.6 % of Fatty acid methyl esters (FAME) in milk (Schmid et al.,2006; Jahreis et al., 1999). Most recommendations vary for optimal human CLA supplementation, being anywhere from 1.7 to 6.8 grams/day. If the average 251 g steak consists of 48 g of lipid, there would be at most 0.48 g of CLA, which would require the consumer to eat nearly 3.5 steaks per day. Further research is needed to understand how to increase CLA more effectively in animal products.

Growing Interest in Marine Microalgae

Increased Algae Production

In the year 2020, the United exported 8.51 MMb/d and imported about 7.86 MMb/d of petroleum, which includes 5.88 MMb/d of crude oil, the lowest importing of petroleum since

1991 (EIA, 2021). Increased demand for fuel increases the need for renewable and environmentally sustainable fuel sources. This includes biofuels derived from conventional crops and microalgae which have been growing in popularity. Microalgae, which are single celled eukaryotes, are incredibly efficient converting resources such as sugars, carbon dioxide, and sunlight to oil when compared on an oil yield per hectare basis (Christi, 2007). Algae are metabolically flexible with the ability to change their biological pathways to increase protein, lipid or carbohydrate production (Tredici, 2010), making them ideal candidates for biofuel harvest. Metabolic flexibility, as well as increasing interest in algae production, has given rise to another source of energy in the form of supplemental fatty acids, particularly omega-3 fatty acids. Omega-3 fatty acids (n-3) are known to provide significant health benefits both for brain and cardiovascular health and development for both humans and animals (Swanson et al., 2012; Adarme-Vega et al., 2014). Because fish oil has been the traditional n-3 source for human supplementation, aquaculture has been under increased harvesting pressure as the industry continues grow at a rate of 8.3% annually since the 1970s (Adarme-Vega et al., 2014). Increased farmed fishing surpassed a milestone in 2015 by producing as much fish as there were wild fish caught in the oceans (FAO, 2018). Because of increased farmed aquaculture, the demand for n-3 fatty acids have only expanded due to the nearly 140,000 tons of n-3 that is needed annually as a farm fish supplement, which is currently being supplied by wild caught aquaculture (Veramaris, n.d.). In order to reduce pressure on wild caught aquaculture, which is not sustainable, microalgae cultivation has become a popular renewable resource to meet this demand on a sustainable and industrial level, though technology has been a limiting factor (Christi, 2007; Adarme-Vega et al., 2014).

Methods of Production

The main challenge facing phototrophic microalgae cultivation and harvest in the initial days of the technology, has been the need for vast amounts of land, water, and specific nutrient requirements, generally in the form of non-renewable mineral phosphorus (Borowitzka, M. A. and Moheimani, 2013; Christi, 2007). Operating costs and energy requirements of harvesting the desired product from algae have also limited production (Christi, 2007). Much of the demand for land, water, and nutrients is due to the photosynthetic needs of the algae in order to convert CO_2, sugar, and sunlight to energy. Over the years, different methods of algae production have slowly increased the practicality of algae as a source of n-3 as well as for biofuels. One method involves a large manmade river called raceway ponds. Christi (2007) describes raceway ponds as closed loop recirculation channels which are powered via paddle wheels to keep the algae flowing through the system. This allows cultured algae to be added ahead of the paddlewheel and then harvested for extraction directly behind the wheel, having made a complete circle. Raceway ponds are generally kept outdoors to allow for photosynthesis. This system, though requiring a sizable portion of land, maximizes its land and water use with lower costs of production because of the simple design. However, in this system there is generally a lower biomass production output compared to other systems such as photobioreactors, due to environmental factors such as evaporation and difficulty in thoroughly mixing the raceways, as well as outside contamination. Photobioreactors or bioreactors are another, albeit more costly industrial method for cultivating microalgae. Again, Christi (2007) explains photobioreactors as being made up of an array of straight and transparent tubes which are exposed to sunlight for photosynthesis. These tubes can be arranged in a horizontal or a vertical fence-like configuration. Algae are circulated to and from a reservoir via these tubes, receiving nutrients in controlled amounts and allowing for the

whole culture to be thoroughly mixed. This system has the ability to culture single algal species for prolonged durations of time and are much more easily harvested. This ability contributes to the greater biomass output (Carvalho et al., 2006). However, this production system has a much greater cost of production, intense and expensive maintenance, and other costly operations connected with photobioreactors.

Microalgae Fermentation

Eukaryotic heterotrophs have the ability to perform anaerobic respiration, and can ferment sugars. Some phototrophic microalgae also have the capacity to perform anaerobic respiration and anaerobic fermentation. Catalanotti et al. (2013) described the process of a phototrophic algae fermenting sugars as an alternate, anoxic (without oxygen), and light limiting method for producing products such as ethanol, acetate, and lactate. Under anoxic conditions, eukaryotes have two methods of coping: first, anaerobic respiration, which utilizes either sulfate or nitrate as electron acceptors; and second, fermentation which uses substrate-level fermentation (Atteia et al., 2013). To provide some background, the fermentation process happens under anaerobic conditions to produce ATP and to recycle NADH and $FADH_2$ in order to sustain life (Catalanotti et al., 2013). During periods of fermentation, microalgae utilize reserved polysaccharides as an energy source, which are broken down to generate the needed ATP and allowing NADH to be re-oxidized (Catalanotti et al. 2013). Again, the products from anoxic fermentation are products such as ethanol and acetate which are excreted into the environment. However, and depending on the species of algae, there are instances where the products of fermentation are not excreted; rather they are stored as a reserve in the microalgae to be used when conditions are more favorable, i.e. aerobic conditions (Atteia et al., 2013; Catalanotti et al.,

2013). In species such as e*uglena gracilis*, wax esters are formed from paramylon, a carbohydrate similar to starch, as the product of fermentation. These waxes are then stored in the cell. Under aerobic conditions the waxes are converted to paramylon, for normal respiration, similar to how lactate is used in the liver of animals. However, this process of converting paramylon to wax esters happens in the same cell (Inui et al., 1982).

Harvesting DHA and EPA

Methods for extracting either biofuel or the stored nutrients within the microalgae are also improving, and involve a series of sedimentations, agitations, centrifugations, or chemical extractions (Milledge and Heaven, 2013; Coons et al., 2014). With a better understanding of stored secondary metabolites, the harvest of n-3 fatty acids such as DHA and EPA, are more economical via specific methods of fermentation. Batch, fed-batch, and continuous fermentation are the major production types. These systems cultivate and then allow the algae to ferment the sugar while providing adequate nutrients for both processes. Batch systems are limited; however, by the risk of initial substrates being too high which can inhibit growth of the organism (Ganuza et al., 2008). Fed-batch systems allow for a culture to be grown and ferment together as a single batch with the substrates being added to the system as they are needed. Due to the ease of operation and the minimal risk of contamination associated with the batch or fed-batch systems, many producers of n-3 via microalgae have opted for these types of system (Huang, et al., 2012).

Of these two systems, there tends to be greater variation between each batch with a much lower production volume compared to photobioreactors (Guo et al., 2018). To help lower the variation and increase production volume, continuous fermentation systems focus on stages of development and usually involve one or more fermenting chambers. Increased chambers have

the potential to increase volumetric productivity and stability of product quality, allowing substrate and more seed organisms to be added as fermenting is occurring. Products are then extracted as they are produced. Substrate can be controlled at specific levels and stages of development are continuously moved down the line until harvest, thus maintaining volume in the fermentation chambers for a continuous flow (Xie et al., 2017). Shuler and Kargi (2002) explain further that continuous fermentation can be either as a single-stage or multi-stage process, with single-stage continuous fermentation being better suited when the product, biomass or non-biomass, production rate is proportional to the growth rate. While multi-stage continuous fermentation is better suited for targeting secondary metabolites such as DHA, product concentration, conversion yield, or both are generally decreased (Guo et al., 2018).

Continuous Fermentation

To improve the multi-stage continuous fermentation system to extract DHA, Guo et al. (2018) evaluated two different multi-stage continuous fermentation processes, a two-stage continuous fermentation system (2S), and a three-stage continuous fermentation system (3S), compared to the standard fed-batch (FB) process. In their experiment, Guo utilized the strain *schizochytrium sp*., a species associated with producing DHA as a byproduct of fermentation. The fermentation of *schizochytrium sp.* is stage-dependent and needs enough nitrogen for cellular growth in the first stage, so that in the second stage the cells can be nitrogen starved to promote lipid accumulation as was noted earlier.

Every 12 hours samples were collected to measure the concentrations of glucose and nitrogen, and also to determine the dry cell weight (DCW), total lipid content, and fatty acid profile. Cell dry weight, total lipid content, fatty acid composition and fatty acid methyl ester

(FAMEs) were prepared and analyzed, and the fatty acids were identified by comparison with related external standards (Sigma, USA) and then quantified using internal standard method (Sigma, USA). Kinetic modeling was then incorporated to analyze both the FB and multi-stage continuous fermentation systems. Guo et al. (2018) reported that both the 2S and 3S both produced DHA g/h at higher rates than the FB system. The 3S had increased lipid, DHA content, and increased DHA productivity by 47.6, 64.3, and 97.1%, respectively, when compared to the 2S. They concluded that the 3S was most suitable for DHA production using the *schizochytrium sp*s.

Feeding Algae to Cattle

After extraction, oil is marketed often as a residue biomass, which is being explored as a feed supplement for livestock. Typically, algae used for biofuel tend to be high in crude protein (CP), low in crude lipid (CL) and are high in ash (Becker, 2007; Drewery, 2012; Lee Chang et al., 2014; Madeira et al., 2017; Morrill et al., 2017). For example, the residue from *chlorella sp*. processing for biofuel is roughly 42.75% CP, and 12% CL (DM basis; Morrill et al., 2017a). Typically, sodium levels average 13.5%, and sulfur can vary form 0.81-1.57% (Drewery et al., 2012; Madeira et al., 2017). Algae harvested for n-3 fatty acids, on the other hand, tend to differ chemically from their biofuel counterparts. In residue from s*chizochytrium sp*. processing for n-3 supplementation, CP tends to be much lower, roughly 12%, and with a much greater concentration of CL ranging from 38.0-71.1% on a DM basis (Lee Chang et al., 2014 Madeira et al., 2017). Sodium levels have been measured to be around 8.52 to 9.96%, and sulfur around 2.54 to 3.5% (Norman et al., 2019; Gibbons et al., 2021). The fatty acid profiles of both Schizochytrium and Chlorella when listed in the literature are generally reported as a percent of

total lipid content on a DM basis. Both s*chizochytrium* and *chlorella* are reported to have 35.23 and 31.84% SFA, 0.86 and 46.23% MUFA, and 57.76 and 10.57% PUFA respectively (Li et al., 2009; Stamey et al., 2012; Morrill et al. 2017b).

Feeding Residual Biomass from Biofuel Algae to Cattle

Researchers evaluated a post extracted algal residual (PEAR), *chlorella sp*., in a series of studies which address the use of extracted algae biomass as a supplement in cattle diets. In their analysis, Drewery et al. (2012) utilized six, ruminally and duodenally cannulated steers in a 3×3 Latin square design, to investigate the effects of PEAR on digestion in low-quality forage diets. Treatments included a base diet of chopped prairie hay (6.7% CP, DM basis) which was fed at 130% of the previous 5d average consumption; with no supplement (CON); or supplemented 100 mg N/kg BW with either PEAR (19.1% CP on a DM basis) or cottonseed meal (CSM; 44.9% CP on a DM basis). All treatment diets except for the control were formulated to be isonitrogenous. Results of this study indicated that there were no differences ($P \geq 0.28$) between PEAR and CSM for forage OM intake, total OM intake, total digestible OM intake, and N intake, though intakes for the CON were decreased compared to both PEAR and CSM ($P \leq 0.05$). There was no difference ($P \geq 0.20$) between CSM and PEAR for fecal and urinary N excretion, though there was a significant increase in absorbed N ($P < 0.01$), with PEAR and CSM having the greatest absorbed N with no difference between the two ($P = 0.20$). There was no difference in total tract OM digestibility ($P \geq 0.33$) for all three treatments, and over-all the results suggested that PEAR was an effective protein source for steers consuming a low-quality forage.

As technology increases, so too the quality of the product. Post extracted algae residue is no exception as the industry expands its market value for PEAR, increasing in CP from 19.1%

CP to nearly 33.8% on a DM basis, while decreasing in ash from 45.5% ash to 12.2% (Morrill et al., 2017a). An experiment was conducted by Morrill et al. (2017a) to evaluate the effects of a more protein rich PEAR in beef steer finishing diets on nutrient utilization and carcass characteristics. They utilized 18 steers with three treatments including 1) PEAR supplemented at 1.0 kg OM/day, roughly 9% of the finishing diet hand mixed into the ration, 2) glucose infused into the rumen (GR) at 1.0 kg OM/day or 3) glucose infused into the abomasum (GA) at 1.0 kg OM/day via cannulas through an infusion line. The two glucose treatments were utilized in order to stop the addition of flavor influencing compounds that would have been in the diet if the treatments were balanced for starch or protein. There was also a high inclusion of forage consisting of cottonseed hulls (13.5%, DM basis) and grass hay (10.0%, DM basis), in this finishing ration, to act as a buffer for the glucose infused treatments to manage intake. In this study, Morrill et al. (2017a) observed greater DMI ($P < 0.05$) for steers that received PEAR compared to the GR and GA treatments, the PEAR treatment reduced ($P \leq 0.01$) DM, OM, NDF, ADF, and gross energy digestibility compared to either GR or GA infused steers, which were not different from each other ($P > 0.38$). When measuring energy, steers fed PEAR had significantly lower ($P \leq 0.01$) digestible energy, and therefore lower metabolizable energy and net energy for both maintenance and gain. Steers which had glucose infused into the rumen and steers which had glucose infused into the abomasum did not differ from one another in this regard ($P \geq 0.62$), even though intakes of OM, digestible OM, NDF, and ADF were not different between the three treatments ($P > 0.10$).

Carcass Characteristics and Consumer Markers

Carcass data were also collected on the steers consuming PEAR by Morrill et al. (2017a). No differences in USDA Yield Grade (YG), hot carcass weight (HCW), 12th rib fat thickness (REA), or longissimus muscle area (LM area) were observed ($P \geq 0.58$). Steers that received PEAR had greater marbling score and quality grade (QG) compared to the other treatments ($P = 0.01$).

Morrill et al. (2017b) also analyzed the fatty acid profile of the beef, as well as sensory characteristics for consumers, utilizing the same steers as described earlier. They utilized various meat cuts which were analyzed for fatty acid profile, tenderness, flavor, and the likeability of the product. There were no differences among the three treatments, GR, GA, and PEAR ($P \geq 0.13$) for sensory tests such as beef identification, bloody/serumy, metallic, umami, sour, salty, bitter, or burnt. However, there were differences in samples being fat-like ($P = 0.02$) with GR samples being greatest, followed by PEAR and then GA. Overall sweetness, and sweetness sensory markers were greater ($P \leq 0.05$) for PEAR, followed by GR and then GA treatments.

