EXAMINING THE POTENTIAL OF THE GALR2 GENOTYPE AS A MARKER-ASSISTED MANAGEMENT STRATEGY TO IMPROVE PRODUCTION EFFICIENCIES AND CARCASS CHARACTERISTICS IN CROSSBRED ANGUS FINISHING STEERS

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Title

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MASTER OF SCIENCE

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ABSTRACT

The objective was to determine how the interaction of implant strategies with the *galanin receptor 2* genotype would influence feeding behavior, production efficiencies, carcass characteristics, and meat quality in finishing steers. Angus steers were selected based on *GALR2-c.-199T>G* genotype (n = 36 TT, 38 TG, and 19 GG). Calves were blocked by body weight and fed a standard feedlot ration, blood and BW were collected every 28 d. Steers were randomly assigned to an implant strategy of Revalor-S (1×) or Revalor-S (2×). Intake and feeding behavior data were individually recorded. There was an effect of genotype on DMI but not feed efficiency. Treatment interactions were observed for several meat quality attributes but not carcass characteristics. Altering implant strategy does not appear to interact with the *GALR2-c.-199T>G* genotype to alter production or carcass characteristics.

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LIST OF ABBREVIATIONS

ADF.....acid detergent fiber

ADG.....average daily gain

ARC .....arcuate nucleus

BF.....back fat

BW .....body weight

CAB .....Certified Angus Beef

CART.....cocaine-and amphetamine-regulated-regulated transcript

CCK .....cholecystokinin

cDNA .....complementary deoxyribonucleic acid

CP.....crude protein

d.....day

DDGs .....dried distillers' grains with soluables

dL .....deccaliter

DMI.....dry matter intake

DMN .....dorsal medial nucleus

DNA.....deoxyribonucleic acid

G: F .....gain: feed

GALR1.....galanin receptor 1

GALR2.....galanin receptor 2

GALR3.....galanin receptor 3

GAPDH.....glyceraldehyde 3-phosphate dehydrogenase

GH.....growth hormone

GI .....gastrointestinal

GLP-1.....glucagon-like peptide

HCW .....hot carcass weight

IGF1 .....insulin-like growth factor 1

kg.....kilogram

LHA .....lateral hypothalamic area

LMA.....longissimus muscle area

mg .....milligram

min .....minute

NDF.....nitrogen detergent fiber

NTS .....solitary tract nucleus

OXM .....oxytimodulin

PCR.....polymerase chain reaction

POMC .....proopiomelancortin

PP .....pancreatic polypeptide

PVN.....paraventricular nucleus

PYY.....peptide tyrosine-tyrosine

QG.....quality grade

RIA.....radioimmunoassay

RNA .....ribonucleic acid

SEM .....standard error of the mean

SNP .....single nucleotide polymorphisms

VMN .....ventral medial nucleus

YG.....yield grade

µg .....microgram

µL.....microliter.

CHAPTER 1. LITERATURE REVIEW

Introduction

Understanding the biological mechanisms that drive metabolism and how these mechanisms can be altered to improve efficiencies has long been a focus for livestock production research. Identifying potential genetic mutations or single nucleotide polymorphisms (**SNP**) that alter the performance of a beef animal is one way to improve the end product quality and profitability of that animal. When precision technologies such as these are implemented in production this is referred to as a marker-assisted management strategy. This review focuses on the biological mechanisms controlled by the neuropeptide galanin and one of its receptors, galanin receptor 2. This review will present complementary production practices used to improve the production of crossbred finishing cattle in feedlot settings along with marker-assisted management strategies.

Appetite Regulation Pathway

Central Nervous System Locations Involved in Appetite Regulation

The center of appetite regulation and energy homeostasis exists in the hypothalamus. The hypothalamus is constructed from several interconnected nuclei: the arcuate nucleus (**ARC**), paraventricular nucleus (**PVN**), lateral hypothalamic area (**LHA**), ventromedial nucleus (**VMN**), and dorsomedial nucleus (**DMN**). Because of the positioning of the ARC, circulating hormones and nutrients are able to enter the ARC without passing through the blood-brain barriers. Through diffusion from the cerebrospinal fluid intro the extracellular fluid, hormones and nutrients can enter the ARC. This anatomical location is the reason the ARC serves as the main sensory point for peripheral metabolic signals. Satiety signals from the gastrointestinal tract are relayed through the solitary tract nucleus, specifically through the sensory vagus nerve. The

sensory vagus nerve serves as a main link between the gastrointestinal tract and the brain; signals regulated through the sensory vagus nerve are involved in control of food intake and eating duration (Yu and Kim, 2012). Within the ARC there are two differing neuronal populations: orexigenic neuropeptides and anorexigenic neuropeptides. Orexigenic neuropeptides include neuropeptide Y and agouti-related peptide while anorexigenic neuropeptides include proopiomelanocortin (**POMC**) and cocaine-and amphetamine-regulated transcript (**CART**). These two classes of neurons are first order neurons where peripheral metabolic signals such as leptin, insulin and ghrelin are transferred. The effects elicited on the GI tract by the CNS's role in appetite regulation is shown in Figure 1.1.

Neurons located in the PVN are charged with synthesizing and secreting neuropeptides that are responsible for a net catabolic effect. These neuropeptides include corticotrophin-releasing hormone, thyrotropin-releasing hormone, somatostatin, vasopressin, and oxytocin. Additionally, the PVN sends sympathetic outflow to peripheral metabolic organs such as the liver and adipose tissue which results in increased fatty acid oxidation and lipolysis (Foster, 2010). The VMN mainly receives neuronal projections from the ARC and extends axons to the ARC, DMN, and LHA and into the brainstem. Neurons in the VMN sense glucose and leptin levels. Insult caused to the VMN has resulted in obesity, hyperphagia, and hyperglycemia. These changes have led to the belief that VMN is a critical region for generating satiety and glucose homeostasis. The DMN contains high levels of neuropeptide Y and α-melanocyte-stimulating hormone terminals from the ARC, thus the degeneration of the DMN also results in hyperphagia and obesity.

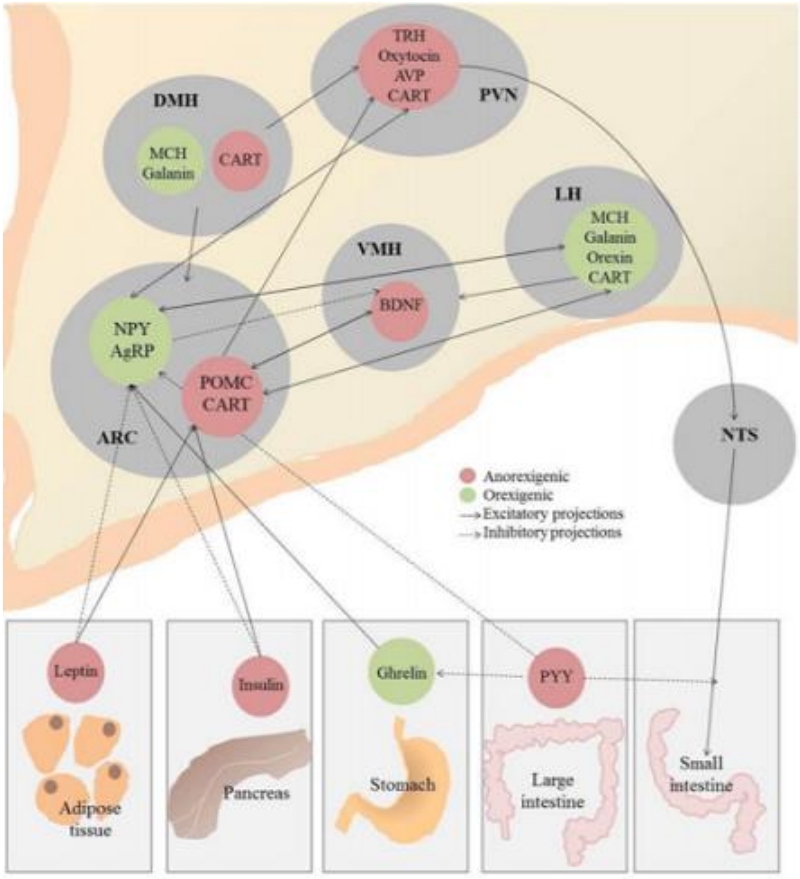

Figure 1.1. Illustrating the role of appetite regulation hormones on the GI tract. Adapted with permission from Farr *et al*. 2016. Schematic of nuclei in the hypothalamus which contribute to the control of eating as well as inputs from the periphery. The arcuate (ARC) nucleus contains NPY/AgRP neurons which are orexigenic and POMC/CART neurons which are anorexigenic. These neurons communicate with the other nuclei and neurons which release other orexigenic or anorexigenic peptides. Please note that the neurons may not release all anorexigenic or orexigenic peptides shown (e.g. a single neuron may not release TRH, Oxytocin, AVP and CART in the PVN), but are shown in groups by whether they are anorexigenic or orexigenic in each nucleus. AgRP, agouti-related peptide; ARC, arcuate nucleus; AVP, arginine vasopressin; BDNF, brain-derived neurotrophic factor; CART, cocaine- and amphetamine regulated transcript; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; NTS, nucleus of the solitary tract; POMC, proopiomelanocortin; PVN, paraventricular nucleus; PYY, peptide YY; TRH, thyroid-releasing hormone; VMH, ventromedial nucleus.

The destruction of LHA however leads to hypophagia and weight loss in comparison to the PVN, VMN, and DMN. Because of this contrast the LHA is thought to be the feeding center. Two neuronal populations exist within the LHA that produce orexigenic neuropeptides: melanin concentrating hormone and orexin, orexin-producing neurons are involved in glucose sensing.

The brainstem also exhibits a crucial role in energy metabolism and the appetite regulation pathway. Satiety signals from the gastrointestinal (**GI**) tract foremost relay to the solitary tract nucleus (**NTS**) following the sensory vagus nerve. The sensory vagus nerve is the major neuronal connection of the brain to the gut. An increase in the signaling of sensory vagal fibers has shown a direct increase in meal size and meal duration; this validates the importance of the vagal afferents transfer satiety signals to the brain (Schwartz, 2000). The NST is located near the circumventricular organ area postrema, creating a central location for receiving hormonal and neural signals. A strong link is present between the hypothalamus and brainstem as supported by the close and widespread linkage of neuronal projections from the PVN that are received by the NST and vice versa (Yu and Kim, 2012). NST neurons produce glucagon-like peptide 1 (**GLP-1**), NPY, and POMC, along with sensing peripheral metabolic signals.

Adiposity Signals

The reward system of the brain is involved in the control of intake of palatable foods. Intake of palatable foods is controlled through metabolic signals (Yu and Kim, 2012). Peripheral adiposity signals are heavily controlled by leptin and insulin. Leptin was first discovered in mice in 1994 through a positional gene cloning of *ob/ob* (Zhang, 1995). Leptin is produced in white adipocytes and released into systemic circulation, while plasma leptin concentrations increase in proportion to body fat mass as such leptin serves as biomarker of adiposity. Circulating levels of leptin enter the brain through the blood brain barrier and blood-cerebrospinal fluid barriers through receptor-controlled mechanisms. Insulin on the other hand is released from pancreatic β-cells at a rapid rate following a meal and then transported to the brain. Fasting insulin levels exhibit a positive relationship to body fat mass. Similar to leptin, insulin acts through binding to receptors present in ARC neurons that signals a downstream activation of POMC neurons and

inhibition of NPY and agouti-related protein neurons through the insulin receptor substrate, the phosphatidyl inositol-3-kinase AKt-FoxO1 signaling pathway (Taniguchi, Emanuelli, and Kahn, 2006).

Appetite Regulating Hormones

The GI tract is the largest endocrine organ, serving a versatile role within the body. Along with the function of digestion and absorption the gut also plays a critical role in energy homeostasis and regulation of food intake. The role of the GI tract in energy homeostasis and food intake is aided by the work of appetite regulating hormones. Appetite regulating hormones include: cholecystokinin (**CCK**), pancreatic polypeptide (**PP**), peptide tyrosine-tyrosine **(PYY**), GLP-1, oxytimodulin (**OXM**), ghrelin, and galanin. An illustration mapping the central nervous system and the role of hormones in appetite regulation is shown in Figure 1.2.