Morrill et al. (2017b) also analyzed the fatty acid profile of the ground beef between the PEAR and the GR treatment, and ground chuck compared to ground round. They observed a treatment × primal (chuck or round) interaction, with the PEAR treatment containing greater C16:1 n-7 concentrations in ground round than in ground chuck compared to the GR treatment, though the interaction was a difference of size, not direction. The increase in C:16:1 n-7 was not observed by Stokes et al. (2015) who reported a decrease in C:16:1 n-7, though this was attributed to the type of algae that was utilized with Stokes utilizing a heterotrophic algae while the PEAR product is derived from a phototrophic algae. There were no significant differences between the two treatments for any of the fatty acids compared, though there was a slight

numerical increase in the amount of SFA and UFA in the GR treatment (48.95 and 49.91% total fat, respectively) compared to the PEAR treatment (47.02 and 49.01% total fat, respectively). There was also a slight numerical increase of PUFA content in PEAR (3.75% total fat) compared to the GR treatment (3.58% total fat). There were significant differences ($P \leq 0.02$) between the chuck and the round samples, with chuck having higher concentrations of SFA and PUFA, 48.21% and 3.84% total fat compared to 44.78% and 3.45% total fat in the round respectively. Morrill et al. (2017b) concluded that supplementing PEAR had no significant effects on flavor in strip steaks, in the likability of the ground beef, or in the tenderness of the loin steaks, most likely due to only a small change in the fatty acid profile of the beef.

Microalgae for Increasing Omega-3 Content in Dairy Cattle

Algae as an n-3 fatty acid supplement is relatively new in the beef industry, much of which centers on costs and supply. However, there has been increasing interest in the dairy industry to utilize microalgae to increase n-3 content of the milk and to help with fetal development (Gulliver et al., 2012). In the past, the dairy industry has utilized fish oils along with plant based n-3 sources to increase the UFA concentration in milk, though this was difficult due to the toxicity of the UFA to the rumen microbes (Pantoja et al., 1994). Research evaluating the effects of protecting the supplemented oil have suggested that protecting the oil is effective against biohydrogenation in the rumen (Storry et al. 1974; Franklin et al. 1999; Dunne et al. 2011; Scollan et al. 2014). Storry et al. (1974) evaluated cod liver oil on milk fatty acid secretion by protecting the fat in formaldehyde-treated casein. They reported an increase in the amount of UFA in the milk, with increased 20 and 22 carbon fatty acids, without influencing the total milk fat but formaldehyde treated feeds is prohibited in the United States for food safety concerns.

Franklin et al. (1999) protected *schizochytrium sp.* algae with xylose and fed 910g/d of either protected or unprotected algae to 9 Brown Swiss and 21 multiparous Holsteins to evaluate the conjugated linoleic acid (CLA), DHA, and transvaccenic acid in milk. They observed a non-significant increase in total SFA in the milk of cows fed the unprotected algae, with a slight non-significant decrease in total UFA. There was a significant ($P < 0.05$) increase in the amount of DHA for the protected algae treatment compared to the unprotected and the control. Both total SFA and UFA for the protected and unprotected algae treatments were greater than the control ($P < 0.05$). They also observed that milk yield was not affected by treatment ($P > 0.50$), though milk fat yield decreased ($P < 0.05$) from 0.84 kg/d for the control compared to 0.65 and 0.69 kg/d for protected and unprotected algae, respectively. Fat percentage also decreased ($P < 0.05$) when algae was included in the diet from 3.7% for the control, to 2.95% for both protected and unprotected algae treatments. There were no differences in flavor ($P > 0.05$) for any treatment. Stamey et al. (2012) also used a lipid encapsulated protected algae biomass as a supplement top-dressed to provided 29 g/d or 19.5 g/ d of DHA, fed to 4 Holstein cows, to evaluate the effects of algae oil as a supplement for dairy cattle. Stamey et al (2012) reported no significant ($P > 0.05$) change in milk production or milk fat yield, with an increase ($P < 0.01$) in DHA concentration in the milk fat in either treatment diet compared with the control.

Microalgae are becoming an important source of protein and omega-3 fatty acids within the beef and dairy industry. Algae can be used as a protein or lipid supplement, though use of the algae is often limited by the high ash content, in particular the sodium and sulfur content. Further research and development could help to lower the ash content of the algae product. Research suggests that utilizing microalgae in feedlot diets may improve the fatty acid profile of the beef and may increase the value of the product.

Heifer Implants

Brief History of Hormone Implants

Implanting cattle is no longer a new technology, as it was developed in the 1950's (FDA, 2020) to increase body weight gain when compared to non-implanted animals at the same body fatness. Research on this topic is continually expanding, with data suggesting that use of hormone implants in beef cattle improves average daily gains, with improved gains ranging from 8 to 28% and 6 to 10%, roughly 0.25 kg/d and 0.08 kg/d for steers and heifers, respectively, and feed efficiency ranging from 5 to 20% and 4 to 7% for steers and heifers, respectively (Lusby and Gill, 1985; Duckett and Andrae, 2001; Wileman et al. 2009; Smith and Johnson, 2020; Johnson and Beckett, 2014). Hormone types that most implants use are either as a single hormone or combination, are androgens, estrogens, and progestins. Androgens include testosterone propionate (T) and trenbolone acetate (TBA); estrogens are estradiol-17β (E2) and estradiol benzoate (EB); and progestins such as progesterone (Smith and Johnson, 2020).

Mode of Action

Estrogenic hormones act on the anterior pituitary gland which increases concentrations of somatotropin (ST) or growth hormone, which in turn stimulates insulin-like growth factor-1 (IGF-1) in the liver. The increase of IGF-1 alters the way nutrients are utilized for muscle and bone growth and decreases fat deposition and protein degradation, resulting in a heavier and leaner carcass (Griffin and Mader, 1997; Clemmons, 2012). Androgenic hormones directly increase the amount of IGF-1 via the liver without influencing ST levels (Hayden et al., 1992; Griffin and Mader, 1997). Both hormones act to increase mature body composition more slowly

compared to non-implanted cattle, thus increasing mature body weight when a similar body fat composition is reached (Guiroy et al, 2002). By combining both estrogenic and androgenic hormones within the implants, studies demonstrate an increase in mature body weight by an additional 14 to 42 kg in steers and 30 to 39 kg in heifers when fed to the same body composition as non-implanted animals, with 15% improved feed efficiency (Johnson et al., 1996;Guiroy et al. 2002). Johnson et al. (1996), evaluated a combined TBA and E2 implant, Revalor-S, (120 mg TBA and 24 mg of E2; Merck Animal Health, Madison, NJ) compared to non-implanted steers and serial slaughtered all steers to measure carcass protein and fat gains. They observed a 38.52% increase in protein gain (g/d) and improved feed efficiency and dry matter intake (DMI) over an average feeding period compared to non-implanted steers. They reported a reduction in marbling scores in implanted cattle with decreased Choice and increased Select carcasses.

Re-Implanting

There is an optimal level of hormone in the bloodstream for an optimal animal response. Once the animal is implanted, the hormone immediately begins to be released into the bloodstream which then acts on the pituitary gland and or the liver. The response to the hormone as BW gain increases rapidly and then steadily begins to wane over roughly 60 to 120 day after the initial implant (Mader, 1998). Johnson et al. (1996), also reported that during the first 40 d after implanting there was a rapid increase in protein gain compared to the control, which had then decreased by 115 d post implanting to same level of non-implanted cattle. In order to maintain elevated levels of hormone in the bloodstream, another implant needs to be given to prevent the hormone levels from dropping below the optimal point. Mader (1998) suggested a re-implant window of 70 to 80 days.

Implants may also be synthetically coated to delay the release of the hormones, extending the payout life of the hormone (Lee et al. 2000; Cady et al. 2002). As explained, the implant increases blood E2 and TBA concentrations which is then followed by a gradual decline (Mader, 1998), though with the administration of a second implant there is another increase in blood E2 and TBA concentrations. Coated implants however, extend the life payout of E2 and TBA by gradually increasing blood E2 and TBA over 200 d after implanting (Smith and Johnson, 2020), albeit the blood concentration of hormone may not be as great as in traditional non-coated implants. Coulson et al. (2019) evaluated different re-implanting time events, utilizing steers implanted with Revalor-IS (80 mg TBA, 16 mg E2; Merck Animal Health, Madison, NJ) as the initial implant on d 1 and Revalor-200 (200 mg TBA, 20 mg E2; Merck Animal Health, Madison, NJ) as the terminal implant given 160, 120, 100, 80, or 40 d before to slaughter, when steers were fed 180 d. They reported that the greatest overall weight gain was achieved when steers were re-implanted 99 d before to harvest and greatest overall feed efficiency was achieved when steers were re-implanted 87 d after the initial implant. Coulson et al. (2019) reported that the optimal time on the terminal implant for improved growth performance and carcass characteristics was 96 d before to harvest.

Parr et al. (2011) evaluated different implant strategies over 4 experiments. Exp. 1 compared 1) no implant, 2) Revalor-S, 3) Revalor-IS followed by Revalor-S (Revalor-IS/S) on d 68 to 74, or 4) Revalor-X (200 mg TBA and 40 mg of E2; Merck Animal Health, Madison, NJ) fed for 174 d. The Revalor-X is comprised of 4 uncoated pellets for immediate release and 6 polymer coated pellets for a delayed release approximately 70-80 d post implanting (Merck Animal Health, 2021). Exp. 2 compared 1) Revalor-S, 2) Revalor-IS/S (re-implanted on d 44 to 47), or 3) Revalor-X, administered to steers fed 131 d. Exp. 3 compared, 1) Revalor-IS/S (re-

implanted on d 90 to 103), or 2) Revalor-X, administered to steers fed 131 d. Exp 4 compared 1) Revalor-IS/S (re-implanted on d 68 to 71) or 2) Revalor-X, administered to steers fed 243 d. For Exp. 2, 3, and 4, there were no differences in DMI, carcass adjusted final BW, ADG, feed efficiency, and HCW, though there was in Exp. 3, there was a tendency ($P < 0.10$) for greater feed efficiency. In Exp. 1 there was also no difference in DMI or ADG, though Revalor-X and Revalor-IS/S treatments had greater carcass adjusted final BW, feed efficiency, HCW, and LM area, compared to the Revalor-S and non-implanted steers. In Exp. 2 Revalor-IS followed by a Revalor-S decreased Choice and increased Select carcasses in steers. In Exp. 4 increased percentage of Choice carcasses and reduced Select carcasses for Revalor-X than for Revalor-IS/S. Parr et al. (2011) concluded that the coated implant performed similarly to Revalor IS/S, and that when the TBA and E2 hormone dose was equal, the coated implant improved performance.

Implanting Feedlot Heifers

Implanting heifers does improve gain and feed efficiency by 4 to 10%, when fed to the same body composition as non-implanted animals (Adams et al., 1990; Cleale et al., 2013; Guiroy et al. 2002; Mader, 1997). Smith et al. (2007) analyzed the effects of implants on intramuscular lipid deposition in finishing beef steers and heifers. The researchers implanted both steers and heifers with Synovex-Plus (28 mg EB and 200 mg of TBA; Zoetis; Fort Dodge Animal Health, Overland Park, KS). They reported a 23% increase in LM area for implanted steers and a 6.6% increase in implanted heifers compared to the non-implanted controls, though there was no change in the intramuscular deposition of the LM area. The increase in LM area supported the observed increase in HCW, with a 10.9% and 7.8% increase in HCW for steers

and heifers, respectively compared to the control. Another experiment conducted by Schneider et al. (2007) evaluated the effects of various heifer implants including single hormone and combination hormone implants on carcass characteristics and longissimus tenderness. They utilized 500 hundred heifers over 140 days on feed, and 12 treatments utilizing Finaplix-H, Revalor-IH, Revalor-H, and Revalor 200 (Intervet Inc., Millsboro, DE). Treatments varied by total mg TBA and mg E2 concentrations, administered either a single implant or as a two implant, re-implant strategy. Treatments included 1= 0:0/0:0 (control); 2=0:200/0:0; 3=8:80/0:0; 4=14:140/0:0; 5=20:200/0:0; 6=0:200/0:200; 7=8:80/8:80; 8=8:80/14:140; 9=14:140/14:140; 10=8:80/20:200; 11=14:140/20:200; 12=20:200/20:200. They observed that heifers that received one implant during the finishing stage had increased HCW compared to heifers that were not implanted. When heifers received two implants compared to one implant, HCW was further increased along with an increase in LM area, with reduced kidney/pelvic/heart fat (KPH) and increased USDA yield grade. They observed that the heifers that received combination implants had greater LM area with a decreased marbling score, compared to the heifers implanted with TBA alone, with the same days on feed.

The benefits of implanting are clear, though research indicates that the technology of implanting heifers is variable, especially when compared to steers, which is attributed in part to factors related to estrus (Adams et al., 1990) which can be managed through surgery or feeding melengestrol acetate. Naturally, higher estrogen levels in heifers also promotes the closure in the growth plates and increased ossification, which could explain the smaller frame size of heifers (Owens et al., 1993).

Heifer Implant strategy

Selecting a heifer implant strategy should consider how aggressive the implant program should be, i.e. concentration of TBA and estradiol, and how many implants administered over the feeding period. An experiment conducted by Hilscher et al (2016), evaluated three different heifer implant strategies over 173 d. Treatments included (1) Revalor-IH (80 mg TBA and 8 mg E2; Merck Animal Health, Madison, NJ) followed by a Revalor-200; (2) Revalor-H (140 mg TBA and 14 mg E2; Merck Animal Health, Madison, NJ) followed by Revalor-200; (3) Revalor-200 followed by Revalor-200. Hilscher et al. (2016) observed no differences in live performance or carcass characteristics, though there was a significant decrease in marbling score as the initial implant increased in TBA and E2 concentrations. There were more Choice and fewer Select carcasses for the Revalor-IH/200 combination treatment, while the Revalor-H/200 and 200/200 treatments did not differ from each other.

In contrast to Hilscher's findings, Folmer et al. (2009) compared feedlot heifers implanted with either a Revalor-IH followed by Revalor-200 to a Synovex-H (200 mg TBA and 20 mg EB; Zoetis; Fort Dodge Animal Health, Overland Park, KS) followed by a Revalor-200 for an average of 177 d. Folmer et al. (2009) observed that heifers implanted with the Revalor-IH/200 combination compared to the Synovex-H/Revalor-200 combination greater carcass adjusted feed efficiency (0.190 compared to 0.186) and a tendency for a 2.5% improvement in carcass adjusted ADG (1.66 kg compared to 1.62 kg), and a tendency for a 3.6% improvement in marbling scores (552 compared to 533). Additionally, a higher number of Choice carcasses were observed for Revalor-IH/200 combination. There was a non-significant numerical decrease in

calculated YG, which is supported by Hilscher et al. (2016). There was no difference in LM area, suggesting that the heifers were also finished to the same body composition.

There are coated and partially coated implants available for use in heifers. One such implant is Revalor-XH (200 mg TBA and 20 mg E2; Merck Animal Health, Madison, NJ) which contains 4 uncoated pellets for immediate release upon implanting while 6 pellets are coated with a polymer coating for delayed release after 70-80d post implanting (Merck Animal Health, 2021). An abstract by Crawford et al. (2018) compared heifers implanted with a total of 280 mg TBA and 28 mg E2 (Revalor-IH followed by a Revalor-200) to 200 mg TBA and 20 mg E2 a single, partially coated Revalor-XH. Heifers were fed for a period of 172, 193, and 214 d. In their study there were no interactions between the implant program and days on feed. They reported that heifers implanted with Revalor-XH had greater DMI and lower ADG and G:F, and they also reported that heifers implanted with the Revalor-IH/200 treatment had greater carcass adjusted final body weight and HCW.

Ohnoutka et al. (2021) in a series of two studies evaluated the use of both a coated and a partially coated heifer implants when heifers were fed for consistent or varying days on feed. For the first experiment, treatments included 1) no implant (control), 2) Revalor-XH (partially coated) on d 1, 3) Revalor-200 on d 1, 4) Revalor-XR (completely coated; 200 mg TBA and 20 mg E2; Merck Animal Health) on d 1, or Revalor-200 on d 70 (D200) administered to heifers fed for an average of 198 d. Ohnoutka et al. (2021) reported no difference between implant treatments for carcass adjusted final BW, ADG, HCW, marbling score or 12^{th} rib fat ($P \geq 0.09$) between implanted heifers. However, heifers implanted with Revalor-XR had greater feed efficiency, and heifers implanted with Revalor-200 on d 1 had the greatest calculated YG. Within

the first 70 d of the trial, the heifers implanted with Revalor-XH when compared to the heifers implanted with Revalor-200 on d 1 and heifers that received Revalor-XR, had greater ADG and feed efficiency. From d 70 to 140 there was a tendency for decreased ($P \leq 0.08$) ADG and a significant ($P \leq 0.01$) decrease in feed conversion in heifers implanted with Revalor-XH compared to the heifers implanted with Revalor-200 on d 1 and heifers that received Revalor-XR. Ohnoutka et al. (2021) concluded that when heifers were fed to the same number of days there was no effect of implant release rate on live performance, also supported by Smith et al. (2020) compared a Revalor-IH on d 1 followed by a Revalor 200 on average d 86, to Revalor-XH over an average of 180 days on feed. However, Schumacher et al. (2019), evaluated heifers implanted with a coated implant on d 1 (Synovex ONE; 200 mg of TBA and 28 mg EB; Zoetis; Fort Dodge Animal Health, Overland Park, KS) compared to heifers implanted with a non-coated implant on d 1 (Synovex Choice) followed by another non-coated implant on d 95 (Synovex Plus), contradict Ohnoutka et al (2021), by reporting a greater DMI, and lower feed to gain. Schumacher et al. (2019) also reported no difference in HCW or 12th rib fat thickness, though heifers implanted with the coated implant had greater marbling scores and YG, with lower LM area.

Conclusions

In summary, lipids stored as energy in beef cattle also provide taste and tenderness to the meat. The lipid profile of the beef may be altered through a combination of dietary lipid source, biohydrogenation, and the desaturase enzyme within the peripheral tissues. Conjugated linoleic acids and omega-3 fatty acids are often targeted in the lipid profile as they provide added health benefits if consumed in adequate quantities. The literature suggests that protecting the lipid CLA

and omega-3 content of the diet as well as increasing omega fatty acid levels in the lipid supplement increases the concentrations in the beef product.

Marine algae and specifically the *schizochytrium sp.* are becoming an important source of omega-3 and omega-6 fatty acids as a supplement for humans, pets and aquaculture. The fermentation process is becoming more efficient, allowing for more concentrated supplements. Both the products and byproducts of microalgae cultivation and harvest are being explored as feed ingredients for dairy and beef cattle. Algae can be used as a protein or lipid supplement, though use of the algae is often limited by the high ash content, in particular the sodium and sulfur content. As technologies for cultivating and harvesting marine microalgae improve, the omega-3 content of the microalgae feeds are becoming more economical to produce. Further development could also help to lower the ash content of the algae product, though research has not explored this. Utilizing heterotrophic microalgae in feedlot diets may improve the fatty acid profile of the beef and may increase the value of the product.

Implanting is an economically important technology that increases body weight and can improve feed efficiency. Combined TBA and estrogen implants are the most beneficial, with prolonged exposure to the hormones giving greater influence to performance. Length of time on feed and increasing the amount of hormone can improve gains and feed efficiency further. When implants are coated or partially coated for delayed release, performance is similar when compared to moderate implant strategies utilizing a single or multiple non-coated implants throughout the feeding period.

The objective of this thesis was to evaluate the feeding value and utility of CARS in feedlot diets (Exp. 1), and to evaluate the impact of CARS on digestibility and fatty acid flow in a feedlot finishing diet (Exp. 2). In addition, this thesis also focuses on the effects of a re-implant

program utilizing 3 implants, given as single implants over the feeding period for a total of 420 mg TBA and 42 mg E2, compared to a single dose delayed payout implant with a total of 200 mg TBA and 20 mg E2 over the feeding period, on live performance and carcass characteristics after similar days on feed (Exp. 3).

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CHAPTER II. Evaluation of Condensed Algal Residue Solubles as an Ingredient in Cattle Finishing Diets and Its Effects on Digestibility and Fatty Acid Flow

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Abstract

Two experiments were conducted to 1) evaluate the energy value of a novel liquid feed, condensed algal residue solubles (CARS), in feedlot diets; and 2) to evaluate the impact of CARS on dry matter and organic matter digestibility and fatty acid flow in cattle diets. In Exp. 1, 480 crossbreed steers (initial BW = 432 kg; S.D. = 40 kg) were utilized in a 2x3 factorial treatment design and fed 148 d. Steers were fed three inclusions of CARS (0, 2.5, 5% of diet DM) displacing corn in the diet, in 2 different base diets representing Northern and Southern Plains finishing diets. Southern Plains diets contained steam flaked corn with 15% dry distillers grains plus solubles, while the Northern Plains diets contained a 50:50 dry rolled corn:high moisture corn blend, with 15% wet distillers grains plus solubles. All diets contained 8% alfalfa haylage and 4% supplement on a DM basis. There were CARS inclusion × diet type interactions for live shrunk final BW and live ADG ($P \leq 0.05$). Main effects of CARS included quadratic responses ($P \leq 0.01$) for greater carcass adjusted final BW, ADG, G:F, HCW, 12th rib fat, and yield grade when CARS was fed at 2.5%, with 5% CARS reducing feedlot and carcass measurements. There was a linear increase in marbling score ($P < 0.01$) and a linear decrease in DMI and LM area ($P \leq 0.01$) were observed as CARS level increased. Main effects of diet indicated Southern diets had greater ($P \leq 0.02$) G:F, HCW, and 12th rib fat compared to Northern diets. In Exp. 2, feeding 0, 2.5, or 5% CARS was evaluated for digestibility and fatty acid flow when included in the Southern plains diets used in Exp. 1, utilizing 6 ruminally cannulated steers in a 3×3 replicated Latin design. Inclusion of CARS in the diet did not affect DM or OM intake ($P \geq 0.17$), though there was a linear decrease in NDF intake ($P = 0.07$). There were no differences in total tract DM, OM, and NDF digestibility ($P \geq 0.52$) due to CARS inclusion. Total fatty acid flow at the duodenum was unaffected ($P = 0.18$) as CARS increased in the diet.

The fatty acid profile was impacted, with a linear decrease ($P = 0.06$) in saturated fatty acids, correlated with a linear increase ($P \leq 0.07$) in unsaturated, mono-unsaturated, and poly-unsaturated fatty acids as CARS inclusion increased in the diet. There was a linear increase ($P < 0.01$) in C16:0, C18:0, C18:1T, C18:1 Vaccenic, and C22:6ω3 Docosahexaenoic fatty acids. There was also a quadratic response for C18:3ω3 α-Linoleic acid concentrations ($P = 0.01$) with CARS included at 2.5% having the greatest concentration of α-Linoleic acid. Flow of both omega-3 and omega-6 fatty acids at the duodenum increased linearly ($P \leq 0.02$) as CARS inclusion increased. Including 2.5% CARS in finishing diets improved feed efficiency in both Northern or Southern diets, but did not affect diet digestibility. Feeding CARS increased unsaturated fatty acids in the total duodenal fatty acid flow composition.

Key words: algae, novel feed, cattle, metabolism, fatty acid flow, omega-3, DHA and EPA

Introduction

Wild caught fish is an important source of omega fatty acids for farmed aquaculture such as salmon, and as a supplement for both human and pets with important brain and body benefits (Swanson et al. 2012). In 2016, an estimated 16 million metric tons of wild caught fish were harvested to produce fish oils and fish meal (Veramaris, n.d.). Overall, production of omega-3 fatty acids has been increasing by an average of 8.3% each year with an estimated $34.7 billion market value of packaged products containing n-3 fatty acids (Adarme-Vega et al., 2014). Marine microalgae, which have the ability to harvest sugars and carbon dioxide to convert them into metabolites such as n-3 fatty acids (Christi, 2007; Tredici, 2010), have been proposed as a "green" solution to reducing pressure on wild caught aquaculture (Ji et al. 2015), with 1 ton of algae oil estimated to replace 60 tons of wild caught fish (Veramaris, n.d.).

Today, the algae used in n-3 FA cultivation are from the Thraustochytriaceae and Crypthecodiniaceae families with the S*chizochytrium sp* from the Thraustochytriaceae family being one of the few algae specifically cultivated for n-3 docosahexaenoic acid (DHA) production on an industrial scale (Lee Chang et al., 2014; Ji et al, 2015). As technology improves, the cattle industry has begun to explore algae products or byproducts as a source of protein and n-3 supplements. S*chizochytrium sp.* as a feed supplement has limitations, being high in ash, with minerals such as sodium ranging from 8.52 to 9.96% (Norman et al., 2018). While dietary inclusions may be low, the literature supports that S*chizochytrium sp.* in particular can be incorporated into dairy cattle diets without detrimental effects (Franklin et al. 1999; Stamey et al; 2012; Bragaglio et al. 2015).

In 2018, production of *schizochytrium sp* began near Blair, NE as a n-3 supplement for pets and aquaculture. Residue from the production process, known as condensed algae residue solubles (CARS; Veramaris, The Netherlands) has been granted generally recognized as safe (GRAS) status after a trial by Norman et al. (2018). In this experiment, CARS was included up to 7.5% of diet DM, replacing corn, utilizing 20 steers and 20 heifers fed for 100 d. While the trial was not to evaluate cattle performance, they observed a linear decrease in DMI and G:F, with a quadratic response in ADG, with CARS included up to 5% having the greatest ADG and HCW. Increasing CARS up to 7.5% of diet DM increased dietary net energy. The authors concluded that CARS had no adverse effect on cattle, and improved performance when fed up to 5.0% of diet DM (Norman et al., 2018).

In a follow up experiment, the nutrient digestibility of CARS in finishing diets was evaluated to determine an optimal inclusion level (Norman, 2021). Utilizing 6 steers, fed 3 levels of CARS (0, 5, and 10% diet DM) and 2 inclusions of wet distillers grains (0 or 20% diet DM), the author reported a linear decrease in DM, OM and NDF intakes as CARS was included in the diet. However, there was no effect of CARS on DM or OM digestibility. Norman (2021) concluded that CARS, when included up to 5% of the diet DM, had limited effect on DM and OM intake and digestibility. Thus, the objective of Exp. 1 was to evaluate the impact of CARS on finishing performance and carcass characteristics, and to determine the energy value. The objective of Exp. 2 was to evaluate the impact of CARS on digestibility and fatty acid flow at the duodenum.

Material and Methods

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved all animal care and management procedures (protocol 1282).

Exp. 1

A total of 480 Crossbred steers (initial BW = 432 kg; S.D. = 40 kg) were fed for 148 d. Research was conducted at the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE. Steers were received at ENREC November 2018 before initiation of the trial. Upon arrival, the steers were vaccinated against bovine respiratory syncytial virus, parainfluenza-3, bovine viral diarrhea Types I and II, infectious bovine rhinotracheitis and Mannhemia haemolytica (Bovi-Shield Gold One Shot, Zoetis , Kalamazoo, MI), and clostridial infections caused by Clostridium chauvoei, Cl. septicum, Cl. novyi, Cl. sordellii, and Cl. perfringens Types B, C and D (Ultrabac 7, Zoetis). Steers also received an injectable anthelmintic (Dectomax, Zoetis). Steers were revaccinated approximately 14 to 28 d after initial processing against bovine respiratory syncytial virus, parainfluenza-3, bovine viral diarrhea Types I and II, and infectious bovine rhinotracheitis (Bovi-Shield Gold 5, Zoetis) and against clostridial infections and Hisophilus somnus bacteria (Ultrabac 7/Somubac, Zoetis). During the period between arrival and trial initiation, steers were utilized in forage based diet trials or grazed corn residue. At trial initiation steers were limit fed for 5 days at 2% of body weight using a diet comprising of 50% alfalfa and 50% Sweet Bran (Cargill Wet Milling, Blair, NE). A common diet was fed to minimize differences in gut fill and steers were weighed on two consecutive days, d 0 and d 1 at 0700 h before feeding, for initial weight. Steers were blocked and stratified by initial body weight into 4 body weight blocks and assigned randomly to 48 pens after the first day of weight

collections (d 0). Pens contained 10 steers/pen and were assigned randomly to 1 of 6 treatment combinations, with 8 replications per treatment.

Treatments were designed as a 2 × 3 factorial with 3 inclusions of CARS at 0, 2.5, and 5% of the diet DM displacing corn in the diet and 2 base diets representing Northern Great Plains diets (Northern) and Southern Great Plains diets (Southern). The CARS used in this study contained 25.4% DM, 19.3% CP, 8.3% crude fat, 0.02% ADF and 9.96% Na, 3.05% S, and 0.53% P on DM basis. Samples were sent to Ward Laboratories Inc. (Kearney, NE; Table 2.1) for analysis. Dry matter was calculated using the NFTA Method 2.1.4 - Dry Matter by Oven Drying for 3 hours at 105oC (Shreve B., Thiex N., Wolf., 2006). Crude protein was determined by the combustion method (AOAC, 1996) using a combustion N analyzer. Crude fat was analyzed by extraction with petroleum ether (Ankom. 2017). The ADF was measured using the Method for Determining Acid Detergent Fiber, ANKOM 200/220 Fiber Analyzer (ANKOM Technology, Fairport, NY.). For analysis of Ca, P, K, S, Na, Mg, Zn, Fe, Mn, Cu, and Mo. The procedure used nitric acid and hydrochloric acid to degrade all OM followed by hydrogen peroxide to dissolve any fats and oils. Samples were then diluted, filtered, and analyzed using Inductively Coupled Plasma-Atomic Emission Spectroscopy (iCAP 6500 ICP Emission Spectrophotometer; Thermo Fisher Scientific, Waltham, MA) with 6 standards of varying mineral concentration (Plank and Campbell, 1992; Peters, 2003).