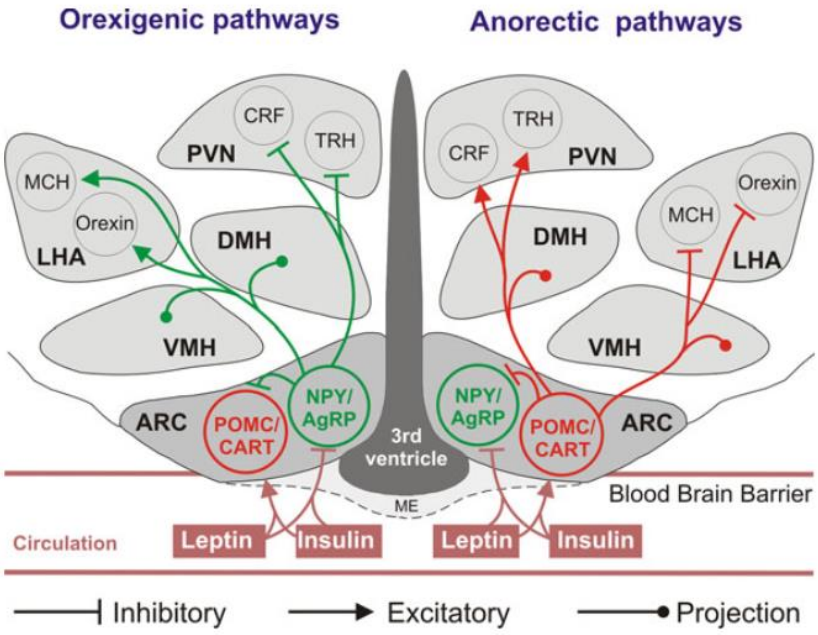

Figure 1.2. The neural pathways that regulate appetite through hormonal secretion. Adapted with permission from Prior *et al*. 2011. Schematic of the hypothalamus illustrating neural pathways that stimulate appetite and reduce energy expenditure (orexigenic pathways, green) or that suppress appetite and increase energy expenditure (anorectic pathways, red). Signals from the circulation including leptin, insulin, and ghrelin access the brain in areas such as the median eminence (ME) that lacks a blood–brain barrier. Hormones such as leptin and insulin stimulate neurons co-expressing pro-opiomelanocortin (POMC)/cocaine and amphetamine transcript (CART) (colored red) and inhibit neurons co-expressing neuropeptide Y (NPY)/agouti-related transcript (AgRP) (colored green) within the arcuate nucleus (ARC). These neurons are proposed to relay these signals to the paraventricular nucleus (PVN), lateral hypothalamic area (LHA), ventromedial hypothalamus (VMH), and dorsomedial hypothalamus (DMH). Neurons in the PVN contain the anorexigenic neuropeptides corticotrophin releasing factor (CRF) and thyroid-releasing hormone (TRH), while neurons in the LHA contain the orexigenic neuropeptides melanin-concentrating hormone (MCH) as well as the orexin peptides. These areas project to higher cortical areas to modulate behavior such as hunger, satiety, and food seeking and hindbrain centers to increase sympathetic outflow to skeletal muscle, brown adipose tissue, and the kidney

One of the first gut hormones to be identified for its role in appetite regulation was CCK. The hormone CCK is associated with an orexigenic action (Gibbs, 1997). With food intake PP secretion is activated from pancreatic islet PP cells through a vagal-mediated mechanism. Elevated circulating levels of PP are directly proportional to caloric intake of a meal for up to six hours after consumption in humans (Adrian *et al*., 1976). PYY is secreted from the L cells of the

ileum, colon, and rectum as a form of PYY_{1-36} (Adrian *et al*., 1985) which is then metabolized to PYY_{3-36} this allows for binding to the Y2 receptor on the presynaptic terminals of hypothalamic NPY and agouti related protein neurons with increased binding affinity (Michel *et al*., 1998). The infusion of PYY_{3-36} has induced a decrease in food consumption in humans when provided a test diet while obese subjects exhibit lower plasma levels of PYY_{3-36} levels when compared to lean test subjects (Batterham, 2003). It is therefore suggested that PYY secretion may contribute to depressed satiety signaling. The production of GLP-1 is from a large precursor peptide preproglucagon found within L cells of the ileum and colon. Secretion of GLP-1 occurs by systematic circulation and is rapidly inactivated by dipeptidyl peptidase-4 (Mentlein, 1993). GLP-1 exhibits an anorexigenic effect through the GLP-1 receptor which is distributed throughout the brain, GI tract, and pancreas (Yamato, 1997). An intravenous introduction of GLP-1 led to reduced food intake in lean and obese humans (Verdich, 2001). Similar to GLP-1 OXM is produced by preproglucagon in intestinal L cells and induces anorexigenic actions rodents and humans (Dakin, 2001). Ghrelin was first isolated from the rat stomach and has been associated with a growth hormone releasing effect. Ghrelin was identified as an orexigenic hormone that stimulates food intake and weight gain when administered both centrally and peripherally (Kojima, 1999).

The neuropeptide galanin is distributed throughout the central nervous system, located in the PVN, hypothalamus, and adrenal medulla. In addition, the presence of galanin has been found throughout the GI tract (Crawley, 1990). Synthetic administration of galanin to the CNS of rats has been shown to increase feed intake in rats (Crawley, 1990). The effect of galanin on appetite satiety have been conflicting depending on diet composition. When rats were fed a pure fat diet central administration of synthetic galanin did increase ad libitum food intake in rats

(Tempel, 1988) however when rats were fed a mixed nutrient diet food intake did not increase (Smith, 1994). Administration of synthetic galanin at the following locations have been found to increase food intake in rats: the PVN, hypothalamus, and the central nucleus of the amygdala (Corwin, 1993 and Kyrkouli 1990). The effect of galanin on feeding behavior are shown to be mediated through the binding affinity of the protein to its receptors. The binding of galanin to its receptors specifically within the PVN of the hypothalamus is shown to elicit a release in norepinephrine leading to increased food consumption (Kyrkouli, 1992).

The appetite regulation pathway is controlled by a complex array of proteins, genes, and hormones throughout various tissues. In livestock production of food animals, the goal is to produce the optimal amount of food animal product with the least amount of inputs possible to improve profitability. As feed often represents upwards of 70% of input costs in beef cattle operations (Nielsen *et al*., 2013) identifying potential ways to control appetite is a valuable avenue for research investigation as a means to improve beef cattle production.

Galanin

Galanin, a neuropeptide named for the N- and C-terminal residues, glycine and alanine was first discovered by isolation from the porcine intestine in the Tatemoto lab in 1983 (Tatemoto *et al.,* 1983). Galanin is distributed through the central and peripheral nervous system with a variety of physiological effects. Galanin is a 29 amino acid neuroendocrine peptide in mammalian vertebrates excluding humans that exhibit 30 amino acids (Crawley, 1995). The galanin gene has been characterized in various species, including the porcine adrenal medulla (Rökaus and Brownstien, 1986), bovine adrenal medulla (Rökaus and Carlquist, 1988), rat hypothalamus (Kaplan, 1988) and human lymphocytes (Evans, 1993). Homologies of the N-terminal amino acids exist across species for the first 15 N-terminal amino acids (Table 1.1).

Table 1.1. Species differences in amino acid sequences of galanin peptide.

Species	Sequence
Human	Gly-Trp-Thr-Leu-Asn-Scr-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-**val**-**gly**-**asn**-His-Arg-Ser-Phe-**ser**-Asp-Lys-asn-Gly-Leu-**thr**-**ser**
Rat	Gly--Trp-Thr-Leu-Asn-Scr-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-**ile**-**asp**-**asn**- His-Arg-Ser-Phe-**ser**-Asp-Lys-asn-Gly-Leu-**thr**-NH_2
Pig	Gly--Trp-Thr-Leu-Asn-Scr-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-**ile**-**asp**-**asn**- His-Arg-Ser-Phe-**his**-Asp-Lys-asn-Gly-Leu-**ala**-NH_2
Cow	Gly--Trp-Thr-Leu-Asn-Scr-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-**leu**-**asp**-**ser**- His-Arg-Ser-Phe-**gln**-Asp-Lys-asn-Gly-Leu-**ala**-NH_2
Chicken	Gly--Trp-Thr-Leu-Asn-Scr-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-**val**-**asp**-**asn**-His-Arg-Ser-Phe-**asp**-Asp-Lys-**his**-Gly-**phe**-**thr**- NH_2

Non-homologous amino acids bolded.

The effects of ^{125}I-galanin(1-29) have been identified through high-affinity binding sites located throughout the central nervous system. Galanin's location within the central nervous allows for control of the actions of classical neurotransmitters like acetylcholine, noradrenaline, serotonin and dopamine. In addition, galanin can inhibit the secretion of insulin, somatostatin, and glucagon in pancreas; stimulate the release of growth hormone (**GH**) and prolactin in the pituitary. Galanin regulates processes including feeding behavior and energy metabolism (Crawley, 1990). An increased presence of galanin within the central nervous system stimulates increasing levels of GH secretion in mice (Crawley, 1995). Galanin triggers cellular responses by acting on specific G-protein couples receptors which initiate the signal to intracellular effectors mainly via the inhibitory G_1/G_o^- subfamily of G-proteins (Langley-Pourmir and Epelbaum, 1992). High concentrations of high-affinity binding sites for galanin have been identified in the amygdala, prefrontal cortex, hypothalamus, thalamus, ventral hippocampus, and dorsal spinal cord (Kar and Quirion, 1994; Köhler *et al*., 1989; Melander *et al*., 1988).

Studies of pharmacological effects of galanin suggest the presence of not only the three currently identified receptor subtypes but also the existence of unidentified galanin receptors possessing a preference for galanin fragments rather than galanin itself (Lang *et al*., 2015).

Galanin Receptors

Galanin has three identified receptors: galanin receptor 1 (**GALR1**), galanin receptor 2 (**GALR2**), and galanin receptor 3 (**GALR3**) (Chen *et al*., 1992). While each receptor is a G-protein coupled receptor, they differ in their response to signaling pathways activated by the galanin peptide. Figure 1 illustrates the variations of downstream affects the three galanin receptors have on biological mechanism via varied pathways. Receptors GALR1 and GALR3 behave similarly by inhibiting adenylate cyclase and the opening of potassium channels (Branchek, 1998). However, GALR2 activation catalyzes an increase in intracellular calcium concentrations by increasing phospholipase C (Pang *et al*., 1998). All three receptors are located in the hypothalamic region of the central nervous system, the binding affinity of galanin to its respective receptors has been linked to the presence of or deletion of the N-terminal portion of Galanin(1-13) (Waters and Krause, 2000). GALR1 does not tolerate deletions of the N-terminal end of galanin while GALR2 and GALR3 show an increased binding affinity. Using synthetic ligands of the three galanin receptors it was discovered the binding of GALR2 leads to increased food consumption in mice (Saar, 2011).

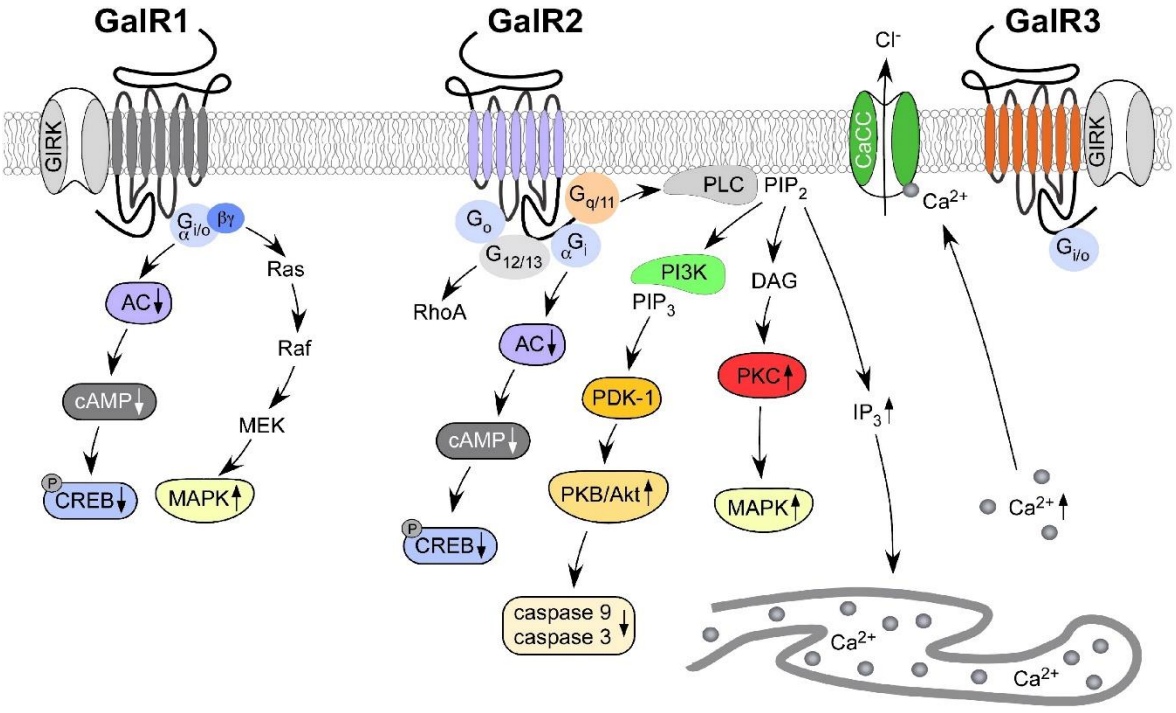

Figure 1.3. Signaling pathways of galanin receptor subtypes.
Adapted with permission from Lang 2007. Abbreviations: AC, adenylate cyclase; CaCC, Ca^{2+}-dependent chloride channel; cAMP, 3′,5′-cyclic adenosine monophosphate; (p)CREB, (phosphorylated) cAMP response element binding protein; 3′,5′-cAMP response element-binding protein; DAG, diacylglycerol; IP_3, inositol triphosphate; MEK, mitogen-induced extracellular kinase; PDK-1, phosphoinositide-dependent protein-kinase 1; PIP_2, phosphatidylinositol bisphosphate; PIP_3, phosphatidylinositol trisphosphate; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PLC, phospholipase C.

Galanin Receptor 2 Gene

Like galanin, *GALR2* is widely expressed throughout the central nervous system and peripheral tissues of the gastrointestinal tract. Galanin receptor 2 is expressed in the hypothalamus, spinal cord, skeletal muscle, duodenum, and jejunum (Smith et al. 1997; Waters and Krause, 2000; Lang et al. 2007).The binding of galanin to *GALR2* directly stimulates feeding behavior in mice with an increase in feed intake positively correlated with an increase in binding affinity of galanin to GALR2 (Kalra, *et al.*, 1999; Saar, 2011). *GALR2* activity has been associated with stimulation of contraction within small intestine, specifically the jejunum as well as initiating the release of GH (Kalra *et al.*, 1999).

A SNP has been identified in cattle at *GALR2c.-199T>G* (Duncombe, 2016). The expression of *GALR2* is thought to be affected by a change in the level of transcription with the presence of a T allele rather than a G allele at the promoter region. This mutation creates a Sp1 transcription factor-binding site (Dynan and Tjian, 1983).

This SNP is associated with carcass traits in beef cattle, where increasing marbling and back fat was exhibited by a dominate effect of the T allele. Conversely, increasing longissimus muscle area (**LMA**) and hot carcass weight (**HCW**) was exhibited by cattle possessing the GG genotype (Duncombe, 2016). The mutation of this genotype increases the amount of *GALR2* present and is thus thought increase the effects of galanin. This mutation is thought to decrease feed intake (Duncombe, 2016). Further research is needed to determine what effects the *GALR2c.-199T>G* has on feeding behavior and energy metabolism.

Cattle Management and Marketing

Assessing Feed Efficiencies in Beef Cattle through Feeding Behavior

A major determinate of profitability in beef cattle is feed efficiency, with feed costs represent up to three-quarters of direct costs (Nielsen *et al*., 2013). To better measure intake efficiencies and quantitatively describe feeding behaviors multiple automated electronic feeder systems have been created to capture data. These systems are controlled by radio frequency identification tags. These electronic feeders allow for the special dietary treatments to be specific animals intermixed within pens. This also allows for individual feed intake to be tracked along with feeding behaviors. Feeding behavior and overall DMI can be described in three quantitative summaries of intake events, time spent eating, and eating rate; each category is further broken down into more descriptive subsets (Montanholi *et al*., 2010; Swanson *et al*., 2014). Events can be defined as number of visits per day and number of meals consumed per day. A visit is further

defined as each time the electronic feeder detects an animal at the bunk. A meal is defined as an eating period that can include breaks separated by intervals no greater than 7 minutes (Forbes 1995; Montanholi *et al*., 2010).

Grid Pricing: Marketing Feedlot Cattle

Grid pricing is a determined using a price figured based on industry and/or slaughter abattoir averages that creates a base price. Premiums or discounts are then applied to the carcasses dependent on if the carcass meets standard specifications or excels beyond base parameters or fails to meet the standard (Schroeder *et al*., 1997). Grid pricing allows for each individual animal to receive a price as opposed to "live weight" or "dressed weight" pricing where a price is determined by the lot average. Premiums are placed on carcasses meeting quality grades above the pre-determined base while cattle not meeting the quality grade average for that abattoir receive a discount. Premiums are also often applied to carcasses with exceptional marbling and/or those meeting specifications for marketing programs, such as Certified Angus Beef (**CAB**). Discounts are applied to carcasses exhibiting hide damage, bruising, light or heavy weight ranges, excess fat, and other undesirable characteristics that may require additional labor to market the end product (Feuz, Ward, and Schroeder, 2002).

Grid pricing is comprised of two main components: quality grade (**QG**) and yield grade (**YG**). A quality grade is a composite of evaluation factors that influence the palatability of meat including: tenderness, juiciness, and flavor. The following factors determine quality grades of beef: maturity, firmness, texture, color of lean, and the amount and distribution of marbling within lean muscle. Yield grades are an estimate on a scale of 1-5 to predict the amount of boneless, trimmed retail cuts from the high-value portions of the carcass (USDA, 1989).

The potential for superior profits when marketing on the gird as opposed to the "live weight" marketing can exist. However, it is pertinent that the cattle feeders are well versed in how grid pricing works and are aware of the quality of cattle they possess prior to marketing. All purchasing decisions should be considered, including diet composition throughout the finishing phase, length of the finishing period and implant strategies (DiCostanzo and Dahlen, 2000).

Implant Strategies

Of the cattle in feedlots in the United States 90.4% of them received some form of hormone implant during the finishing phase (USDA-APHIS, 2013). Anabolic implants are used as a management strategy to improve the growth rate and feeding efficiency of cattle during the finishing phase. There are different modes of actions for anabolic implants commonly used in beef production: estrogenic, androgenic, or a combination of estrogenic and androgenic.

In cattle muscle accretion is controlled by the response to GH. The receptors for GH are regulated by steroids. Estrogenic and estrogen-like compounds increase protein accretion by elevating the levels of GH and insulin. Androgenic implants increase protein deposition by catalyzing muscle protein synthesis (Trenkle, 1983). In comparison synthetic androgen trenbolone acetate increases protein by increasing the rate of protein synthesis in relation to the rate of protein degradation (Buttery, 1978). Combination implants containing both trenbolone acetate and estradiol cause muscle to produce greater concentrations of insulin like growth factor 1 (**IGF1**) (Johnson *et al*., 1998). It must be noted not all muscles respond to the effects of implants. Function and location of a muscle influences the effect implants have on protein accretion. The greatest muscle accretion occurs in extensor muscles attached to long bones; the direct effect the hormones elicit on the muscle increases the rate of cell division originating at the bone growth plate. Conversely, it has been noted that the psoas major and semitendinosus

muscles do not exhibit a response to implants (Elasser *et al*., 1998). Additionally, muscle fiber type contributes to rate of protein accretion with the influence of anabolic implants. Type 1, red muscle fibers have a greater accretion rate in comparison to type 2, white fiber types when implant strategies are implemented (Pell and Bates, 1978).

Average daily gain (**ADG**), feed conversion ratio (**G: F**), and dry matter intake (**DMI**) have all been reported to improve when estradiol implants were used in finishing steers (Enright, 1990). When steers received two separate implants of trenbolone acetate throughout finishing there was a 7.4% improvement in feed efficiency in comparison to control steers (Trenkle, 1987). Conflicting reports about implants containing trenbolone acetate in combination with estrogen have been shown for effects on efficiency when steers were allowed ad libitum access to feed. Where Comerford *et al.* (2001) reported an increase while Apple *et al.* (1991) reported an increased rate of gain with no effect on feed efficiency when implants were administered to Holstein steers. Steers receiving anabolic implants and confined to a feedlot with restricted access to feed exhibited improved F: G in comparison to control steers receiving no implant (Foutz *et al*., 1997; Herschler *et al*., 1995; Hermesmeyer *et al*., 2000).

Marker-assisted Management Strategies

Marker-assisted management can be defined a variety of ways. Specifically, in a feedlot setting marker-assisted management is a strategy often combining genetic information of a beef animal using a SNP of interest with live animal evaluation. The goal of marker-assisted management is to generate decision making based on the genetic background of a beef animal specifically targeting a SNP of interest (Kolath, 2009). An array of decisions can be made utilizing genetic information and current body conformation of the beef animal upon arrival at

the feedlot. Days spent on feed, implant strategy, and diet composition are all management decisions that can be influenced by genetic background information.

Genetic markers do not directly influence profit but rather they influence growth and carcass traits that determine profit (Thompson *et al*., 2014). Due to the large scale of operation size in feedlots, managing cattle individually is cost prohibitive and cattle are therefore managed in pens, or lots, often determined based on arrival date and size (Kolath, 2009). Upon arrival at a feedlot, cost of purchase and live weight are the only known variables where as ADG, YG, and QG are functions of management decisions, growth and carcass composition. Many feedlots operate on forward contracts to guarantee a future price grid however, carcass premiums and discounts are still not known until harvest. Information from genetics testing allows feedlots the ability to differentially manage and/or select cattle based on genetic potential (Stigler, 1961).

Utilizing genotyping technology for 47 different SNPs associated with growth, carcass traits, and tenderness, two different implant strategies were applied to various contemporary groups of steers and heifers at the United States Department of Agriculture Meat Animal Research Center (King *et al*., 2012). Of the SNPs associated with increased LMA, the cattle possessing the wild type SNPs exhibited not only, increased LMA, but also increased final body weight (**BW**), improved ADG, and increased HCW upon receiving the aggressive implant strategy (King *et al*., 2012). When utilizing SNP markers associated with tenderness it is reported that cattle exhibiting the mutated SNP present increasing shear force values correlated with a positive linear correlation to number of implants given (Schneider *et al.*, 2007; King *et al*., 2012).

GALR2 Effects on Production Efficiencies in Beef Cattle

The *GALR2* SNP mutation of replacing a T allele with a G allele at the 199th position of the coding region of the *GALR2* gene is thought to decrease feed intake (Duncombe, 2016). However, research has not adequately been conducted to properly quantify these findings. Because of the large economic effect of diet on potential profitability of beef cattle, further research is needed to determine what effects the *GALR2c.-199T>G* has on feeding behavior and energy metabolism.

Meat Quality Attributes

Eating satisfaction of beef is known to be influenced by three traits: tenderness, juiciness, and flavor, as well as the interaction of these traits (O'Quinn *et al*., 2018). It is thought that a steak may exceed a consumer's eating experience expectations by greatly exceling at a single trait or conversely fail due to an undesirable experience attributed to one trait (O'Quinn *et al*., 2018). Tenderness, juiciness, and flavor can often be positively or negatively influenced by live cattle management and/or the slaughter and post-mortem process.

Tenderness

Tenderness has been identified as the largest contributor to eating satisfaction in a variety of studies (Savell *et al*., 1987; Miller *et al*., 1995; Savell *et al*., 1999; Egan *et al*., 2001). However, in more recent studies tenderness has been identified as the second most important factor influencing eating satisfaction (Chail *et al*., 2016; Wilfong *et al*., 2016; Chail *et al*., 2017; McKillip *et al*., 2017). Tenderness can be influenced by a variety of factors including biological, genetic, and environmental.