Southern diets contained on a DM basis 73, 70.5, and 68% steam flaked corn (SFC), and 15% dry distillers grains plus solubles (DDGS), while Northern diets contained 73, 70.5, and 68% of a 50:50 dry rolled corn:high moisture corn (DRC:HMC) blend, and 15% wet distillers grains plus solubles (WDGS; Table 2.3) on a DM basis. The CARS replaced either DRC:HMC or SFC in the diets. All diets included 8% alfalfa haylage and a 4% supplement. Two

supplements were formulated, one for each of the 0% and 5% treatments and then blended (50:50) for the 2.5% treatment because sodium was decreased in the supplement from 0.3% in 0% CARS diets to 0% sodium in 5% CARS diets. Diets were formulated to provide similar Ca and appropriate Ca:P ratios. Supplements were formulated to provide 33 mg/kg of diet DM of monensin (Rumensin; Elanco Animal Health) and provide 90 mg/animal daily of tylosin (Tylan; Elanco Animal Health). All supplements contained 0.5% urea to ensure RDP requirements were met. Cattle were adapted to the finisher diet in 4 steps over a period of 25 days. All diets in each step consisted of a decreasing amount of grass hay fed (28.5%, to 8% grass hay) while increasing the amount of corn (34% to 64% SFC or DRC:HMC). The CARS was added to the diet on day 18 at the beginning of step 4, displacing corn. Feed refusals were collected as needed throughout the trial and analyzed for DM in order to adjust feed offered to actual dry matter intake (DMI). Cattle were fed once daily at ad libitum intake.

Steers were implanted on d 1 with 80 mg trenbolone acetate and 16 mg estradiol (Revalor-IS, Merck Animal Health, Madison, NJ) and on d 70 steers were re-implanted with 200 mg trenbolone acetate and 20 mg estradiol (Revalor-200, Merck Animal Health). On d 120 to 148 ractopamine (Optaflexx, Elanco Animal Health) was included in the diet at 300 mg/steer daily for a total of 28 d and all blocks were harvested on d 148 at Greater Omaha (Omaha, NE). At harvest, hot carcass weight (HCW), liver abscess scores, and kill order were recorded. Carcass adjusted final body weight (BW) was calculated from HCW using a common 63% dressing percentage. Carcass adjusted final BW was used to calculate average daily gain (ADG) and gain to feed (G:F). Dietary energy for maintenance (NEm) and for gain (NEg) were calculated utilizing initial BW and final BW for the pen, and the BW at target endpoint (heaviest pen average BW by block), ADG and DMI (NRC, 1996 equations) as described by Vasoncelos

and Galyean (2007). Carcass characteristics including marbling score, 12th rib back fat thickness, longissimus muscle (LM) area, and yield grade were recorded after a 48 hour chill.

Performance and carcass data and economic analysis were analyzed using the MIXED procedure of SAS as a 2×3 factorial with CARS inclusion, base diet, and the interaction between CARS and base diet, as well as body weight block included as fixed effects. Pen was the experimental unit. There were CARS×diet type interactions for live final shrunk BW, live ADG, and a tendency for an interaction in live G:F and dressing percentage. However, there were no interactions for carcass adjusted performance and the main effects of CARS inclusion and base diet were evaluated and presented to evaluate the energy value of CARS. Orthogonal contrasts were used to test linear and quadratic effects of CARS inclusion. Six treatments were evaluated with 8 replications per treatment. The probabilities were considered significant at $\alpha \leq 0.05$.

Exp. 2

To evaluate the impact of feeding CARS on diet digestibility and fatty acid flow, 6 ruminally and duodenally cannulated steers were used in a 3 × 3 replicated Latin Square design. The steers were housed individually in 2.2 × 3.7 m concrete slatted-floor pens and were provided water with ad-libitum access. The room ambient temperature was maintained at 25ºC during the duration of the study. Treatments (n = 3) differed by increasing inclusion of CARS at 0, 2.5, and 5% of diet DM, replacing SFC. All diets and supplements were formulated identical to the Southern Great Plains diet in Exp. 1 (Table 2.3). Steers were assigned randomly to 1 of the 3 treatments over 3 collection periods. Diets were mixed twice weekly and were stored in a cooler to maintain fresh feed. Steers were adapted to a new treatment diet by blending diets from the

previous period and the new period over 4 d by feeding blended amounts of the current diet, while increasing the new diet in a 75:25, 50:50, and 25:75 ratio.

Steers were fed *ad libitum* and feed was delivered once daily at 0700 h and the feed refusals were collected daily before feeding. Intakes were calculated from d 16 to 21, and samples of the feed refusals were collected for d 16 to 21, and diet ingredient samples were collected on d 18. Feed refusals and ingredient samples were dried for 48-hr in a forced air oven at 60^{o}C (AOAC, 1999; method 4.1.03) to calculate DMI and the ingredient DM. Subsamples of feed ingredients were composited by period and subsamples of feed refusals were composited by period and by steer, then both ingredient and refusal samples were frozen at -20^{o}C. At the completion of the trial, ingredient and refusals samples were freeze dried (Virtis Freezemobile 25EX, SP industries, Warminster, PA) and ground to pass through a 1-mm screen in a Wiley Mill (Thomas Scientific, Swedesboro, NJ).

Periods lasted 21 d with 16 d for diet adaption and 5 d of collections from d 17 to 21. Steers were dosed directly into the rumen twice daily at 0800 and 1600 h with 5 g of titanium dioxide (TiO_2) as an insoluble marker on d 7 to 20 for a total of 10 g / d of TiO_2. Duodenal (approximately 250 ml) and fecal grab samples (approximately 300 g) were collected four times / d at 0700, 1100, 1500 and 1900 h on d 17 to 20. Samples were immediately frozen at -20^{o}C. After the period collections, fecal samples were composited by weighing out 100 g from each sample for a 400 g daily sample of thawed wet feces by steer. Fecal and duodenal samples were then freeze-dried (Virtis Freezemobile 25EX, SP industries, Warminster, PA) and ground to pass through a 1-mm screen using a Wiley Mill (Thomas Scientific). The freeze-dried (Virtis Freezemobile 25EX, SP industries, Warminster, PA) and ground fecal and duodenal samples were composited using 2 tablespoons of sample per day by period for each steer.

Feed, fecal, refusals and duodenal samples were analyzed for DM, OM, and NDF. The composited samples were dried at 100^{o}C for 24 h to determine DM. Samples were burned in a cool muffle furnace at 600^{o}C for 6 h to determine OM. The samples were analyzed for NDF as described by Van Soest et al. (1991), with the addition of alpha-amylase which was added in 0.5 mL increments at the beginning and 30 min into reflux for all fecal, duodenal, orts, SFC as a high starch feed ingredient, and supplement composites. Sodium sulfite was added to all samples at 0.5 g before refluxing. Additionally, ingredient samples of DDGS and CARS had fat extracted before NDF analysis using the fat and NDF procedure described by Buckner et al. (2013). Gross energy was determined for feed and fecal samples using a Parr 6400 oxygen bomb calorimeter (Parr Instrument Company, Moline, IL) and digestible energy was calculated using the gross energy intake and gross energy in feces.

Fecal and duodenal samples were ground again to pass through a ½ mm screen (Cyclotec 1093, Foss Tecator AB, Hoganas, Sweden) to measure TiO_2 concentration (Spectra MAX 250, Moleculare Devices, LLC, Sunnyvale, CA) using the procedure described by Myers et al. (2004). The TiO_2 concentration was used to calculate both fecal output and feed flow through the duodenum. Feed ingredient and duodenal samples were analyzed for total fat, which was outlined by Bremer (2010) using a bi-phasic fat extraction method utilizing a 1:1 ratio of hexane and diethyl ether. However, due to difficulty in separating the fat from CARS, crude fat was analyzed by extraction with petroleum ether (Ankom. 2017; Ward Laboratories, Inc. Kearney, NE; Table 2.1)

Fatty acid profile analysis was conducted on all feed ingredient and duodenal samples. Each sample was combined with 10 mL of a 2:1 solution of chloroform and methanol, vortexed, and allowed to sit for 1 h at room temperature. Samples were then centrifuged at 1000 × g for 5

minutes. Two mL of 0.74% potassium chloride solution was then added to the samples, vortexed, and again centrifuged at 1000 × g for 5 minutes. Following centrifugation, the liquid portion was syphonedoff and samples were placed in a heating block and evaporated to dryness under nitrogen at 60°C. Once dried, 1 mL of 0.5 M sodium methoxide was added, and samples were vortexed then heated for 10 minutes at 100°C. After 10 minutes, 1 mL of boron trifluoride in 14% methanol was added, samples were vortexed, and then heated for 5 minutes at 100°C. After heating, 2 mL of saturated salt solution and 2 mL of hexane were added, vortexed, and the samples centrifuged 1000 × g for 5 minutes. After centrifugation, the liquid hexane layer was removed and analyzed via gas chromatography (TRACE 1310 Gas Chromatography; ThermoFisher Scientific, Waltham, MA). Fatty acid separation was done utilizing a capillary column (Chrompack CP-Sil 88 – 0.25 mm x 100 m, inlet temp. 270°C, Oven, 140°C hold for 5 minutes, increase at 4°C/minutes to 240°C and hold for 15 minutes. FID temp: 280°C. Injected at a 30:1 ratio) and were identified by comparing their retention times to known commercial standards (NU-Check Prep, Inc., Elysiam, MN; #GLC-68D, GLC-79, GLC-87, GLC-455, and GLC-458). Fatty acid percentage was calculated as the peak areas in the chromatograph as a percent of total fat flow through the duodenum.

On d 14, wireless pH probes (Dascor Inc; Escondido, CA) were placed directly into the rumen and were removed on d 21. Probes were programmed to record rumen pH every minute. Once removed, the probes were washed and the data immediately retrieved. Rumen pH averages were evaluated from d 16 to 20 for each period and the pH was measured at the same time for all steers. On d 21 at 1500 h, a whole rumen fluid sample was collected in order to correct microbial protein supplied in the duodenal samples by obtaining a purine:N ratio for rumen microbial biomass. Samples were collected using a scoop directly from the rumen via the rumen cannula

and were mixed with 2 L of 10% formalin solution, then frozen at -20°C. Whole rumen samples were blended in a blender for approximately 1 minute and strained through four layers of cheesecloth. The liquid portion was poured into 250 ml bottles and centrifuged at 500 × g for 20 minutes. The liquid portion of was poured off into tubes and centrifuged at 30,000 × g for 20 minutes. Following this step, the liquid portion was siphoned off so that the remaining bacteria pellet was exposed. The pellet was then washed into a container and frozen. Samples were freeze dried (Virtis Freezemobile 25EX, SP industries, Warminster, PA). Both the duodenal and whole rumen bacteria samples were analyzed for purine content. The purine procedure used was outlined by Zinn and Owens (1986). However, this procedure was modified using more dilute $HCLO_4$ as described by Crawford et al. (2008). Dilution consisted of adding 167 ml of 12 M (70%) $HCLO_4$ to 833 ml deionized distilled H_2O in a 1 L volumetric flask. With a spectrometer set at 260 nm, purine concentration was determined so that true ruminal digestibility could be calculated by subtracting the microbial fraction from the concentration of nutrient intake and the nutrient concentration in the duodenal sample. Ingredient and whole rumen bacteria samples were analyzed for nitrogen and crude protein using the nitrogen content combustion (DUMAS) method (TruSpec N, LECO Corporation, St. Joseph, MI). The purine:nitrogen ratio was determined for each animal within period and used to determine flow of nutrients to the duodenum.

Intakes, digestibility, and duodenal fatty acid flow were analyzed using the MIXED procedure in SAS (SAS Inst., Cary, NC) with treatment and period as fixed effects and steer as a random effect. Orthogonal contrasts were used to test linear and quadratic effects of CARS inclusion on all data. Ruminal pH data were analyzed as a repeated measure using the MIXED procedure of SAS. The model consisted of the fixed effects of treatment and period with steer in

each period being random effect. Day was treated as a repeated measure. The probabilities were considered significant at $\alpha < 0.10$ for metabolism results.

Results and Discussion

Exp. 1

Steers fed the Southern diet with 0 or 5% CARS had greater ($P \leq 0.05$) shrunk final BW and live ADG then steers fed the Northern diet when compared at the respective CARS level (Table 2.4). Steers fed 2.5% CARS had similar shrunk BW and live ADG, regardless of diet type. These differences were due to changes in dressing percent. There were no other interactions between CARS inclusion and diet type ($P \geq 0.46$) for any other variable tested, therefore main effects of carcass adjusted performance and carcass characteristics are discussed.

CARS inclusion main effects

Dry matter intake decreased linearly ($P < 0.01$; Table 2.5) as level of CARS increased. For carcass adjusted final BW and ADG, there was a quadratic response ($P < 0.01$), with 2.5% having greater carcass adjusted final BW and daily gains, compared to the 0 and 5%, while 5% had the least final BW and ADG. Due to decreased DMI and increased ADG, carcass adjusted G:F was greatest ($P < 0.01$) in cattle fed 2.5% CARS, while G:F for 0 and 5% were similar. Norman et al. (2018) also observed a linear decrease in intake and a quadratic response in final BW and ADG as CARS inclusion increased in the diet up to 2.5% CARS then decreasing at the 5% and 7.5% CARS inclusion. Norman et al. (2018) observed a decrease in feed efficiency as CARS was increased in the diet, though their observed response was linear. The decrease in intake and the decrease in ADG and G:F could be due to the high sulfur content of CARS, which can reduce intake (Sarturi et al., 2013; Zinn et al., 1997) In forage diets, Costa et al. (2016)

observed an increase in both DMI and ADG when an algae product was fed as a protein supplement, and Morrill et al. (2017) observed an increase in DMI when post extracted algae residue (PEAR) was included in a feedlot finishing diet, though their rations had a greater roughage content based on their study design. Stamey et al. (2012) observed no effect on DMI when including algae biomass or oil in dairy diets.

There was a quadratic response for both dietary NEm and NEg ($P < 0.01$), with 0% and 5% CARS having similar dietary energy for maintenance and gain, while diets with 2.5% CARS had the greatest NEm and NEg. Zinn et al., (1997) reported a decrease in NEg as sulfur increased in the diet. This could explain the reduction in calculated NEg at 5% CARS. Norman et al. (2018) observed a linear increase in their dietary NEm and NEg as CARS was included, though Morrill et al. (2017) observed a decrease in dietary NEm and NEg as an algae supplement was included.