Tenderness can vary among different muscles due to the variation in sarcomere length, muscle fiber diameter, the concentration of stromal proteins, size of elastin fibrils, and solubility

of collagen (Brady, 1937; Stolowski *et al*., 2006). Live cattle handing and transportation have been identified as factors that influence tenderness at well. One of the most widely known influences of tenderness is associated with the amount and distribution of marbling (Berry, 1993). Marbling distribution can be influenced by diet, genetic potential and environment.

Genetics can have an influence on the potential of an animal to yield more or less tender meat. A widely known genetic mutation in *myostatin* known to cause 'double muscling' causes cattle who exhibit this mutation to yield greater muscle mass as well as more tender meat due to a decrease in collagen content (Grobet et al., 1997; Arthur 1995).

It has been well established the importance of tenderness in consumer acceptability of beef. However, predicting tenderness through sensory panels or objective measures can be difficult (Peachey, 2002). Differences in correlation of tenderness when evaluated by both sensory panels and objective measures have been widely variable in the past (Brady and Hunecke, 1985; Crouse *et al*., 1985; Dransfield *et al*., 1984; Otremba *et al*., 1999). Much of this variation is thought to be attributed to differences in sensory panels (trained vs. untrained), cooking method, final temperature and degree of doneness to which steaks were cooked, and the specific muscle the steaks were cut from. Sensory panels can often be a more cumbersome method of identifying tenderness because of the need to source participating panelists for a span of time. In addition, if a trained panel is being used time is needed to train the panel. One of the most common forms of objective measure of tenderness is the Warner-Bratzler shear force method. By utilizing an objective measure of tenderness this allows researchers a way to quantify if an independent variable such as diet, breed, implant strategy, or genetics has an effect on the tenderness of the meat.

Tenderness can also be affected by an array of factors throughout the slaughter and post-mortem process including stunning method, chilling rate, rate of pH decline post-mortem (Belk, 2002; King et al., 2003; Watanabe *et al*., 1995). Each of these factors can be controlled to help ensure optimal quality of the meat harvested from each carcass is achieved.

When evaluating tenderness, degree of doneness is critical for accurate comparison. The accepted degree of doneness is considered 71 °C (Warner-Bratzler Shear Force protocol, 1994). Degree of doneness is one of the biggest points of variation when considering consumer consumption due to a wide array of preference and this can often be why various programs to establish 'certified tender' meat have often struggled to garner much retail traction. Overall, it is evident that a variety of influences should be considered when evaluating tenderness. Considerations should be made to determine if subjective, objective and/or both types of traits should be utilized to measure tenderness.

Protein degradation from the calpain/calpastatin activity post-slaughter may also contribute to tenderness. The calpain/calpastatin(calcium-dependent) system, proteosomal and lysosomal systems has been identified for its' role in proteolytic degradation and meat tenderization (Kemp *et al*., 2010; Koohmaraie *et al*., 1996). The presence of calpastatin in meat influence the calpain by acting as inhibitor. Calpastatin is a marker used determine the tenderness of meat. Whipple *et al.* (1990) found the activity of the calpastatin in meat at 24 hours was highly related to shear force value after 14^{th} day after post mortem; this study showed that an early event after the animal being slaughter could be predictive of ultimate shear force because of the low activity of calpastatin (Whipple *et al*., 1990).

Flavor

Flavor is another attribute responsible for consumer acceptance and enjoyment of meat products. Like tenderness, flavor can be influenced by a variety of live and post-mortem factors of beef. Diet is one of the first factors associated with flavor; greater concentrate diets or grain-fed cattle are associated with greater levels of marbling as opposed to cattle who are grass finished (Hwang, 2017). Volatiles within lipids are a major contributor of flavors within beef animals (Calkins, 2007).

Flavor is often the most difficult meat quality attribute to quantify as it influenced through combination of supporting sensations that may alter the consumer's perception such as: texture, juiciness, mouthfeel, and aroma. Some pre-harvest factors known to be associated with flavor include: animal handling, diet, genetics, age and sex of the beef animal (Jeremiah, 1988; Hwang, 2017; Dikeman *et al*., 2005; Cross, 1984).

Water Holding Capacity

As meat is the post-mortem product of muscle it is comprised of 73-75% water (Predersen *et al*., 2013). A majority of the water held in muscle is held between the thick and thin filaments post-mortem. An accumulation builds between fiber bundles and in between individual fibers and a minute portion is held through electrostatic attraction between proteins (Bond *et al*., 2004). The water holding capacity of meat can be indicative not only of consumer satisfaction but also shelf-life stability of the product. With increased water holding capacity consumers often identify cuts of meat as being more palatable with increased flavor and juiciness. As with other meat quality factors water holding capacity can be affected by numerous pre- and post-mortem factors. Genetics have been known to influence water holding capacity (Cheng, 2008). Additionally, post-slaughter chilling time and rate and aging of the product have

been known to affect a water holding capacity (Blakely *et al*., 2019). Because of the relationship of water holding capacity and consumer satisfaction with cooked products it is pertinent to investigate the influence the *GALR2c.-199T>G* genotype may have on water holding capacity.

Color

The color of meat is the biggest driver in purchasing decisions beyond economics (Danner, 1959). Consumers view the color of meat as the best indicator of freshness and quality (Priko and Ayres, 1957). While discoloration of packaged meat is known as a 'loss of bloom' within the meat industry, many consumers view it as an indicator of bacterial growth even if this may not always be the case (Seideman *et al*., 1983). The color of meat as perceived by a consumer is determined by the concentration and chemical state of myoglobin, morphology of the muscle structure and the ability of the meat to absorb or reflect light (Walters, 1975). Hemoglobin is the primary pigment in blood; myoglobin is relatively close to one of the four subunits of hemoglobin and a majority of myoglobin is located within muscle cells.

Once an animal is stunned and exsanguinated the associated hemoglobin within the blood leaves the carcass as well, myoglobin then becomes the primary principle for meat color. Myoglobin is a globular protein comprised of a heme group enveloped by a globin moiety. A centrally located atom of iron (Fe) within four pyrollic rings creates a heme. The valance state of the iron (Fe) atom and the ligand bond to the free binding site of the heme are the main factors contributing to the color of hemoglobin and myoglobin, and consequently the color of meat (Clydesdale and Francis, 1971).

The intensity of color has been found to be associated with species, stress, sex and age of the animal. Additionally, postmortem pH decline and the ultimate pH of meat are significantly associated ($P < 0.05$) with color (Seideman *et al*. 1983). An effect of genotype × age interaction

has been found to significantly influence color in pigs ($P < 0.0001$) (Franco *et al*., 2016). Due to the influence on consumer perception, and consequently purchasing decisions, understanding the influence the GALR2 genotype on meat color is pertinent.

GALR2 Effects on Meat Quality

The *GALR2c.-199T>G* genotype has been found to elicit a significant effect on marbling and back fat accumulation for cattle possessing the TT genotype. The *GALR2c.-199T>G* genotype was also found to influence overall protein accretion with the GG genotype exhibiting increased LMA and heavier HCW than the TT cohorts (Duncombe, 2016). Therefore, investigating the effects of the *GALR2c.-199T>G* genotype on meat quality attributes is an important goal for further understanding the potential of the *GALR2* SNP to serve as a marker-assisted management strategy.

Statement of the Problem and Experimental Objectives

Feeding cattle to the most uniform pen finish of quality cattle is the goal when marketing cattle on the grid. When feedlots and/or individual producers choose to market on the grid it is important to consider all marketing factors that can be controlled from the initial purchase of cattle, their genetic background, diet, management style(s), implant strategy, feeding period, diet, (DiCostanzo and Dahlen, 2000) and when and where to market the cattle. The goal of marker-assisted management is to help alleviate some of the potential unknowns the genetic background of cattle.

Galanin and the biological mechanisms associated with the binding of galanin to its receptors GALR1, GALR2, and GALR3 have been identified within the CNS and GI tract. Specifically, GALR2 has been associated with feeding behaviors in a variety of species through altering the signals sent and received by the gut-brain axis (Smith et al. 1997; Waters and

Krause, 2000; Lang et al. 2007).The binding of galanin to *GALR2* directly stimulates feeding behavior in mice with an increase in feed intake positively correlated with an increase in binding affinity of galanin to GALR2 (Kalra, *et al.*, 1999; Saar, 2011). *GALR2* activity has been associated with initiating the release of GH and insulin (Kalra *et al.*, 1999). These changes alter feeding behavior and energy metabolism.

A SNP has been identified at *GALR2c.-199T>G*; this SNP is associated with carcass traits in beef cattle, where increasing marbling and LMA are exhibited by a dominate effect of the T allele (Duncombe, 2016). In the case of *GALR2* this is the mutation of a G in place of a T allele. The expression of *GALR2* is thought to be affected by a change in the level of transcription with the insertion of a G allele rather than a T allele at the promoter region. This mutation creates a Sp1 transcription factor-binding site (Dynan and Tjian, 1983). This is believed to change the amount of GALR2 present within cattle possessing the G allele. This mutation is thought to decrease feed intake (Duncombe, 2016). Further research is needed to determine what effects the *GALR2*c.-199T>G SNP has on feeding behavior and energy metabolism.

Therefore, we sought to examine the potential of the *GALR2* genotype as a marker-assisted management strategy when implementing two differing implant strategies (one implant strategy vs. two implant strategy) on crossbred Angus finishing steers. This objective was explored with the hypothesis that by utilizing two different implant strategies we would be able to help manage two divergent growth patterns and production efficiencies of the *GALR2* genotypes in crossbred Angus finishing steers; with the GG genotype cattle exhibiting a greater propensity for overall protein accretion and benefiting from the conservative implant strategy and the TT genotype cattle exhibiting a greater affinity for increased marbling and back fat benefiting from an aggressive implant strategy and the heterozygote acting as an intermediate.

The objectives of this study were to 1) Examine the effects of the *GALR2* genotype × implant strategy on feeding efficiencies and behaviors of crossbred Angus finishing steers through utilization of Insentec ® Feeders (Hokofarm Group B.V., Markness, The Netherlands) to quantify intake and feeding behaviors, 2) Evaluate the effects of *GALR2* genotype × implant strategy on blood metabolites for serum glucose and serum urea nitrogen levels as well as hormone analysis of GH and IGF1 throughout the finishing period, and 3) Determine the effects of the interaction of the *GALR2* genotype × implant strategy on carcass characteristics and meat quality attributes of crossbred Angus finishing steers.

The following chapters of this thesis will be divided by these following objectives: 1) Examine the effects of the *GALR2c.-199T>G* genotype × implant strategy on feeding efficiencies and behaviors of crossbred Angus finishing steers through utilization of Insentec ® Feeders (Hokofarm Group B.V., Markness, The Netherlands) to quantify intake and feeding behaviors, 2) Evaluate the effects of *GALR2c.-199T>G* genotype × implant strategy on blood metabolites for serum glucose and serum urea nitrogen levels as well as hormone analysis of GH and insulin-like growth factor 1 (**IGF1**) throughout the finishing period, in chapter 2, 3) Determine the effects of the interaction of the *GALR2c.-199T>G* genotype × implant strategy on carcass characteristics and meat quality attributes of crossbred Angus finishing steers in chapter 3, and lastly, 4) Concluding with a general discussion and future implications in chapter 4.