The response to CARS was quadratic for HCW and linear for LM area ($P < 0.01$). Steers that received either 0% or 2.5% CARS treatment had similar HCW and LM area while steers that received 5% CARS had the lowest HCW and LM area. There was no difference ($P = 0.12$) in dressing percentage for any inclusion of CARS. Marbling score was linearly increased as CARS was included in the diet ($P < 0.01$). There was a quadratic response for 12^{th} rib fat thickness and USDA YG ($P < 0.01$), with 2.5% CARS having the greatest values. Morrill et al. (2017) also reported greater marbling scores; however, in their study they saw no difference in 12^{th} rib fat thickness, LM area, or USDA YG. Morrill et al. (2017) reported no difference in HCW when algae supplement was included in the diet.

Diet type main effects

Steers fed the Northern and Southern diets had similar ($P = 0.72$) DMI (Table 2.6). Steers fed the Southern diets had greater carcass adjusted final BW compared to steers fed the Northern diets ($P < 0.01$) increasing by 16 kg. Southern diets also had a greater ADG compared to the Northern diets ($P < 0.01$) with a 5.6% increase in gain. Gain to feed was greater in the Southern diets ($P < 0.01$) with a 5.8% difference. This difference between SFC and DRC not reflecting the 12% difference established by Owens et al. (1997), can be explained in part by the diets themselves, with the Northern diets containing a 50:50 DRC:HMC blend, which gives added efficiency to the diet. The diets are also composed of only 73% corn on a DM basis, which also decreased the amount of energy in the diet. Inclusion of distillers grains in either diet also has an effect on feed efficiency, with DDGS only being 110 to 112% the feeding value of corn, while WDGS is 131 to 145% the value of corn (Bremer et al., 2011). Dietary NEm and NEg were different between base diets ($P < 0.01$) with Southern diets having greater energy concentration than Northern diets, with 4.6% and 6.2% difference for NEm and NEg, respectively. Corrigan et al. (2009) reported that DMI was least when steers were fed SFC compared to either DRC or HMC based diets, and the intakes decreased as WDGS was increased in their rations. They reported no difference in ADG when no byproduct was included; however, Huck et al., (1998) and Cooper et al. (2002) observed that steers fed a SFC based diet had greater ADG when compared to either DRC or HMC based diets when no distillers were added, and Huck et al (1998) also observed a greater feed efficiency in the SFC diets. Both dietary NEm and NEg were greater in SFC diets, when compared to HMC or DRC diets. (Cooper et al., 2002).

Steers fed the Southern diets had greater HCW compared to steers fed the Northern diets ($P < 0.01$), though there was no difference ($P = 0.94$) in dressing percentage. The LM area was

similar for both diets, though there was a tendency ($P = 0.09$) for Southern diets to have a greater area. The 12th rib fat thickness was greater for Southern diets compared to the Northern ($P = 0.02$), while there was a tendency ($P = 0.06$) for Southern diets to have greater marbling scores compared to Northern. USDA Yield grade was different ($P = 0.01$) with Southern diets having a greater YG compared to Northern diets. Huck et al. (1998) reported increased HCW in SFC diets, though they reported no difference for 12th rib fat or marbling score. Corrigan et al. (2009) reported greater HCW, marbling scores 12th rib fat, and YG for DRC based diets compared to SFC diets, though LM area was not different.

Exp. 2

Total fatty acid content of the diets numerically increased with the addition of CARS (Table 2.7), with n-3 fatty acids increasing from 0.13 to 0.33% of diet DM for 0% and 5% diets, respectively. Costa et al. (2016) reported an increase in crude lipid content in *Schizochytrium sp.* compared to other algae species.

Intake of both DM and OM were not affected by CARS inclusion ($P \geq 0.17$; Table 2.8), though there was a linear decrease ($P = 0.07$) in NDF intake as CARS was included in the diet. Norman (2021) reported a linear decrease in DM, OM and NDF intake as they included CARS at 0, 5, and 10% of the diet DM. Though their intakes were similar when CARS was include at 0 and 5%, which supports the lack of difference and tendency in intake for this experiment. Inclusion of CARS had no effect on total tract DM, OM, or NDF digestibility ($P \geq 0.52$). Gross energy (GE) DM intake and digestible energy (DE) DM intake were not different ($P \geq 0.32$). Apparent ruminal DM, OM or NDF digestibility were not affected by treatment ($P \geq 0.20$). True ruminal DM and OM digestibility were not affected by treatment ($P \geq 0.38$). Norman (2021) also

reported no differences in DM and OM digestibility regardless of treatment, though they did detect a quadratic response in NDF digestibility, with CARS included at 5% of diet DM having the greatest NDF digestibility and CARS included at 10% being the least digestible. Morrill et al. (2017) reported a decrease in DM, OM, and NDF digestibility when an algae by-product was included at roughly 9% the finishing diet.

Total fatty acid flow at the duodenum was unaffected ($P = 0.18$ Table 2.9) as CARS increased in the diet, though the fatty acid profile was impacted, with a linear decrease ($P = 0.06$;) in saturated fatty acids (SFA), correlated with a linear increase ($P \leq 0.07$) in unsaturated (UFA), mono-unsaturated (MUFA), and poly-unsaturated fatty acids (PUFA) as CARS increased in the diet. There was a linear increase ($P < 0.01$) of C16:0, C18:0, C18:1T, and C18:1 Vaccenic acid in the fatty acids flow to the small intestine. There was also a quadratic response for C18:3ω3 α-Linoleic acid concentrations ($P = 0.01$) with CARS included at 2.5% having the greatest g/d flow of α-Linoleic acid, increasing from 1.54 g/d flow at the 0% CARS to 2.07 g/d flow at the 2.5% CARS, decreasing at 5% CARS to 1.87 g/d flow to the small intestine. This supports the claim made by Morrill et al. (2017) for greater C18:3 when feeding an algae residue. Franklin et al. (1999) also reported an increase in both UFA and SFA in the milk concentration of dairy cows as algae was included at 3.97 g/100 g diet DM.

Both n-3 and n-6 fatty acid content of total flow to the small intestine also increased linearly ($P \leq 0.02$) as CARS increased in the diet, with 5% having the greatest n-3 and n-6 content and 0% having the least n-3 and n-6 fatty acids. The DHA content of fat available for post rumen digestion was significantly different ($P < 0.01$) with 5% CARS treatment having the greatest DHA content at 7.75 g/d flow to the small intestine compared to 2.5% CARS having 5.12 g/d and 0% CARS having the least at 4.57 g/d. There was not enough EPA in the samples to

analyze. Doreau and Chilliard (1997) reported a low DHA and EPA concentration in the duodenal flow of dairy cows supplemented with fish oil, and Stamey et al (2012) reported an increased DHA content in milk when protected algae was compared to fish oil. These findings suggest that biohydrogenation could affect the flow of ruminal PUFA, thereby decreasing the amount of DHA and EPA for digestion in the small intestine.

Average ruminal pH (Table 2.10) linearly increased ($P < 0.01$) as CARS was included in the diet. There was a linear decrease ($P \leq 0.01$) in both time and area under pH of 5.6 as CARS was included. A rise in pH could be due to the increase in S in the CARS diet. While intake was not different, there was a numerical decrease, which would be expected with high S content of feed (Zinn at al. 1997). Drewery et al. (2014), when feeding chopped prairie hay with either no supplement, or a supplement comprising of post extracted algae residue, or cotton seed meal (CSM), supplemented at 100 mg N/kg of BW reported a decrease in ruminal pH when the algae or CSM was supplemented, compared to the control.

The results from the current experiments indicate that including CARS at 2.5% of diet DM may improve feed efficiency, HCW, 12th rib fat, and yield grade. For diets utilized in Exp. 1, there was greater feed efficiency and hot carcass weight in Southern diets compared to the Northern base diets which tends to be consistent in the literature. Morrill et al. (2017) also reported greater marbling scores and suggested that an increase in post-rumen C18:1 absorption could have contributed to the accumulation of body fat. This is also supported by the duodenal fatty acid profile in Exp. 2 which shows that there were more MUFA in the 5% CARS treatment. Replacing up to 5% steam-flaked corn with CARS did not impact DM, OM, or NDF intake or total tract digestibility in Exp. 2. With a small numerical increase in fatty acid content of the diet and small numerical decrease in DMI, there was no effect on total fatty acid flow to the small

intestine. However, concentration of PUFA, including n-3 and DHA in the duodenal flow, increased with CARS inclusion in the diet. Thus, CARS can effectively be included in feedlot finishing diets up to 2.5% of the diet DM without affecting digestibility and can influence duodenal fatty acid flow composition. Including CARS at 2.5% of diet DM, in both SFC and HMC:DRC based finishing diets, improved feed efficiency compared to the control.

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Table 2.1. Nutrient composition of Condensed Algal Residue Solubles (CARS) as an ingredient in cattle finishing diets

	Exp. 1	Exp. 2
Dry Matter (DM), %	25.43[1]	22.7[2]
	Dry Basis	
Organic Matter	-	63.0[2]
Crude Protein	19.30[1]	21.7[2]
Neutral Detergent Fiber	-	1.59[2]
Acid detergent fiber	0.20[1]	-
Fat[3]	8.30[1]	6.10[1]
Calcium	0.44[1]	0.44[1]
Phosphorus	0.53[1]	0.53[1]
Potassium	0.80[1]	0.80[1]
Sulfur	3.05[1]	3.05[1]
Sodium	9.96[1]	9.96[1]
	Mg/kg, DM Basis	
Magnesium	0.45[1]	
Zinc	55.4[1]	
Iron	168[1]	
Manganese	13[1]	
Copper	8.2[1]	
Molybdenum	1.18[1]	

[1]Nutrient Composition of CARS analyzed by Ward Laboratories, Inc. (Kearney, NE).
[2]Analysis conducted on site at the University Nebraska, Lincoln (Lincoln, NE)
[3]Total fat was analyzed using acid hydrolysis method.

Table 2.2. Fatty acid profile of Condensed Algae Residue Solubles (CARS) as an ingredient in cattle finishing diets (Exp. 1)

***Fatty Acid*[1]**	*% of Fat*
SFA	28.3
UFA	68.1
MUFA	1.45
PUFA	66.7
ω3	64.8
ω6	1.82
C16:0	22.9
C18:0	1.42
C18:1T	nd[2]
C18:1 Oleic	0.101
C18:1 Vaccenic	nd[2]
C18:2 Linoleic	0.054
C18:3ω3 α-Linoleic	nd[2]
C20:5ω3 (EPA)	0.157
C22:6ω3 (DHA)	49.1

[1] Fatty acid analysis was conducted utilizing hexane and a 2:1 solution of chloroform and methanol. The liquid hexane layer was removed and analyzed via gas chromatography (TRACE 1310 Gas Chromatography; ThermoFisher Scientific, Waltham, MA). Fatty acid separation was done utilizing a capillary column (Chrompack CP-Sil 88) and were identified by comparing their retention times to known commercial standards (NU-Check Prep, Inc., Elysiam, MN; #GLC-68D, GLC-79, GLC-87, GLC-455, and GLC-458). Fatty acid percentage was calculated as the peak areas in the chromatograph as a percent of total fat flow through the duodenum

[2] nd signifies no fatty acid was measured

Table 2.3. Dietary treatment compositions (DM basis) in the evaluation of Condensed Algae Residue Solubles (CARS) as an ingredient in cattle finishing diets and inclusion on diet digestibility (Exp. 1)

	Treatment					
	Northern			Southern		
Ingredient, %diet DM	**0%**	**2.5%**	**5%**	**0%**	**2.5%**	**5%**
Dry Rolled Corn	36.5	35.25	34.0	-	-	-
High Moisture Corn	36.5	35.25	34.0	-	-	-
Wet Distillers Grains Plus Solubles	15.0	15.0	15.0	-	-	-
Steam Flaked Corn	-	-	-	73.0	70.5	68.0
Dried Distillers Grains Plus Solubles	-	-	-	15.0	15.0	15.0
CARS	0.0	2.5	5.0	0.0	2.5	5.0
Alfalfa Haylage	8.0	8.0	8.0	8.0	8.0	8.0
Supplement	4.0	4.0	4.0	4.0	4.0	4.0
Supplement						
Limestone	1.69	1.69	1.69	1.69	1.69	1.69
Fine Ground Corn	1.32	1.47	1.62	1.32	1.47	1.62
Urea	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.3	0.15	-	0.3	0.15	-
Tallow	0.1	0.1	0.1	0.1	0.1	0.1
Trace Mineral	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin ADE	0.015	0.015	0.015	0.015	0.015	0.015
Rumensin-90	0.016	0.016	0.016	0.016	0.016	0.016
Tylan-40	0.009	0.009	0.009	0.009	0.009	0.009
Nutrient Composition, %						
DM	73.1	73.4	73.7	77.4	73.0	68.8
OM, % DM	94.8	94.1	93.2	95.4	94.6	93.7
NDF, % DM	15.6	15.3	15.1	16.9	16.6	16.4
CP, % DM	14.2	14.4	14.6	14.2	14.4	14.6
Fat, % DM	4.85	4.90	4.96	3.38	3.46	3.54
Na, % DM						
S, % DM						

[1] Treatments were arranged as a 2×3 factorial and included CARS at 0, 2.5, and 5% of diet DM in both Northern and Southern Great Plains diets.

Table 2.4. Simple effects and interactions of CARS and diet type in the evaluation of Condensed Algae Residue Solubles (CARS) as an ingredient in cattle finishing diets (Exp. 1)

	Treatments[1]							P-Value[2]
	Northern			Southern				Interaction
	0%	2.5%	5%	0%	2.5%	5%	SEM	CARS*Diet
Performance, live								
Initial BW, kg	431	431	431	431	431	431	0.4	0.97
Shrunk Final BW, kg	701 ^{b}	711 ^{b}	678 ^{c}	730 ^{a}	708 ^{b}	698 ^{b}	15.8	0.05
DMI, kg/d	11.9 ^{a}	11.6 ^{bc}	10.9 ^{d}	11.9 ^{ab}	11.5 ^{c}	10.8 ^{d}	0.12	0.99
ADG, kg	1.82 ^{b}	1.89 ^{b}	1.67 ^{c}	2.02 ^{a}	1.87 ^{b}	1.81 ^{b}	0.048	0.05
G:F	0.153 ^{b}	0.164 ^{ab}	0.154 ^{b}	0.171 ^{a}	0.162 ^{ab}	0.167 ^{a}	0.0044	0.07
Performance, Carcass Adjusted								
Final BW, kg[3]	701 ^{b}	707 ^{b}	676 ^{d}	720 ^{a}	724 ^{a}	689 ^{c}	4.0	0.75
ADG, kg[3]	1.83 ^{b}	1.86 ^{b}	1.66 ^{d}	1.95 ^{a}	1.97 ^{a}	1.74 ^{c}	0.028	0.79
Gain:Feed[3]	0.153 ^{c}	0.161 ^{b}	0.153 ^{c}	0.164 ^{b}	0.171 ^{a}	0.161 ^{b}	0.002	0.72
NEm, Mcal/kg[4]	1.91 ^{a}	1.98 ^{bc}	1.93 ^{ac}	2.01 ^{b}	2.07 ^{a}	2.00 ^{b}	0.018	0.68
NEg, Mcal/kg[4]	1.26 ^{a}	1.33 ^{bc}	1.28 ^{ac}	1.35 ^{b}	1.41 ^{a}	1.34 ^{b}	0.016	0.70
Carcass Characteristics								
HCW, kg	442 ^{b}	446 ^{b}	426 ^{d}	454 ^{a}	456 ^{a}	434 ^{c}	2.5	0.75
12th rib fat, cm	1.55 ^{bc}	1.65 ^{ab}	1.50 ^{c}	1.63 ^{ab}	1.73 ^{a}	1.57 ^{bc}	0.041	0.99
Marbling Score[5]	551 ^{ab}	572 ^{bc}	592 ^{a}	575 ^{abc}	585 ^{ab}	603 ^{a}	10.4	0.81
LM area, cm^{2}	95.5 ^{ab}	94.2 ^{bc}	92.3 ^{c}	97.4 ^{a}	96.8 ^{ab}	92.3 ^{c}	1.03	0.46
Dressing Percent	63.0 ^{ab}	62.6^{ab}	62.8 ^{ab}	62.1 ^{b}	64.3^{a}	62.1^{b}	0.01	0.10
Yield Grade	3.53 ^{bc}	3.62 ^{ab}	3.47 ^{c}	3.61 ^{ab}	3.71 ^{a}	3.55 ^{bc}	0.038	0.99

[1] Treatments were arranged as a 2×3 factorial and included CARS at 0, 2.5, and 5% of diet DM in both Northern and Southern Great Plains diets

[2] Main effects included CARS inclusion in the diet and diet type (Northern or Southern Great Plains). CARS*Diet is the interaction between these factors. Linear and quadratic orthogonal contrasts are shown for CARS inclusion in the diet

[3] Calculated from hot carcass weight, adjusted to a common 63% dressing percentage

[4] Calculated utilizing initial BW, adjusted final BW, heaviest block wt, ADG and DMI (NRC, 1996 equations)

[5] Marbling Score 400-Small00, 500 = Modest00

^{abc} Means within a row that lack a common superscript are different ($P < 0.05$)

Table. 2.5 Main effects of CARS in the evaluation of Condensed Algal Residue Solubles (CARS) as an ingredient in cattle finishing diets (Exp. 1)

	Treatment[1]				*P*-Value[2]		
	0%	2.5%	5%	SEM	CARS	Linear	Quadratic
Pen, n	16	16	16				
Performance, Carcass Adjusted							
Initial BW, kg	431	431	431	0.3	0.81	0.55	0.80
DMI, kg/d	11.9 [a]	11.6 [b]	10.8 [c]	0.08	< 0.01	< 0.01	0.05
Final BW, kg[3]	710 [a]	715 [a]	682 [b]	2.9	< 0.01	< 0.01	< 0.01
ADG, kg[3]	1.88 [a]	1.92 [a]	1.70 [b]	0.020	< 0.01	< 0.01	< 0.01
Gain:Feed[3]	0.159 [b]	0.166 [a]	0.157 [b]	0.0015	< 0.01	0.72	< 0.01
NEm, Mcal/kg[4]	1.96 [b]	2.03 [a]	1.97 [b]	0.018	< 0.01	0.67	< 0.01
NEg, Mcal/kg[4]	1.31 [b]	1.37 [a]	1.31 [b]	0.016	< 0.01	0.70	< 0.01
Carcass Characteristics							
HCW, kg	447 [a]	450 [a]	430 [b]	1.8	< 0.01	< 0.01	< 0.01
12th rib fat, cm	1.59 [b]	1.69 [a]	1.54 [b]	0.028	< 0.01	0.20	< 0.01
Marbling Score[5]	563 [b]	579 [ab]	597 [a]	7.5	0.01	< 0.01	0.87
LM area, cm[2]	96.5 [a]	95.5 [a]	92.3 [b]	0.74	< 0.01	< 0.01	0.28
Dressing Percent	62.6	63.4	62.5	0.005	0.29	0.90	0.12
Yield Grade	3.57 [b]	3.67 [a]	3.51 [b]	0.028	< 0.01	0.16	< 0.01

[1] Treatments were arranged as a 2×3 factorial and included CARS at 0, 2.5, and 5% of diet DM in both Northern and Southern Great Plains diets

[2] Main effects included CARS inclusion in the diet and diet type (Northern or Southern Great Plains). CARS*Diet is the interaction between these factors. Linear and quadratic orthogonal contrasts are shown for CARS inclusion in the diet

[3] Calculated from hot carcass weight, adjusted to a common 63% dressing percentage

[4] Calculated utilizing initial BW, adjusted final BW, heaviest block wt, ADG and DMI (NRC, 1996 equations)

[5] Marbling Score 400-Small00, 500 = Modest00

[abc] Means within a row that lack a common superscript are different ($P < 0.05$)

Table. 2.6 Main effects of diet in the evaluation of Condensed Algal Residue Solubles (CARS) as an ingredient in cattle finishing diets (Exp. 1)

	Treatment[1]			*P*-Value[2]	
	Northern	**Southern**	**SEM**	**CARS*Diet**	**Diet**
Pen, n	24	24			
Performance, Carcass Adjusted					
Final BW, kg[3]	695	711	2.4	0.75	< 0.01
DMI, kg/d	11.4	11.4	0.07	0.99	0.72
ADG, kg[3]	1.78	1.88	0.017	0.79	< 0.01
G:F[3]	0.156	0.165	0.0012	0.72	< 0.01
NEm, Mcal/kg[4]	1.94	2.03	0.018	0.68	< 0.01
NEg, Mcal/kg[4]	1.29	1.37	0.016	0.70	< 0.01
Carcass Characteristics					
HCW, kg	438	447	1.5	0.75	< 0.01
12th rib fat, cm	1.57	1.65	0.023	0.99	0.02
Marbling Score[5]	571	588	6.18	0.81	0.06
LM area, cm^2	94.1	95.5	0.61	0.46	0.09
Dressing Percent	62.8	62.8	0.004	0.10	0.94
Yield Grade	3.54	3.62	0.028	0.99	0.01

[1] Treatments were arranged as a 2×3 factorial and included CARS at 0, 2.5, and 5% of diet DM in both Northern and Southern Great Plains diets

[2] Main effects included CARS inclusion in the diet and diet type (Northern or Southern Great Plains). CARS*Diet is the interaction between these factors. Linear and quadratic orthogonal contrasts are shown for CARS inclusion in the diet

[3] Calculated from hot carcass weight, adjusted to a common 63% dressing percentage

[4] Calculated utilizing initial BW, adjusted final BW, heaviest block wt, ADG and DMI (NRC, 1996 equations)

[5] Marbling Score 400-Small00, 500 = Modest00

[abc] Means within a row that lack a common superscipt are different ($P < 0.05$)

Table 2.7. Fatty acid profile of diets including Condensed Algal Residue Solubles (CARS) (Exp. 2)

	Treatment[1]		
Fatty Acid, % diet DM	**0%**	**2.5%**	**5%**
Total Fat	3.38	3.46	3.54
		% of fat	
SFA	18.7	19.0	19.4
UFA	81.0	80.6	80.1
MUFA	26.3	25.6	24.9
PUFA	54.7	54.9	55.2
ω3	4.15	5.73	7.30
ω6	0.003	0.007	0.011
C16:0	0.358	0.361	0.354
C18:0	0.240	0.241	0.232
C18:1T	24.7	24.1	23.4
C18:1 Oleic	0.488	0.473	0.467
C18:1 Vaccenic	50.3	48.9	47.5
C18:2 Linoleic	3.96	3.93	3.89
C18:3ω3 α-Linoleic	0.427	0.427	0.427
C20:5ω3 (EPA)	0.153	1.38	2.59
C22:6ω3 (DHA)	14.8	15.0	15.3

[1]Treatments varied in CARS inclusion, 0%, 2.5%, and 5% of the diet DM replacing steam flaked corn; CARS = condensed algae residue solubles

Table 2.8. Effect of Condensed Algal Residue Solubles (CARS) inclusion on diet digestibility (Exp. 2)

	Treatment[1]				*P*-Value[2]	
Item	**0%**	**2.5%**	**5%**	**SEM**	**Linear**	**Quadratic**
DM						
Intake, kg/d	7.89	7.80	7.39	0.313	0.27	0.67
Apparent Rumen digestibility, %	26.2	22.2	25.6	2.18	0.86	0.20
True Rumen digestibility, %	53.6	51.8	55.1	2.405	0.66	0.38
Total Tract digestibility, %	81.2	81.3	82.2	1.45	0.58	0.80
OM						
Intake, kg/d	7.53	7.39	6.94	0.293	0.17	0.67
Apparent Rumen digestibility, %	34.3	31.4	33.3	2.318	0.77	0.42
True Rumen digestibility, %	62.3	61.2	64.1	2.51	0.62	0.53
Total Tract digestibility, %	83.6	83.7	84.3	1.349	0.68	0.87
NDF						
Intake, kg/d	1.27	1.22	1.13	0.049	0.07	0.75
Apparent Rumen digestibility, %	14.2	14.7	13.3	4.98	0.87	0.85
Total Tract digestibility, %	53.4	51.1	54.4	4.885	0.83	0.52
Energy						
Gross Energy Intake, Mcal/d	31.1	30.9	29.4	1.25	0.32	0.66
Digestible Energy Intake, Mcal/d	24.9	25.0	24.3	1.34	0.71	0.78
Digestible Energy, Mcal/kg of DM	3.15	3.21	3.27	0.063	0.15	0.94

[1]Treatments varied in CARS inclusion, 0%, 2.5%, and 5% of the diet DM replacing steam flaked corn; CARS = condensed algae residue solubles

[2] Linear and quadratic orthogonal contrasts are shown for CARS inclusion.

[abc] Means in a row with different superscripts differ ($P < 0.05$)

Table 2.9. Effect of Condensed Algal Residue Solubles (CARS) inclusion on fatty acid profile of duodenal flow (Exp. 2)

	Treatment[1]				*P*-Value[2]	
Fatty Acid, g/d[3]	**0%**	**2.5%**	**5%**	**SEM**	**Linear**	**Quadratic**
Total Duodenal Fat Flow	438.4	493.2	458.2	25.817	0.60	0.18
SFA	315 [ab]	332 [a]	238 [b]	25.449	0.06	0.10
UFA	123 [b]	158 [b]	212 [a]	11.469	< 0.01	0.52
MUFA	73.6 [c]	107.0 [b]	154.2 [a]	10.375	< 0.01	0.60
PUFA	49.2 [b]	51.2 [ab]	58.0 [a]	3.095	0.07	0.54
ω3	11.0 [b]	12.1[b]	15.4[a]	4.22	< 0.01	0.09
ω6	3.92 [b]	4.02 [b]	4.59 [a]	1.32	0.02	0.28
C16:0	63.6[c]	93.7[b]	111.2[a]	5.49	< 0.01	0.36
C18:0	236.9[a]	218.5[a]	108.1[b]	23.16	< 0.01	0.13
C18:1T	31.5[c]	59.5[b]	108.5[a]	9.66	< 0.01	0.39
C18:1 Oleic	34.8	37.0	33.1	2.05	0.59	0.24
C18:1 Vaccenic	4.26[b]	5.92[a]	7.36[a]	0.60	< 0.01	0.88
C18:2 Linoleic	40.5	40.3	39.3	2.72	0.77	0.90
C18:3ω3 α-Linoleic	1.54[b]	2.07[a]	1.87[a]	0.101	0.04	0.01
C20:5ω3 (EPA)	.	.	.	.	.	.
C22:6ω3 (DHA)	4.57[a]	5.12[a]	7.75[b]	3.99	< 0.01	0.19

[1]Treatments varied in CARS inclusion, 0%, 2.5%, and 5% of the diet DM replacing steam flaked corn; CARS = condensed algae residue solubles

[2] Linear and quadratic orthogonal contrasts are shown for CARS inclusion.

[3] Fatty Acids given as g/d of fat flowing to the duodenum.

[abc] Means in a row with different superscripts differ ($P < 0.05$)

Table 2.10. Effect of Condensed Algal Residue Solubles (CARS) inclusion on rumen pH (Exp. 2)

	Treatment[1]				*P*-Value[2]	
Item	**0%**	**2.5%**	**5%**	**SEM**	**Linear**	**Quadratic**
Average, pH	5.76 [b]	5.83 [b]	6.06 [a]	0.105	< 0.01	0.10
Minimum, pH	5.17 [b]	5.23 [b]	5.38 [a]	0.062	< 0.01	0.21
Maximum, pH	6.64 [b]	6.57 [b]	6.83 [a]	0.108	< 0.01	< 0.01
Time						
Time under pH 5.6, min/d	661 [a]	555 [a]	324 [b]	113.5	< 0.01	0.35
Time under pH 5.3, min/d	271	186	223	70.5	0.45	0.23
Area[3]						
Area Under 5.6	156 [a]	127 [a]	68.9 [b]	32.48	< 0.01	0.49
Area Under 5.3	26.2	16.8	23.2	8.55	0.73	0.25

[1]Treatments varied in CARS inclusion, 0%, 2.5%, and 5% of the diet DM replacing steam flaked corn; CARS = condensed algae residue solubles

[2] Linear and quadratic orthogonal contrasts are shown for CARS inclusion.

[3] Area < 5.6, 5.3 and 5.0 = ruminal pH units below 5.6 and 5.0 by minute.

[abc] Means in a row with different superscripts differ ($P < 0.05$)

CHAPTER III. Comparison of Revalor-XH with a Revalor-IH/Revalor-H/Revalor-200 Re-implant Program on Feedlot Cattle

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Abstract

A commercial feedlot trial evaluated a 3-implant re-implant program including Revalor-IH/Revalor-H/Revalor-200 compared to a single Revalor-XH implant on feedlot performance and carcass characteristics of calf fed heifers. A total of 1,356 heifers (initial body weight (BW) = 293; SD = 19.1 kg) were utilized in a generalized randomized block design. Treatments consisted of either 1) a Revalor-IH given on d 1, followed by a Revalor-H given on d 67, followed by a Revalor-200 given on d 137 (IH/H/200); or 2) a Revalor-XH given on d 1 (XH). Heifers were fed for an average of 215 d. The IH/H/200 treatment comprised of a total of 400 mg trenbolone acetate (TBA) and 52 mg of estradiol (E2) administered in segments over the course of the trial. The XH treatment involved a total of 200 mg TBA and 20 mg E2 given in a single dose on d 1. The XH implant is comprised of four noncoated pellets containing 80 mg TBA and 8 mg E2 for immediate release and six polymer coated pellets containing 120 mg TBA and 12 mg E2 to release approximately 70 to 80 d after implanting. There were no significant differences ($P \geq 0.21$) between implant treatments for either deads-in or deads-out, live or carcass adjusted final body weight, dry matter intake, average daily gain, or feed efficiency. There were no significant differences ($P \geq 0.81$) between treatments for hot carcass weight, 12^{th} rib fat, dressing percent, or calculated yield grade. There was a tendency ($P \leq 0.09$) for greater NEm, NEg, and marbling score in heifers that received the Revalor-XH treatment. There was also tendency ($P = 0.09$) for a greater LM area in heifers that received the Revalor-IH/H/200. There was no influence of treatment ($P \geq 0.19$) on USDA yield grade or USDA quality grade distributions. Heifers that receive a single Revalor-XH implant, when fed to 215 d, perform similarly to heifers on a 3-implant Revalor-IH/H/200 program.