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CHAPTER 2. EXAMINING MARKER-ASSISTED MANAGEMENT AS A STRATEGY IN PRECISION AGRICULTURE TO MAXIMIZE PRODUCTION EFFICIENCIES IN FINISHING STEERS

Abstract

The objective of this study was to determine how the interaction of different implant strategies with the *galanin receptor 2* (*GALR2*) genotype influences intake, efficiency, and feeding behavior of finishing steers. Angus crossbred steers (n = 93) were selected based on their *GALR2-c.-199T>G* genotype with n = 36 TT, 38 TG, and 19 GG. Calves were blocked by initial body weight and fed a standard finishing diet for 166 d or 202 d. Body weight and jugular blood samples were collected every 28 d. Steers were randomly assigned to one of two growth promoting implant strategies; 1) a single Revalor-S (Merk, New Jersey) on d 77 (1×), or 2) a Revalor-S on d 0 and another on d 77 (2×). Intake and feeding behavior were recorded using RFID tags and Insentec feeders (Hokofarm Group, B.V., Marknesse, The Netherlands). Total DMI and feeding behavior including events per day, time spent eating and eating rate were captured and analyzed. Serum samples were analyzed for blood urea nitrogen (BUN) and glucose concentration. Data were analyzed using the mixed procedure in SAS with a 2 × 3 factorial arrangement of treatments. No genotype by implant strategy interactions were observed for intake and growth measurements. Genotype had a significant effect on DMI ($P = 0.05$), number of meals/day ($P \leq 0.0001$), and intake/minute ($P = 0.002$). While there was a greater DMI with steers of the GG genotype there is no corresponding improvement in feed efficiency. The main effect of implant had a significant effect on G: F ($P = 0.0008$) and intake/visit ($P \leq 0.0012$). Day × Implant interaction had a significant effect on urea-N ($P = 0.0035$), and day × genotype had a significant effect on serum glucose concentration ($P = 0.0049$). The effect of

GALR2c.-199T>G genotype influenced GH levels ($P = 0.006$). Insulin-like growth factor 1 levels were different based *GALR2c.-199T>G* genotype × implant strategy ($P = 0.04$). Based on these data we conclude that there is no interaction effect of *GALR2c.-199T>G* genotype × implant strategy on intake, metabolism or overall gain: feed. However, there was an effect of genotype on DMI which may influence performance and could potentially serve as a candidate for developing a marker-assisted management strategy, but further research is needed.

Introduction

Feedlots have the opportunity to capitalize on increased profit margins through optimizing the quality and yield grades of cattle they manage. Regardless of whether cattle are marketed on a live weight, carcass weight, or grid-basis, increased muscling and marbling are key to increasing profitability. In addition, value-based markets provide a premium price for well marbled carcasses. Therefore, the use of marker assisted management has become a growing practice in many successful feedlot production systems. There is an opportunity cost of underfed animals when uniform pen weights are not achieved (Woronuk et al., 2012). The North American beef industry provides premiums for well-marbled carcasses without excessive fat cover (DiCostanzo and Dahlen, 2000). It is because of these market incentives, this study evaluates the effects of a target for genetic marker assisted management on finishing cattle production.

Galanin receptor 2 (GALR2) is a neuropeptide receptor that is associated with feeding behavior, insulin release, and growth hormone secretion in mice (Smith et al., 1997; Waters and Krause, 2000). A previous study in cattle examined the effect of a mutation in *GALR2* (*GALR2c.-199T>G*) on carcass characteristics (Duncombe, 2016). The GG genotype was associated with greater longissimus area, whereas the TT genotype was associated increased

marbling score. This divergence between muscle and fat growth between the genotypes presents a unique opportunity for marker assisted management. Utilizing different management practices based on genotype, such as different implant strategies, could improve carcass uniformity and profitability, for beef producers. Additionally, gaining insight into the mechanisms of how *GALR2c.-199T>G* genotype affects muscle growth and fat deposition could lead to a development of additional management strategies that could be implemented to improve beef production.

Materials and Methods

All animal procedures performed in this study were approved by the North Dakota State University Institute for Animal Care and Use Committee (Protocol #A18062). Procedures for this study were conducted at the North Dakota State University Central Grasslands Research Extension Center and North Dakota State University Beef Cattle Research Complex.

Experimental Design and Treatments

Cattle for this experiment were sourced from the North Dakota State University Central Grasslands Research Extension Center herd near Streeter, ND. Ninety-five steers were initially selected for the study but two steers were removed from the study for an inability to train to the feeding system so 93 steers were used for this experiment. The distribution of the *GALR2c.-199T>G* genotype for the study was: 36 TT, 38 TG and 19 GG based on *GALR2c.-199T>G* genotype. Cattle were assigned to one of four pens based on initial body weight with an average of 255 kg (± 80 kg) and were allowed a two-week training period to adapt to the Insentec ® Feeders (Hokofarm Group B.V., Markness, The Netherlands). Upon initiation of the study cattle were randomly assigned within *GALR2c.-199T>G* genotype to one of two growth promoting implant strategies; Revalor-S (120 mg trenbolone acetate and 24 mg estradiol; Merk Animal

Health, Summit, NJ) on d 77 of finishing (1×) or Revalor-S on d 0 and d 77 of finishing (2×). Forty-seven of the steers were assigned randomly within genotype to the aggressive (2×) strategy and 46 to the conservative (1×).

DNA Extraction and Genotyping

Blood samples were collected via jugular venipuncture, using 10 mL EDTA vacutainer tubes (Fisher Scientific, Walmath, MA, U.S.), from all bull calves (n = 191) born at the North Dakota State University Central Grasslands Research and Extension Center cattle herd in 2018. The blood samples were centrifuged for 15 minutes at 3,000 × g. The buffy coat was collected for each sample and stored at -80 ºC, until DNA extraction was performed. DNA extraction was performed using the Qiagen DNAeasy® Blood & Tissue Kit (Qiagen Germantown, MD).

Genotyping of all samples was performed using the KASP genotyping system (LCG Genomics, Beverly, MA, Aliquot ID 116365958) with target sequence CGCCTCGGCCGCCA[G/T]CGGGATCATCCCCCC. PCR conditions were 94 º C for 15 minutes and 10 cycles at 94 º C for 20 seconds, 68-62 º C for 1 minute dropping 0.6 º C per cycle, 94 º C for 20 seconds, 62 º C for one minute for 26 cycles, then 94 º C for 20 seconds, 57 º C for 1 minute for 3 cycles. Genotyping were performed on an initial population of 191 samples using the ABI 7500Fast real time PCR machine (Applied Biosystems, Waltham, MA, US) to identify the *GALR2c.-199T>G* genotype of each calf. Out of the 191 samples genotyped only 19 GG calves were identified, thus there was a lower number of steers for this genotype on study. Steers were then selected for the study at weaning based on *GALR2c.-199T>G* genotype, weaning weight, and date of birth to create the most uniform group.

Feed Intake and Performance

The sample population was shipped from the Central Grasslands Research Extension Center to the Beef Cattle Research Complex in Fargo, ND. After successfully training to the Insentec system (Insentec Roughage Intake Control System, Hokofarm Group B.V., Markness, The Netherlands), they were fed a step up diet over 30 d to reach a 90% concentrate, 15.5% crude protein finishing diet. Steers began on the standard finishing diet (Table 2.1) on d 0. Samples of the total mixed ration were obtained and analyzed for chemical composition including N using official method 988.05 (AOAC 1990) with a Kjeltec Auto 1030 Analyzer (Foss Tecator AB, Höganäs, Sweden), NDF and acid detergent fiber (ADF) (Van Soest et al. 1991). Dry matter, ash, and ether extract concentrations were analyzed using standard procedures (procedure numbers: 934.01, 942.05, and 920.39 respectively; AOAC 1990).

By using Insentec Feeders (Hokofarm Group B.V.) feed intake was determined. Upon conclusion of the experiment, feed intake data were downloaded and used to calculate the average daily feed intake (DMI; kg/d) over the finishing period. Feed intake data were filtered first to exclude outlier records or days where mechanical problems may have occurred. The gain to feed ratio was calculated as a ratio of ADG:DMI throughout the finishing period. Feeding behavior and overall DMI can be described in three categories: intake events, time spent eating, and eating rate (Montanholi *et al*., 2010; Swanson *et al*., 2014). Events are defined as number of visits per day and number of meals consumed per day. A visit is defined as each time the electronic feeder detects an animal at the bunk. A meal is defined as an eating period that can include breaks separated by intervals no greater than 7 minutes (Forbes 1995; Montanholi *et al*., 2010). Two-day body weights were recorded at the beginning and the end of finishing (d 166 and d 201 for group 1 and group 2 respectively). Additionally, BW were collected every 28 d.

Table 2.1. Ingredient and nutrient composition (DM basis) of TMR finishing diet.

Ingredient	%
Corn	60
DDGS[1]	20
Corn Silage	10
Hay	5
Premix[2]	5
Nutrient Composition	
CP[3]	15.58
NDF[4]	30.56
ADF[5]	10.45

[1]Dried distiller's grains with soluables.
[2]Premix contained ground corn, limestone, urea, salt, monensin (176.4 g/kg Elanco, Greenfield, IN), tylosin (88.2g/kg Elanco, Greenfield, IN).
[3]Crude protein.
[4]Nitrogen detergent fiber.
[5]Acid detergent fiber.

Muscle Biopsies

A muscle biopsy (~0.05 cm long, 1.0 cm diameter) was collected from the longissimus dorsi muscle between the 12th and 13th ribs of each steer 14 days prior to slaughter. The steers were individually restrained in a squeeze chute. Flunixin meglumine (Merk Animal Health) was administered at 1.1 mg/kg body weight via intravenous injection to serve as an analgesia and a prophylactic dose of penicillin G procaine (Pfizer, Chesterfield, MO) via intramuscular injection at 2.5 mL/100 kg body weight was administered. The surgical site was clipped of hair, scrubbed with 7.5% povidone-iodine scrub, and was sterilized with 70% isopropanol. A 10mL injection of lidocaine hydrochloride (Pfizer) was then administered via subcutaneous injection in a circular pattern around the surgical site to provide a local anesthetic. The surgical site was then further sterilized with 10% povidone-iodine solution and 70% isopropanol. An approximate 4 cm horizontal incision was made through the skin and subcutaneous tissue on the left side of the animal between the 12th and 13th rib. A 10 mm Bergstrom biopsy instrument (Agnthos, Lidingö, Sweden) was then introduced into the incision and used to collect the biopsy (~ 30 mg). The

tissue sample was then preserved in RNAlater (Qiagen) and stored at -80ºC until RNA extraction could be performed. The incision was closed with surgical staples and allowed to heal for 14 d, staples were then removed.

RNA Extraction and cDNA Synthesis

Muscle samples collected from longissimus via the biopsy procedure previously had the RNA extracted from 30 mg samples of longissimus samples using a Trizol extraction method. One mL of TRIzol (Qiagen) and a 5 mg stainless steel bead was added to the muscle sample and homogenized for five minutes using a Tissuelyser (Qiagen). Chloroform was then added at 200µL/reaction and vortexed thoroughly and samples were incubated on ice for 15 minutes. Samples were then centrifuged at 12,000 × g for 10 minutes at 4 °C. The aqueous phase was transferred to a spin column (Qiagen), 500 µL of cold 70% isopropanol was then added, and samples were once again incubated on ice for 10 minutes. Samples were centrifuged at 12,000 × g for 10 minutes at 4 °C. The supernatant was discarded and 1mL of 70% ethanol was added as a wash step and samples were centrifuged at 7500 × g for 10 minutes at 4 °C. The supernatant was discarded, and excess ethanol was allowed to evaporate for approximately 10 minutes. Finally, the samples were resuspended in RNAse-free water (Qiagen).

Samples were quantified for RNA using the Quibit 3 (Fisher Scientific) to calculate dilutions. A total of 2 µg of RNA was used for a 20 µL reaction to synthesize cDNA. cDNA synthesis was performed using the Applied Biosystems High capacity reverse transcriptase cDNA synthesis kit (Applied Biosystems) following manufacturer instructions.

Gene Expression

Quantitative polymerase chain reactions were performed using the cDNA synthesized from muscle samples. The iTaq™ Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA)

kit was utilized. Five different potential reference genes were compared across all treatments to determine the most stably expressed reference gene. This was determined by selecting the reference gene with the lowest M-value as calculated by qbase+ software (Biogazelle, Zwijnaarde, Belgium). Glyceraldehyde-3-Phosphate Dehydrogenase (**GAPDH**) was used as the reference gene. Primer sequences are displayed in Table 2.2. All samples were analyzed in triplicate with a non-template control, reverse transcriptase control and positive control on each plate. A melt curve was run with each plate and a six-point standard curve was calculated to verify amplification efficiency.

Table 2.2. Primer set used for real time quantitative reverse-transcription PCR.