Key words: finishing heifers, implant, coated, payout, re-implanting

Introduction

The literature is consistent in that when implanting heifers there is improved growth performance as well as increased hot carcass weight (HCW) to heifers that received no implant. Adams et al. (1990) implanted heifers with 200 mg trenbolone acetate (TBA) and 20 mg estradiol benzoate (EB) (Synovex-H; Zoetis; Des Moines, IA) compared to non-implanted heifers. They reported a significant 4.2% increase in final BW and 12.2% increase in ADG, 8% improvement in feed efficiency and a 4.7% improvement in HCW in implanted heifers. Crouse et al. (1987) and Cleale et al. (2013) also reported improvements in growth performance and HCW for implanted heifers. Heifers at the same age tend to have increased fat deposition compared to steers, thus more aggressive implant strategies which utilize more than one implant throughout the feeding period and increase the potency and exposure to TBA and estradiol, are typically used in feedlot finishing heifers.

The literature also suggests, that when comparing implant strategies among heifers, the amount of total hormone used can vary in its effect on performance. Hilscher et al. (2016) observed no differences in live performance or carcass characteristics between three different implant strategies. Treatments includied either 1) a two-implant strategy with single implants given throughout the feeding period for a total of 280 mg TBA and 28 mg estradiol (E2); 2) a two implant strategy with single implants given throughout the feeding period for a total of 340 mg TBA and 34 mg E2; or 3) a two implant strategy with single implants given throughout the feeding period for a total of 400 mg TBA and 40 mg E2. There was a significant decrease in marbling score with increasing hormone concentration. Folmer et al. (2009) compared feedlot heifers implanted with 280 mg TBA and 28 mg E2 (2 single implants) to 400 mg TBA and 40 mg estradiol (2 single implants). Folmer reported greater carcass adjusted feed efficiency and

greater live average daily gain (ADG) in heifers that were implanted with the 280 mg TBA and 28 mg E2 treatment, with a tendency for greater marbling scores for heifers implanted with the low dose of TBA and E2.

Though there is variation between heifer implant strategies, single-dose heifer implants with delayed payouts of both TBA and E2 have been shown to perform similar to other heifer implant strategies which use multiple implants over the feeding period. Schumacher et al. (2019) compared a delayed payout implant with 200 mg TBA and 28 mg EB to a two-implant strategy with single implants given throughout the feeding period for a total of 300 mg TBA and 42 mg EB. They reported no differences between strategies for final BW, ADG, or HCW and improved feed efficiency and intake in the single dose delayed payout implant. The delayed release of the hormones eliminates the need for additional implants, which reduces the labor needs associated with implanting events. Thus, the objective of this trial was to compare the effects of a re-implant program utilizing 3 implants, given as single implants over the feeding period for a total of 420 mg TBA and 42 mg E2, compared to a single dose delayed payout implant with a total of 200 mg TBA and 20 mg E2 over similar days on feed on live performance and carcass characteristics after similar days on feed.

Materials and Methods

Crossbred heifer calves (n= 1356; initial body weight (BW) = 293 kg; SD = 19.0 kg) were used in the finishing study at a commercial feedyard in South central Nebraska. The heifers were sourced from sale barns located in Kansas, Nevada, Wyoming, Montana, and Nebraska. Heifers were received over time ranging from October 2019 to May 2020. This study was conducted as a generalized randomized block design, with the blocking factors being the time of

arrival (n = 7) at the feedlot. Treatments inclueded a Revalor-IH (80 mg TBA and 8 mg E2; uncoated; Merck Animal Health, Desoto, KS) given upon trial initiation, followed by a Revalor-H (140 mg TBA and 14 mg E2; uncoated; Merck Animal Health) given 50 to 73 days later, and Revalor-200 (200 mg TBA, 20 mg E2; uncoated; Merck Animal Health; IH/H/200) given 65 to 77 d after the second implant, or utilizing a single Revalor-XH (200 mg TBA and 20 mg E2; partially coated; Merck Animal Health; XH) at trial initiation. The Revalor-XH implant consists of four noncoated pellets (80 mg TBA and 8 mg E2) for immediate release and six polymer coated pellets (120 mg TBA and 12 mg E2) to release approximately 70 to 80 d after initial implanting.

Heifers were held upon arrival for two days before trial initiation and were fed during the two days a grower diet consisting of 15% steam-flaked corn (SFC), 10% mixed hay, 45% corn silage, 26% wet distillers grains plus solubles (WDGS) and 4% liquid supplement. During the second day after arrival, heifers were checked for pregnancy and were removed if found to be pregnant. Heifers were assigned randomly to pen (n = 20) on d 1 of the trial before feeding, by sorting every two heifers into one of two pens within each replication, with each block containing an equal number of pens. Pens were assigned randomly to implant treatment, with 10 pens per treatment, averaging 72 heifers/pen. Blocks one to three had 2 replications per treatment while blocks four to seven had 1 replication per treatment. On d 1, the heifers were sorted, weighed collectively as pens, and processed. During processing, heifers received the assigned treatment implant in the middle-third of the ear under the skin. Each heifer was also vaccinated against infectious bovine rhinotracheitis (IBR) virus and bovine viral diarrhea (BVD) types one and two viruses (Vista 3 SQ, Merck), dosed with a pour-on for external parasite control using moxidectin (Cydectin, Bayer Animal Health, Shawnee, KS), and given an oral dose of internal

parasite control using fenbendazole (Safe-Guard, Merck). Heifers assigned to the IH/H/200 treatment were re-implanted on d 67 with Revalor-H and on d 137 with Revalor-200, alternating which ear received the implant, always in the middle-third of the ear. Heifers that received Revalor-XH were not removed from their pens while the heifers on the 3-implant treatment were re-implanted.

Heifers were housed in open lots, spread throughout the feedyard and had ad libitum access to both feed and water. Pen dimensions were 76.2 × 45.7 m. with 33.5 m. of feed bunk. Pen space per heifer averaged 348.4 m^2 with an average of 47.2 cm of bunk space (range of 36.83 to 67.1 cm) within each replication. Heifers were stepped up to the finishing diet in three steps, each lasting 6 days, with the finishing diets consisting of 67.8% steam-flaked corn, 16.0% wet distillers grains plus solubles, 3.5% mixed hay, 6.0% corn silage, 1.7% tallow and 5.0% supplement. Diets were constant across treatments (Table 3.1). Feed additives were dosed daily by micro machine which was formulated to provide 340mg/heifer daily of monensin (Rumensin; Elanco Animal Health, Greenfield, IN), 70 mg/heifer daily DM of tylosin (Tylan; Elanco Animal Health), 0.45 mg/heifer daily of melengestrol acetate (MGA; Zoetis, Kalamazoo, MI), and 250 mg/heifer of ractopamine (Optaflexx; Elanco Animal Health). Optaflexx was targeted to be fed for 28 d before the end of the feeding period, allowing for a 2 d withdraw before harvest. All blocks received Optaflexx; however, block three (pen = 4; 268 hd) did not receive Optaflexx, due to COVID-19 related marketing issues, for the inability to schedule a harvest date with time to feed Optaflexx within the 28 to 42 d prescribed window.

All heifers from a block were harvested on the same day, which averaged 215 d on feed (range from 196 to 231 d). Heifers were weighed by pen before to loading and were shipped to, and harvested at various commercial abattoirs (Tyson Foods, Lexington, NE; JBS Swift and Co.,

Grand Island, NE; National Beef, Dodge City, KS). Individual HCW were collected at slaughter, and after a 24 - 48 hour chill, 12th rib fat depth, longissimus muscle (LM) area, marbling, USDA quality grade (QG), and USDA yield grade (YG) were collected using camera grading data. Yield grade was also calculated using the equation 2.5 + (0.98425 x 12th rib fat, cm) + (0.2 x 3.0 [KPH, %]) + (0.00837 x HCW, kg) – (0.0496 x LM area, cm^2) where KPH fat was assumed to be 3.0% (Boggs and Merkel, 1993). Dietary NEm and NEg values were calculated utilizing initial BW, adjusted final BW, the BW at target endpoint (heaviest pen average BW by block), ADG and dry matter intake (DMI; NRC, 1996) equations as described by Vasconcelos and Galyean (2017). Dressing percentage was calculated by dividing HCW by a 4% shrunk live final BW. For carcass analysis, 27 heifers were removed from the analysis due to missing carcass data. It was also not possible to collect 12th rib fat depth, LM area, marbling, QG and YG on block three because of COVID-19 related issues, and these animals were removed from the carcass analysis, although hot carcass weights were collected.

For deads-in calculations, the initial BW was calculated by dividing the pen weight by the number of heifers in the pen, and the initial weight was then shrunk 4%. The final average live BW was also shrunk by 4% and was calculated by taking total pen weight on the day of harvest and dividing it by the total number of heifers shipped. This calculation was used for both deads-in and deads-out calculations. At both re-implanting events, interim DMI was calculated by taking the total feed delivered to the pen up to the day of re-implanting, on a DM basis, and dividing the total DM by the total number of deads-in animal days up to the day of re-implanting. To calculate live ADG, shrunk initial BW was subtracted from the shrunk final live BW. The difference was then divided by total number of deads-in animal days. For total DMI, total feed delivered to the pen, on a DM basis, was divided by the total number of deads-in animal days.

The live G:F was calculated by dividing live ADG by the average DMI. Carcass-adjusted final BW was calculated by dividing the treatment average HCW by a standard dressing percent of 64.0% and was used for both deads-in and deads-out calculations. Carcass-adjusted ADG was also calculated by subtracting shrunk initial BW from the carcass-adjusted final BW. The difference was then divided by the total number of deads-in animal days. Carcass-adjusted G:F was calculated by dividing carcass-adjusted ADG by the average DMI.

For the deads-out calculations, shrunk initial BW was calculated by dividing the initial pen weight, shrunk by 4%, by the final head count at harvest. At both re-implanting events, interim DMI was calculated by taking the total feed delivered to the pen up to the day of re-implanting, on a DM basis, and dividing this by the total number of deads-out animal days up to the day of re-implanting. Live ADG was calculated by taking the shrunk initial BW and subtracting it from the final shrunk BW. The difference is then divided by the total number of deads-out animal days. The DMI was calculated by taking the total feed delivered to the pen on a DM basis, divided by the total number of deads-out animal days. Carcass-adjusted ADG was calculated by taking shrunk initial BW and subtracting it from the carcass-adjusted final BW (the same used in the deads-in calculations), then dividing the difference by the total number of deads-out animal days. Carcass-adjusted G:F was calculated as carcass-adjusted ADG divided by DMI.

The percent morbidity was calculated by dividing the heifers treated during the duration of the study by the total number of heifers enrolled; and the percent mortality was calculated by dividing the number of heifers that died or were euthanized by the total number of heifers enrolled. Percent removed was calculated by dividing the total number of cattle removed from the study because of chronic illness, structural unsoundness or injury by the total number of

heifers enrolled. Thus, the total percent of heifers removed was calculated by dividing the number of heifers that were removed or that had died by the total number of heifers enrolled. Percent of morbidity categories were calculated by taking the number of heifers that were treated during the duration of the trial because of respiratory disease, bloat, foot-rot/hairy heel wart, or all other reason, divided by the total number of heifers enrolled. The categories of total removed were also calculated by taking the number of heifers that were removed and which had died because of respiratory disease, lameness, acidosis, or all other reasons divided by the total number of heifers enrolled.

The deads-in and deads-out live performance (initial and final BW, ADG, DMI, and G:F) and carcass characteristics (HCW, LM area, 12^{th} rib fat, marbling score, dressing percent, and calculated YG) were analyzed as a generalized randomized block design using the MIXED procedure of SAS (9.40, SAS Institute INC., Cary, NC). Pen was the experimental unit. Treatment was analyzed as a fixed effect and block was considered random. Treatment averages were calculated using the LSMEANS option of SAS. Frequency data for QG distribution and camera graded YG distribution were analyzed using the GLIMMIX procedure of SAS, with the model specified a logistic link function for the binary response, with the number of animals harvested identified in the denominator. The means and SE of the proportions for the frequency data were determined using the ILINK option. The probabilities were considered significant at $\alpha \leq 0.05$, among treatments.

Results and Discussion

During the interim re-implant periods, there were no differences ($P \geq 0.21$; Table 3.2) in deads-in or deads-out DMI at the time of either event (d1 to 67 and d 67 to 137). This fits well

with the no difference in DMI reported by Ohnoutka et al. (2021) during the first 70 d and from d 70 to d 140, with heifers being fed for 198 d. In the current trial there was a 3.6% and a 2.6% decrease in deads-out and deads in ADG, respectively for the IH/H/200 from d 67 - 137. Feed efficiency likewise decreased 7.1% and 5.6% for deads-out and deads in, respectively, from d 67 - 137. Ohnoutka et al. (2021) reported an increase in feed efficiency in heifers implanted with a single non-coated implant (Revalor 200; Merck Animal Health) on d 1, though feed efficiency decreased with the passage of time. When heifers received a single non-coated implant (Revalor 200; Merck Animal Health) on d 70 instead of d 1, Ohnoutka reported a greater feed efficiency on d 140, though it decreased by the end of the trial. Because XH heifers on the current trial were not removed from their pen, ADG and G:F between treatments could not be compared. Ohnoutka et al. (2021) were able to weigh heifers implanted with a single XH and reported ADG to increase 10.1% compared to non-implanted heifers d 0 to 70, then by 13.0% from d 70 to 140. However, Ohnoutka also reported a 6.1% decrease in ADG compared to non-implanted heifers from d 70 to 140. Ohnoutka reported that gain to feed followed a similar pattern, with heifers implanted with Revalor XH having a 7.4% increased efficiency on d 70 compared to non-implanted heifers and remaining at a 7.4% improved feed efficiency on d 140, and feed efficiency dropped by 19.4% at the end of the trial, with G:F of XH heifers being 0.134 compared to 0.149 for the non-implanted heifers.

In the current study there were no differences in deads-in or deads-out performance ($P \geq 0.98$; Table 3.3) between IH/H/200 and XH for live shrunk final BW, and no difference ($P \geq 0.30$) for either deads-in and deads-out, DMI, live ADG and live G:F. There were also no differences ($P = 0.35$) between treatments for carcass adjusted final BW, and no difference ($P \geq 0.32$) for deads-in and deads-out carcass adjusted ADG or G:F. There was a slight tendency ($P =$

0.09) observed for calculated NEm and NEg dietary values between the two treatments, with Revalor-XH treatment having the greater NEm and NEg. Ohnoutka et al. (2018) observed no differences in live or carcass adjusted performance between heifers implanted with Revalor XH compared to re-implanted heifers. Smith et al. (2020) reported a significant 0.08% increase in DMI in heifers implanted with Revalor-XH on arrival (Merck Animal Health) compared to heifers that had been re- implanted, and fed approximately 175 d. Crawford et al. (2018) reported a greater DMI though decreased ADG and feed efficiency in heifers implanted with Revalor-XH compared to re-implanted heifers. Re-implanted heifers also had greater carcass adjusted final BW (Crawford et al. 2018). Reporting on another type of feedlot heifer coated implant (Synovex-ONE; 200 mg of TBA and 28 mg EB; Zoetis), Schumacher et al. (2019) observed a 2.6% significant decrease in carcass adjusted feed conversion, though live and carcass adjusted performance were otherwise not different from heifers that had been re-implanted, and fed approximately 180 d.

There were no differences ($P \geq 0.21$) in carcass characteristics between the two treatments for HCW, 12th rib fat thickness, calculated YG and dressing percentage. There was, however, a tendency ($P = 0.06$) for the XH heifers to have a greater marbling score. There was a tendency ($P = 0.08$) for LM area to be greater for IH/H/200 heifers. Smith et al. (2020) also reported significant differences in carcass characteristics, including a 2 kg decrease in HCW, a 2.8% improvement in 12th rib fat, and a 0.32% decrease in DP when heifers were implanted with Revalor-XH. Supporting the current trial Smith also reported a significant 2.6% decrease in LM area, though there was no difference in marbling score. Carlson et al. (2020) also observed no differences in carcass characteristics except for a decreased LM area in heifers that received Revalor-XH compared to re-implanted heifers. Ohnoutka et al. (2021) also observed no

differences in carcass characteristics even with LM area in heifers implanted with a Revalor-XH compared to Heifers implanted with a single non coated implant on d 70, though Crawford et al. (2018) reported a decrease in HCW when heifers were implanted with Revalor-XH compared to re-implanted heifers.

There was no influence ($P \geq 0.19$; Table 3.4) of treatment on USDA QG or USDA YG distribution. Crawford et al. (2018) and Carlson et al. (2020) also reported no difference in USDA QG, though they did observe an influence of implant on USDA YG with more grade 4 carcasses when heifers were implanted with Revalor-XH. The data from the current trial are not as well supported by the literature which reports a tendency for implant to affect USDA QG, and USDA YG (Ohnoutka et al. 2021; Schumacher et al. 2019) and a significant influence of implant on both USDA QG and USDA YG, with more grade 3, 4, and 5 carcasses and an increase in Choice and Prime carcasses when heifers are implanted with a single Revalor-XH compared to heifers implanted with a Revalor-IH followed by a Revalor-200 over an average of 180 days on feed (Smith et al., 2020). Smith et al. (2020) reported that heifers were not finished to the same body composition (12^{th} rib fat), which could help to explain why they observed that heifers implanted with Revalor-XH had improved calculated YG and USDA QG and YG.

There was an increase in morbidity ($P = 0.04$; Table 3.5) in heifers that received XH treatment compared to IH/H/200, with more XH heifers being treated for respiratory disease ($P = 0.03$). However, there were no other differences ($P \geq 0.15$) between treatments for animals treated for either bloat, foot-rot/hairy heel wart, or any other disease or injury. There were no difference in total head removed from the trial ($P = 0.71$), and heifer mortality and heifers removed for chronic illness, structural unsoundness, or injury were not different ($P \geq 0.16$) between implant treatments. Likewise, there were no differences ($P \geq 0.27$) between treatments

for heifers that were removed or that died from respiratory disease, lameness, acidosis, or any other disease or injury. Oney et al. (2018) and Hilscher et al. (2016) reported no differences in performance or carcass characteristics regardless of implant strategy or hormone dosage. Gruber et al. (2011) and Munson et al. (2012) each reported that implant potency did not affect morbidity or mortality rates, which supports these data.

Summary

There were no significant differences between a 3-implant re-implant program, utilizing Revalor-IH/Revalor-H/Revalor-200 compared to a single Revalor-XH implant on deads-in or deads-out, live or carcass adjusted performance or on carcass characteristics, when heifers were finished to the same body composition across 215 days on feed. The tendency for greater NEm, NEg and marbling score for heifers that received the Revalor-XH treatment and the tendency for greater LM area in heifers that received Revalor-IH/Revalor-H/Revalor-200 could be attributed to the increased days on feed, being fed an average of 215 d. Crawford et al. (2018) reported an increase of carcass adjusted final BW, HCW, DP, and USDA Prime and Choice carcasses as days on feed increased, ranging from 172, 193, and 214 d. Heifers that received a single Revalor-XH implant, when fed to similar 215 d, performed similarly to heifers receiving 3 implants across the feeding period (Revalor-IH/H/200 program).

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Table 3.1. Diet composition of heifers fed for an average 215 d implanted with Revalor-XH or Revalor-H/IH/200 combination

Item, % DM [1]	
Steam Flaked Corn	67.8
Wet Distillers Grains Plus Solubles	16.0
Mixed Hay	3.5
Corn Silage	6.0
Tallow	1.7
Supplement [2]	5.0
Diet Nutrient composition	
Crude Protein, % DM	14.20
Crude Fat, % DM	6.70
Calcium, % DM	0.70
Phosphorous, % DM	0.36
Potassium, % DM	0.70
Sulfur, % DM	0.22

[1] Treatments included either a 3-implant re-implant treatment utilizing Revalor-IH (80 mg TBA and 8 mg E2, noncoated, Merck Animal Health Desoto, KS), Revalor-H (140 mg TBA and 14 mg E2 noncoated Merck Animal Health), and Revalor-200 (200 mg TBA, 20 mg E2, noncoated (IH/H/200), Merck Animal Health) or utilizing the single implant Revalor-XH (200 mg TBA and 20 mg E2 partially coated (XH), Merck Animal Health)

[2] Supplements were formulated to provide vitamin A at 30,000 IU/d, vitamin D at 3,000 IU/d, and vitamin E at 100 IU/d and 340mg/hd/d DM of monensin (Rumensin; Elanco Animal Health), 70 mg/hd/d DM tylosin (Tylan; Elanco Animal Health), and 0.45 mg/hd/d of melengestrol acetate (MGA, Zoetis)

Table 3.2. Interim pen weights of heifers fed for an average 215 d implanted with Revalor-XH or Revalor-H/IH/200 combination

	Treatment [1]			
	Rev-IH/H/200	Rev-XH	SEM	*F*-Test
d 67 Interim BW, kg [2]	401	-	-	-
d 137 Interim BW, kg [2]	508	-	-	-
Live Performance, Deads-Out [3]	**d 67**			
DMI, kg/d	9.66	9.54	0.2156	0.38
ADG, kg	1.66	-	-	-
Gain:Feed	0.168	-	-	-
	d 137			
DMI, kg/d	10.15	9.98	0.2018	0.21
ADG, kg	1.60	-	-	-
Gain:Feed	0.156	-	-	-
Live Performance, Deads-In [4]	**d 67**			
DMI, kg/d	9.57	9.47	0.119	0.53
ADG, kg	1.57	-	-	-
Gain:Feed	0.160	-	-	-
	d 137			
DMI, kg/d	9.99	9.84	0.123	0.41
ADG, kg	1.53	-	-	-
Gain:Feed	0.151	-	-	-

[1] Treatments included either a 3-implant re-implant treatment utilizing Revalor-IH (80 mg TBA and 8 mg E2, noncoated, Merck Animal Health Desoto, KS), Revalor-H (140 mg TBA and 14 mg E2 noncoated Merck Animal Health), and Revalor-200 (200 mg TBA, 20 mg E2, noncoated (IH/H/200), Merck Animal Health) or utilizing the single implant Revalor-XH (200 mg TBA and 20 mg E2 partially coated (XH), Merck Animal Health)

[2] During re-implanting events on d 67 and d 137, heifers that received XH treatment were not removed from their pens.

[3] Deads-out DMI, ADG and GF were calculated using the total number of deads-out animal days up to the day of each re-implanting

[4] Deads-in DMI, ADG and GF were calculated using the total number of deads-in animal days up to the day of each re-implanting

Table 3.3. Heifer performance and carcass characteristics of heifers fed for an average 215 d implanted with Revalor-XH or Revalor-H/IH/200 combination

	Treatment [1]			
Item	Rev-IH/H/200	Rev-XH	SEM	F-Test
Pen, n (Head Count)	10 (684)	10 (673)	-	-
Days on Feed	215	215	-	-
Live Performance, Deads-Out				
Initial BW, kg	293	294	1.8	0.55
Final BW [2], kg	608	608	5.6	0.98
DMI, kg/d	10.3	10.2	0.21	0.30
ADG, kg	1.46	1.46	0.022	1.00
G:F	0.142	0.144	0.0012	0.21
Carcass-Adjusted Performance, Deads-Out				
Final BW [3], kg	617	613	6.6	0.35
ADG, kg	1.51	1.49	0.028	0.32
G:F	0.146	0.146	0.0009	0.98
NEm, Mcal/kg [4]	1.75	1.78	0.011	0.09
NEg, Mcal/kg [4]	1.13	1.15	0.0101	0.09
Carcass Characteristics				
HCW, kg	395	393	4.2	0.35
Dressing, %	64.9	64.9	0.15	0.92
LM area, cm^2	91.8	89.7	0.12	0.08
Marbling [5]	561	579	5.9	0.06
12^{th} rib fat, cm	2.05	2.11	0.011	0.21
Calculated YG [6]	3.90	4.02	0.064	0.18
Live Performance, Deads-In				
DMI, kg/d	10.1	10.0	0.09	0.42
ADG[2], kg	1.34	1.32	0.026	0.62
G:F	0.133	0.133	0.0015	0.94
Carcass-Adjusted Performance, Deads-In				
ADG, kg	1.39	1.35	0.028	0.37
G:F	0.137	0.135	0.0015	0.44

[1] Treatments included either a 3-implant re-implant treatment utilizing Revalor-IH (80 mg TBA and 8 mg E2, noncoated, Merck Animal Health Desoto, KS), Revalor-H (140 mg TBA and 14 mg E2 noncoated Merck Animal Health), and Revalor-200 (200 mg TBA, 20 mg E2, noncoated (IH/H/200), Merck Animal Health) or utilizing the single implant Revalor-XH (200 mg TBA and 20 mg E2 partially coated (XH), Merck Animal Health)

[2] Final BW is the average pen weight shrunk by 4%. ADG and G:F are calculated from the shrunk BW.

[3] Carcass-adjusted final BW was determined by dividing the average HCW per treatment by the average dressing percent 64 %.

[4] Calculated utilizing initial BW, live final BW, heaviest block wt, ADG and DMI (NRC, 1996 equations)

[5] USDA marbling scores. 400 = small, 500 = modest, 600 = moderate.

[6] Calculated YG = 2.5 + (0.98425 x 12^{th} rib fat, cm) + (0.2 x 3.0 [KPH, %]) + (0.00837 x HCW, kg) – (0.0496 x LM area, cm^2) where KPH fat was assumed to be 3.0%

Table 3.4. Quality grade and yield grade distribution of heifers fed for an average 215 d implanted with Revalor-XH or Revalor-H/IH/200 combination

	Treatment[1]		
Item	Rev-IH/H/200	Rev-XH	*P*-Values
Quality Grade [2]***, %***			
Prime	13.4	17.4	0.81
Choice	82.5	81.4	
Select	4.2	1.3	
Yield Grade Distribution [2]***, %***			
YG 1	2.5	1.4	0.19
YG 2	18.9	15.0	
YG 3	38.6	40.8	
YG 4	29.5	34.1	
YG 5	10.5	8.7	

[1] Treatments included either a 3-implant re-implant treatment utilizing Revalor-IH (80 mg TBA and 8 mg E2, noncoated, Merck Animal Health Desoto, KS), Revalor-H (140 mg TBA and 14 mg E2 noncoated Merck Animal Health), and Revalor-200 (200 mg TBA, 20 mg E2, noncoated (IH/H/200), Merck Animal Health) or utilizing the single implant Revalor-XH (200 mg TBA and 20 mg E2 partially coated (XH), Merck Animal Health)

[2] All numbers are expressed as a percentage. The Yield grade and quality grade values are representative of the proportion of carcasses within each group that received a yield grade and quality grade

Table 3.5. Morbidity, mortality, and removals of heifers fed for an average 215 d implanted with Revalor-XH or Revalor-H/IH/200 combination

Item [2]	Treatment [1]		SEM	F-Test
	Rev-IH/H/200	Rev-XH		
Morbidity, % [3]	8.91	14.12	1.654	0.04
Respiratory	7.99	14.42	1.919	0.03
Bloat	0.31	0.50	0.249	0.57
Foot-Rot/Hairy heel wart	0.98	0.46	0.249	0.15
Other [4]	1.63	2.53	0.560	0.27
Mortality, % [5]	1.27	2.15	0.426	0.16
Removed, % [6]	4.18	3.85	1.007	0.82
Total Removed, % [7]	5.45	6.00	1.057	0.71
Respiratory	2.26	2.53	0.758	0.80
Lameness	0.96	0.60	0.241	0.30
Acidosis	0.59	0.34	0.238	0.45
Other [8]	1.63	2.53	0.560	0.27

[1] Treatments included either a 3-implant re-implant treatment utilizing Revalor-IH (80 mg TBA and 8 mg E2, noncoated, Merck Animal Health Desoto, KS), Revalor-H (140 mg TBA and 14 mg E2 noncoated Merck Animal Health), and Revalor-200 (200 mg TBA, 20 mg E2, noncoated (IH/H/200), Merck Animal Health) or utilizing the single implant Revalor-XH (200 mg TBA and 20 mg E2 partially coated (XH), Merck Animal Health)

[2] Statistical means were used to generate this table, and proportions of the subcategories may not sum to equal the proportions of the main categories.

[3] Morbidity was calculated as the percent of animals treated in the duration of the study with subsequent categories expressed, each as a percent of the total animals enrolled.

[4] This category includes heifers treated for other illnesses and injuries that did not fit into the other categories.

[5] Mortality was calculated as the percent of heifers that died while on trial.

[6] Removed was calculated as the percent of heifers that were taken off study based on chronic illness, structural unsoundness, or injury.

[7] Total removed was calculated as the percent of heifers that died or that were removed based on the subsequent categories expressed, each as a percent of total animals enrolled.

[8] This category includes heifers removed due to injury or death that did not fit into the other categories.