Gene	Product size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')	GenBank Accession No.
GAPDH	20	AGG TCG GAG TGA ACG GAT TC	ATG GCG ACG ATG TCC ACT TT	NM_001034034
GALR2	19	AGG GCC AGG GAG CAG GAA C	CTC GTG TCC TCG GTG TCC	NM_001105010.1

Serum Glucose and Urea Nitrogen Concentrations

Serum samples were collected every 28 d to evaluate serum glucose and urea nitrogen concentrations as well as hormone concentrations. Blood was collected via jugular venipuncture into 10 mL blood collection tubes with no additives (Fisher Scientific). Collected blood was protected from light, held on ice, and allowed to clot for two hours prior to centrifugation. Blood samples were centrifuged for 15 minutes at 3,000 × g and serum were collected. Serum was stored at -80 °C until further analyses could be performed. Serum glucose levels were analyzed using the Infinity Glucose Hexokinase Reagent assay TR15421 (ThermoFisher, San Jose, CA) and serum urea nitrogen levels were analyzed using the QuantiChrom Urea Assay kit DIUR-100 (BioAssay Systems, Hayward, CA). Serum glucose and urea nitrogen levels were quantified using a Synergy H1 Microplate reader, (Biotek, Winooski, VT) quantified at a wavelength of 450 mg/dL.

Hormone Analysis

Serum concentrations of GH were determined in duplicate by radioimmunoassay (**RIA**) using procedures described by Klindt *et al* (1983). Bovine GH (AFP-9884C, National Hormone and Pituitary Program) was used as the radioiodinated antigen and standard. Growth hormone antisera (AFPB55, National Hormone and Pituitary Program) was used at an initial dilution of 1:200,000. Inhibition curves of sera ranging from 50 to 400 mL were parallel to standard curves. Sensitivity of the assay was 17 pg/tube. Intraassay and interassay CV were 10.0% and 15.4%, respectively.

Serum concentrations of IGF-I were determined in duplicate by radioimmunoassay (Echternkamp *et al*., 1990; Funston *et al*., 1995) for all samples. Insulin-like growth factor binding proteins (IGFBP) were extracted from serum using a 1:17 ratio of sample to acidified

ethanol (12.5% 2 N HCl: 87.5% absolute ethanol) (Daughaday *et al*., 1980). Extracted samples were centrifuged (12,000 × *g* at 4°C) to separate IGFBP. A portion of the resulting supernatant was removed and neutralized with 0.855 M Tris base, incubated for an additional 4 h at 4°C, and then centrifuged at 12,000 × g at 4°C to remove any additional IGFBP. When samples of this extract, equivalent to the original serum sample, were subjected to Western ligand blot analysis and subsequent phosphorimagery, no detected binding of ^{125}I-IGF-I to IGFBP was observed. Inhibition curves of the neutralized extracted serum ranging from 12.5 to 50 μL were parallel to the standard curve. Recombinant human IGF-I (GF-050; Austral Biological, San Ramon, CA, USA) was used as the standard and radioiodinated antigen. Antisera AFP 4892898 (National Hormone and Peptide Program, National Institutes of Diabetes, Digestive and Kidney Diseases, Bethesda, MD, USA) was used at a dilution of 1:62,500. Sensitivity of the assay was 11.7 pg/tube. Intra- and interassay CV were 11.7% and 13.4%, respectively.

Statistical Analysis

Data were analyzed as a completely randomized block design with a 2 × 3 factorial treatment of implant strategy (1× vs. 2×) by *GALR2* genotype (TT vs. TG vs. GG) using the mixed procedure of SAS (version 9.4; SAS Inst., Cary, NC). Implant strategy, genotype, and their interaction were used as fixed effects, initial body weight was used to determine slaughter date and acted as the block, and individual animal serves as the experimental unit. Serum urea nitrogen, glucose and hormone levels were analyzed using repeated measures and included day, genotype, implant, implant by genotype interaction and initial body weight block included in the model. Appropriate covariance structures were used (Wang and Goonewardene, 2004). When an interaction was present ($P \leq 0.05$), means were separated using the LSMEANS procedure with

the PDIFF feature in SAS and $P \leq 0.05$ were considered significant and trends were defined at $0.05 < P \leq 0.10$.

Results

Performance and Feeding Behavior

No significant *GALR2*c.-199T>G genotype × implant strategy interactions were detected for performance traits, including DMI, ADG, or G: F ($P = 0.48$). However, there was an effect of *GALR2c.-199T>G* genotype on DMI (Figure 2.1), with the GG and TG groups having 0.8 kg/d greater intakes than their TT counterparts ($P = 0.05$). Gain to feed was not influenced by *GALR2c.-199T>G* genotype, there was an effect of implant ($P = 0.008$), with the 2× implant strategy having an improved G: F efficiency in comparison to the conservative strategy (Table 2.4). There was no interaction of *GALR2*c.-199T>G genotype × implant strategy effect or main effect of *GALR2*c.-199T>G genotype or implant strategy on ADG throughout the feeding period (Table 2.4). Feeding behavior characteristics are listed in Table 2.3

Table 2.3. Effects of *GALR2c.-199T>G* genotype and implant strategy on feeding behavior in finishing steers.

Item	Genotype			Implant		SEM			*P*-value		
Events	GG	TG	TT	1×	2×	Geno	Imp	G × I	Geno	Imp	G × I
Visits day^{-1}	33.8	29.6	32.3	31.7	32.0	1.29	1.20	1.80	0.14	0.89	**0.02**
Meals day^{-1}	8.14$^{\mathbf{a}}$	7.75$^{\mathbf{a}}$	8.71$^{\mathbf{b}}$	8.17	8.23	0.15	0.14	0.21	**0.0001**	0.76	0.23
Time eating											
Min visit^{-1}	33.80	29.62	32.38	31.7	32.2	1.32	1.20	1.85	0.14	0.89	**0.02**
Min meal^{-1}	11.97$^{\mathbf{a}}$	11.33$^{\mathbf{a}}$	10.58$^{\mathbf{b}}$	11.63	10.96	0.34	0.31	0.47	**0.05**	0.14	0.48
Eating rate											
Kg visit^{-1}	0.29	0.33	0.30	0.29	0.32	0.01	0.01	0.02	0.20	0.18	0.07
Kg meal^{-1}	1.15$^{\mathbf{a}}$	1.19$^{\mathbf{a}}$	1.07$^{\mathbf{b}}$	1.11	1.16	0.02	0.02	0.03	**0.002**	0.06	0.41
Kg min^{-1}	0.09	0.10	0.10	0.09$^{\mathbf{a}}$	0.10$^{\mathbf{b}}$	0.002	0.002	0.003	0.19	**0.0008**	0.99

Table 2.3 lists feeding behaviors and their SEMs and *P*-values. All significant *P*-values are denoted in bold. SEM = standard error of the mean, Geno. = *GALR2c.-199T>G* Genotype, Imp. = Implant strategy.

Table 2.4. Effects of *GALR2c.-199T>G* genotype and implant strategy on performance of finishing steers.

	Genotype			Implant		SEM			*P*-value		
Item	GG	TG	TT	1×	2×	Geno	Imp	G × I	Geno	Imp	G × I
DMI, kg/d	11.97$^{\mathbf{a}}$	11.33$^{\mathbf{a}}$	10.58$^{\mathbf{b}}$	11.63	10.96	0.35	0.31	0.47	**0.05**	0.14	0.48
ADG, kg	1.75	1.79	1.78	1.75	1.81	0.02	0.58	0.64	0.15	0.82	0.96
G:F	0.14	0.15	0.16	0.15$^{\mathbf{a}}$	0.16$^{\mathbf{b}}$	0.002	0.002	0.003	0.19	**0.0008**	0.99

Table 2.4 lists feeding performance and their SEMS and P-values. All significant P-values are denoted in bold. SEM = standard error of the mean, Geno. = *GALR2c.-199T>G* Genotype, Imp. = Implant strategy, G × I = Genotype × Implant Strategy interaction.

Number of visits per day was influenced by the interaction of *GALR2c.-199T>G* genotype × implant strategy with steers from the GG2× group having the most visits per day (Figure 2.1). The TT1× and TT2× however have similar number of visits per day as the GG2× group. Meals consumed per day was influenced by genotype with the GG and TT groups consuming a greater number of meals per day in comparison to the heterozygote TG ($P < 0.0001$). When evaluating time eating an interaction of *GALR2c.-199T>G* genotype × implant strategy is present for minutes per visit (Figure 2.2). Similar to the pattern shown for visits per day the GG2× exhibit the greatest time spent per visit with the TT1× and TT2x steers having similar time spent per visit. For minutes per meal the effect of *GALR2c.-199T>G* genotype had an effect with the GG and TG steers spending 0.75 minutes more than the TT steers per meal ($P = 0.05$).

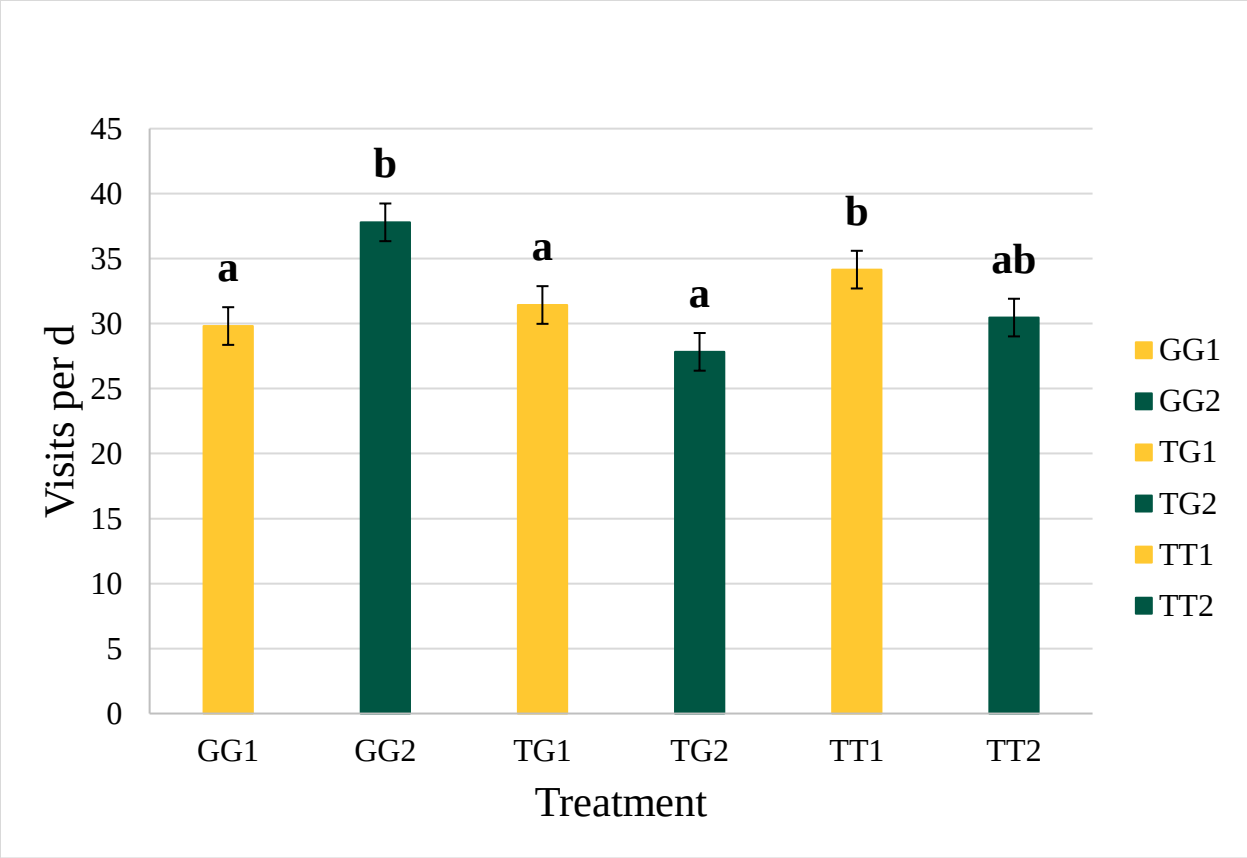

Figure 2.1. Effect of *GALR2c.-199T>G* genotype × implant strategy on number of Visits per d for finishing steers. Where 1 is the 1× implant of Revlor-S on d 77 and 2 is the 2× implant of Revalor-S on d 0 and d77 of the finishing period. Error bars depict SEM. [ab] Bars lacking a common superscript differ (*GALR2c.-199T>G* genotype × implant strategy $P = 0.02$; Genotype $P = 0.14$; Implant strategy $P = 0.89$).

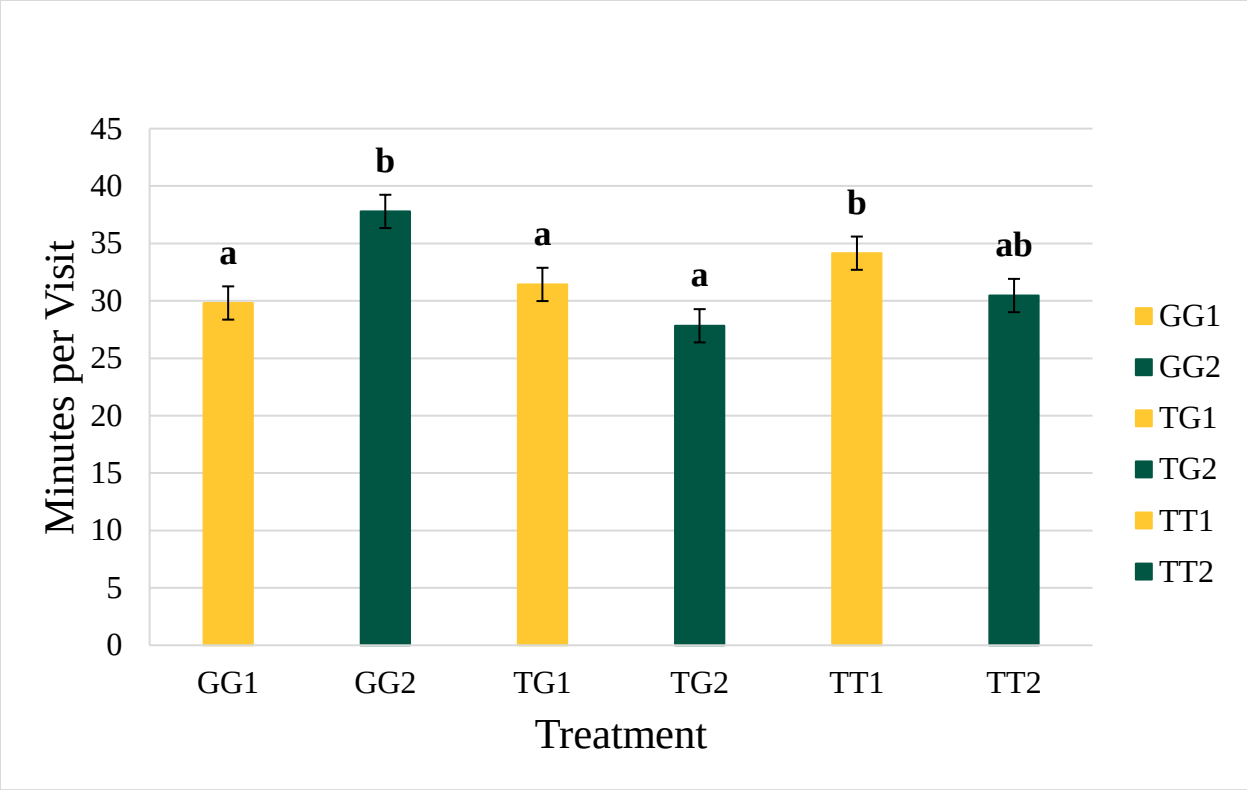

Figure 2.2. Effect of *GALR2c.-199T>G* genotype × implant strategy on minutes per visit for finishing steers. Where 1 is the 1× implant of Revlor-S on d 77 and 2 is the 2× implant of Revalor-S on d 0 and d77 of the finishing period. Error bars depict SEM. [ab] Bars lacking a common superscript differ (*GALR2c.-199T>G* genotype × implant strategy $P = 0.02$; Genotype $P = 0.14$; Implant strategy $P = 0.89$).

Gene Expression

The expression of the *GALR2* gene was evaluated for differences in expression based on treatment. No significant differences were found for the interaction of *GALR2c.-199T>G* genotype × implant strategy ($P = 0.15$) or the main effects of *GALR2c.-199T>G* genotype ($P = 0.62$) or implant strategy ($P = 0.31$) on the *GALR2* expression within skeletal muscle tissue.

Serum Glucose and Urea Nitrogen Concentrations

Serum urea nitrogen concentration was not influenced by the *GALR2c.-199T>G* genotype × implant strategy × day interaction ($P = 0.82$).There was no effect of *GALR2c.-199T>G* genotype × implant interaction for serum urea nitrogen ($P = 0.71$). *GALR2c.-199T>G*

genotype × day had a tendency to influence serum urea nitrogen concentrations (P =0.07). There was a day × implant strategy interaction effect for serum urea nitrogen concentrations (P = 0.0035) (Figure 2.3).

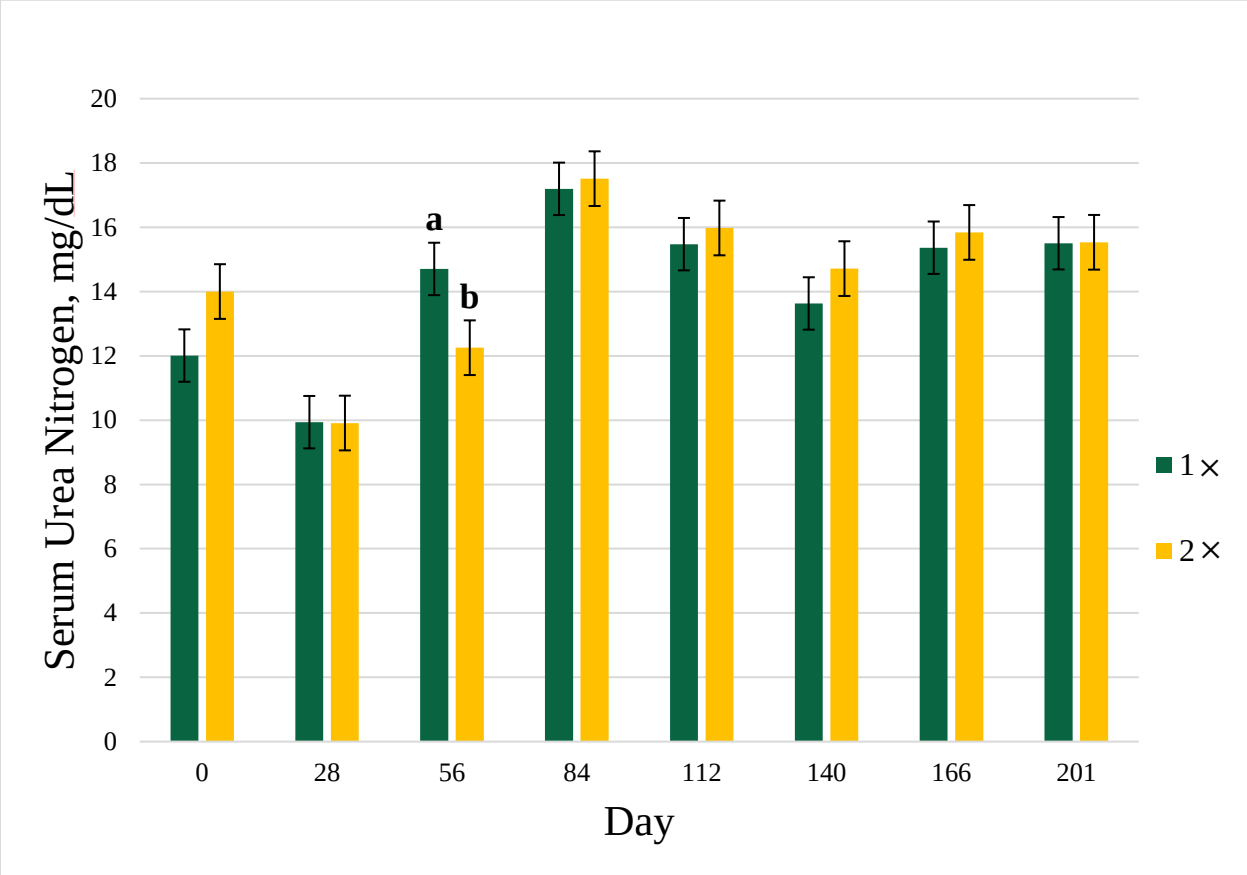

Figure 2.3. Effect of implant strategy on serum urea nitrogen levels across d. Where 1 is the 1× implant of Revlor-S on d 77 and 2 is the 2× implant of Revalor-S on d 0 and d77 of the finishing period. For d 0 through d 166 n = 93 steers while d 201 n = 47 as group one steers were slaughtered after d 166. Error bars depict SEM. [ab] Bars lacking a common superscript differ (Implant strategy × Day P = 0.0035; Implant P = 0.38; Day P < 0.0001).

For serum glucose concentrations the interaction of *GALR2c.-199T>G* genotype × implant strategy × day was not significant (P = 0.19). *GALR2c.-199T>G* genotype × implant strategy did not have an influence on serum glucose levels (P = 0.33). The implant strategy × day interaction had no effect on serum glucose concentrations (P = 0.31). The interaction of day × *GALR2c.-199T>G* genotype had a significant effect on serum glucose concentration (P =

0.0049) (Figure 2.4). On days 84 and 140 glucose concentration was greater in GG steers than TG steers ($P < 0.05$) and tended to be greater than TT steers ($P = 0.06$). At the conclusion of the finishing phase serum glucose levels for GG steers tended to be lowest with the TT steers exhibiting increased serum glucose concentrations and TG steers having intermediate concentrations ($P = 0.09$).

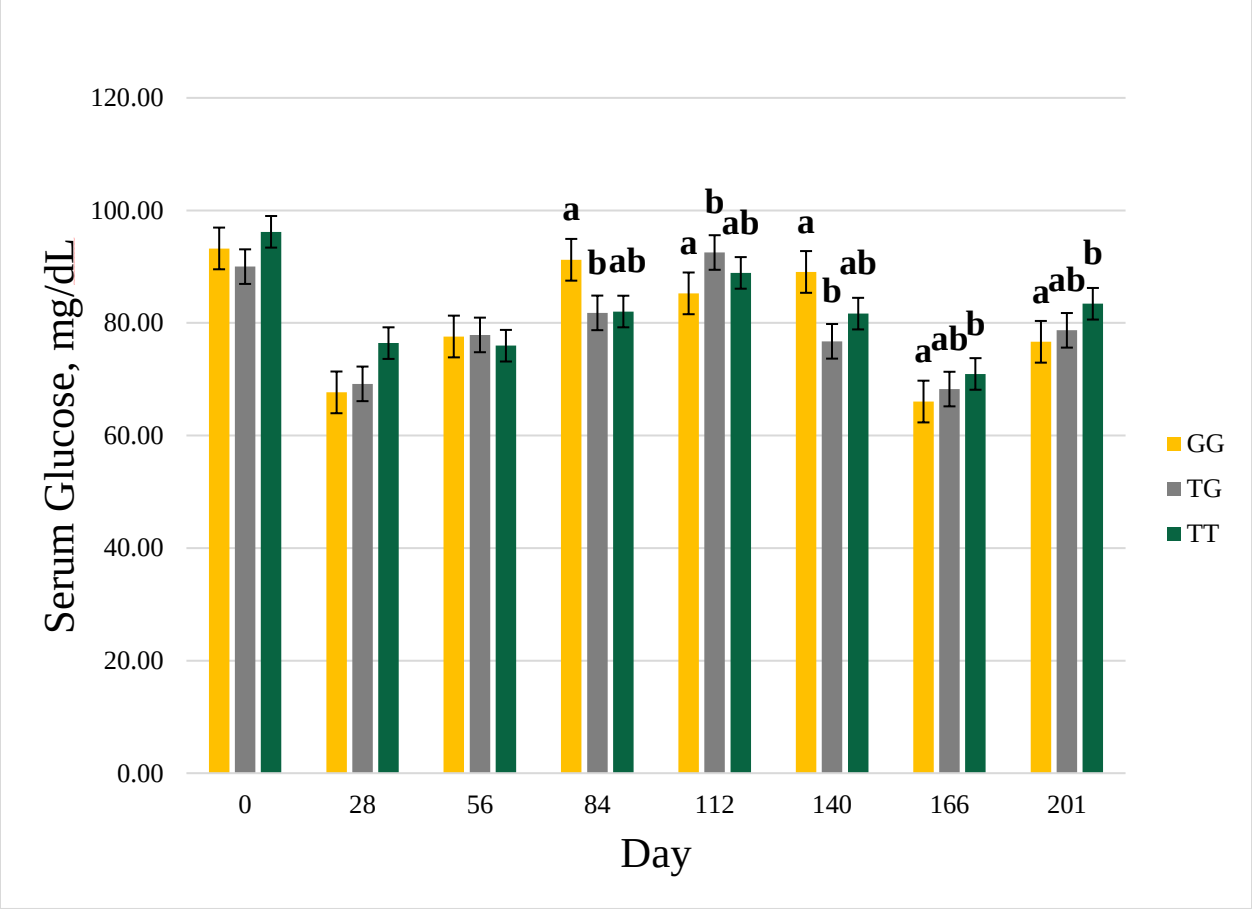

Figure 2.4. Effects of *GALR2c.-199T>G* genotype on serum glucose levels across d. For d 0 through d 166 n = 93 steers while d 201 n = 47 as group one steers were slaughtered after d 166. Error bars represent the SEM. Bars exhibiting significantly different glucose levels within a day possess differing superscripts (*GALR2c.-199T>G* Genotype × Day $P = 0.0049$; Genotype $P = 0.43$; Day $P < 0.0001$).

Growth Hormone and Insulin-like Growth Factor 1

There was no effect of *GALR2*c.*-199T>G* genotype × implant strategy × day ($P = 0.87$) or *GALR2*c.*-199T>G* genotype × implant strategy ($P = 0.75$) on GH concentrations. The

*GALR2*c.*-199T>G* genotype × implant strategy interaction had no effect on GH levels (*P* = 0.75). Additionally, there was no effect of implant strategy × day on GH levels (*P* = 0.81). However, there was an effect of *GALR2*c.*-199T>G* genotype × day on GH levels (*P* = 0.04). The TT steers exhibited elevated GH levels at the beginning of the study and after a convergence of GH concentrations on d 28 they remain elevated over the GG and TG steers until d 140 and d 166 where all genotypes exhibit declining GH levels. The heterozygote steers maintained intermediate levels with the GG seers exhibiting the lowest levels until d 166 where they exhibited the highest concentrations of GH (Figure 2.5).

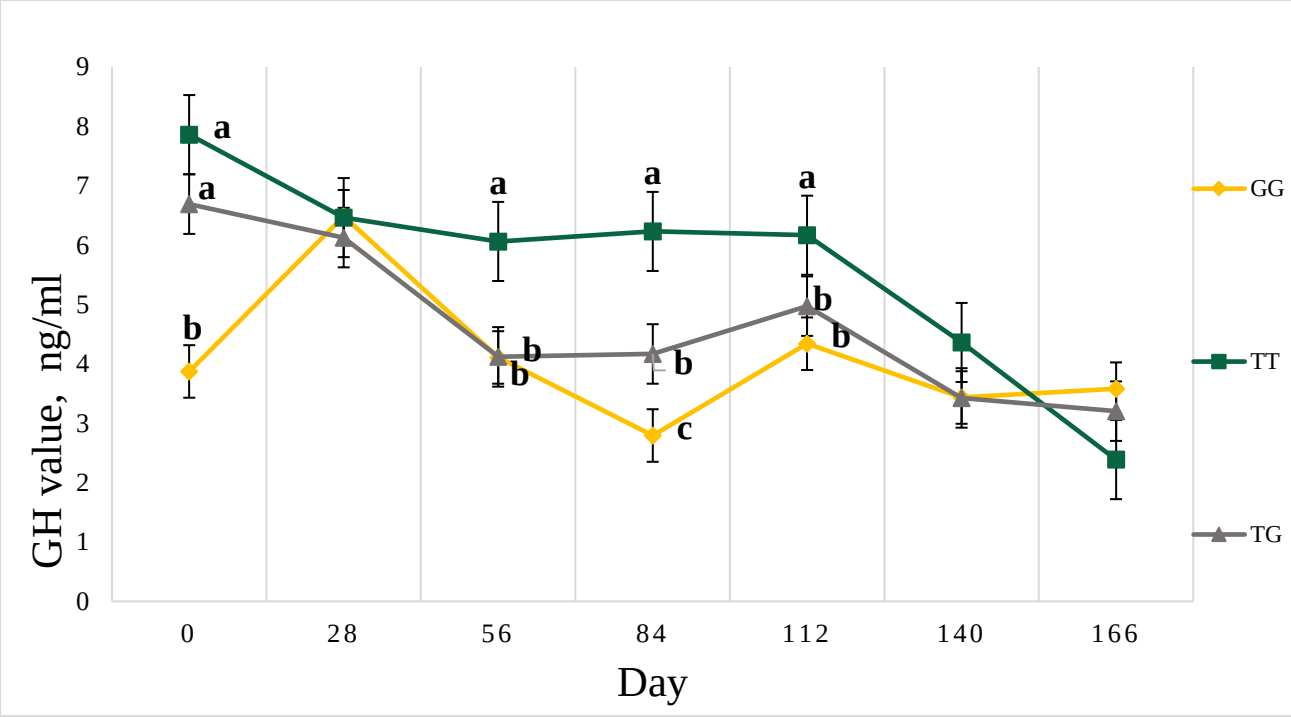

Figure 2.5. Effect of *GALR2c.-199T>G* genotype on GH levels throughout the finishing period. Error bars depict SEM. [abc] Days lacking a common superscript differ (Genotype × Day *P* = 0.04; Genotype *P* = 0.006; Day *P* < 0.0001).

For IGF-1 concentrations *GALR2*c.*-199T>G* genotype × implant strategy × day had no effect (*P* = 0.21). However, a *GALR2*c.*-199T>G* genotype × implant strategy interaction was observed (*P* = 0.04). Steers of the TT2× group have greater concentrations of IGF-1 than the

TG2× and GG2× steers which are greater than that of the other steers (Figure 2.6). There was no effect of *GALR2*c.*-199T>G* genotype × day ($P = 0.31$), there was an implant strategy × day effect with steers assigned to the 2× having elevated IGF-1 levels throughout the finishing phase on d 28, 56, 84, 112, and 140 but not on d 166 ($P = 0.009$).

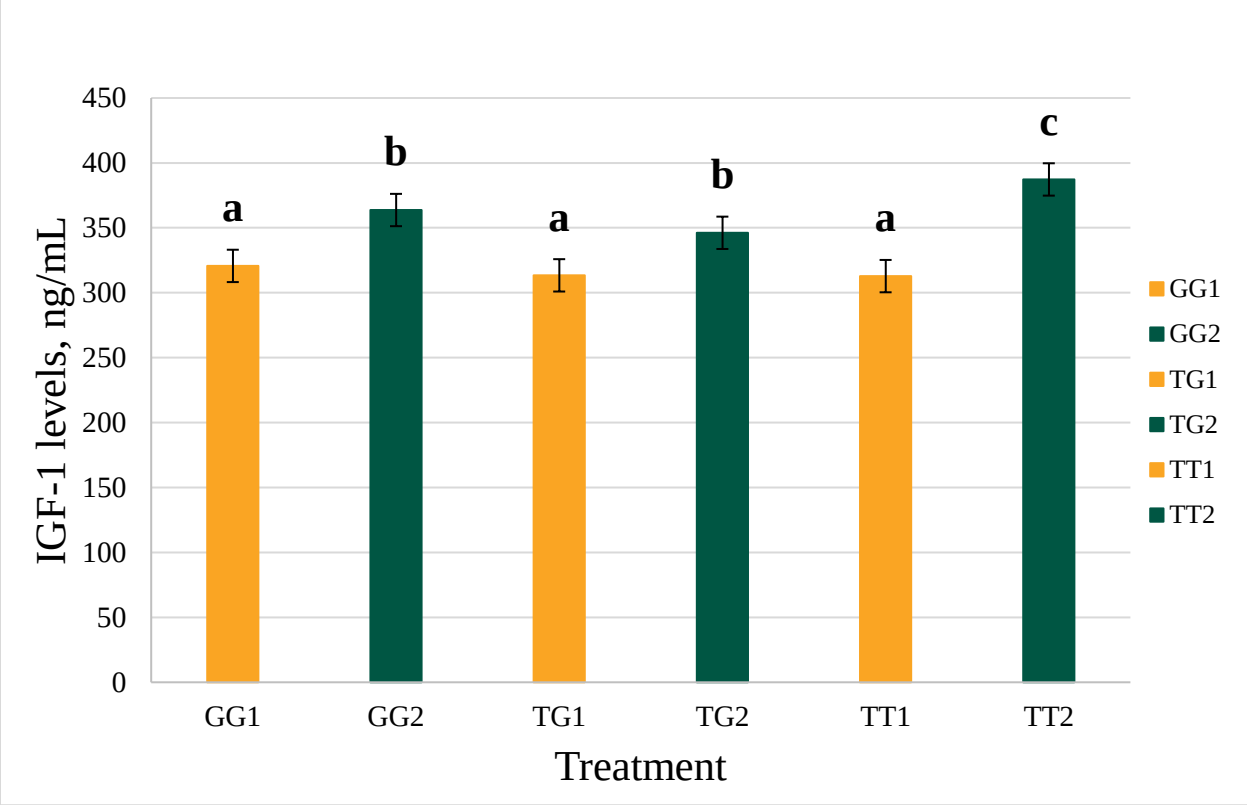

Figure 2.6. Effect of *GALR2c.-199T>G* genotype × implant strategy interaction on IGF-1 concentrations in finishing steers. Geno. = *GALR2c.-199T>G* genotype, Imp. = implant strategy; Where 1 is the 1× implant of Revlor-S on d 77 and 2 is the 2× implant of Revalor-S on d 0 and d77 of the finishing period. Error bars depict SEM. [abc] Bars lacking common superscripts differ (*GALR2c.-199T>G* genotype × implant strategy $P = 0.04$; Genotype P = 0.06; Implant P < 0.0001).

Discussion

Galanin receptor 2 genotype has a significant effect on DMI with the GG group consuming greater DMI over the finishing period. With the previous work from Duncombe (2016) suggesting that the GG genotype exhibits a greater propensity for protein accretion as

supported by heavier HCW and larger LMA this study may support this greater propensity for protein accretion increased nutrient composition. There was an influence of implant on G: F and HCW, with the 2× implant strategy resulting in improved G: F and heavier HCW. This suggests that *GALR2c.-199T>G* genotype does not have a negative impact on the ability for the Revalor-S implant to improve protein accretion in finishing steers.

While there was a significant effect of day × implant interaction on serum urea nitrogen, this interaction was driven by a difference on d 56 and was not present on any other d, suggesting there was not a prevalent pattern throughout the finishing period. Additionally, with the day × *GALR2c.-199T>G* genotype interaction effect on serum glucose concentration the GG genotype had elevated levels on d 84 and 140 and lower concentrations at both slaughter points 166 and d 201. There was no clear trend to suggest divergent blood metabolite levels based on the genotype × implant interaction or the *GALR2c.-199T>G* genotype.

Measuring serum urea nitrogen levels can serve as an indicator of the animal's utilization of amino acids. The movement of nitrogen in the form of NH_3-N and urea via the portal-drained viscera and the liver, in ruminants, is a physiological mechanism known as urea-N salvaging and is critical for the conservation of nitrogen in the body (Lapierre and Lobley, 2001). Additionally, measuring serum glucose levels allows for evaluation of an animal's utilization efficiency of carbohydrates.

The inverse in effect of *GALR2c.-199T>G* genotype on GH levels that is present only on d 166 may represent that the GG steers would benefit from a longer finishing phase and the TG steers peaking during an intermediate finishing phase compared to the GG and TT steers. These results also align with DMI levels, as we saw the GG steers had the greatest amount of DMI over the feeding period and inversely the lowest GH levels. In previous studies, GH levels have been

shown to be decreased with an increased availability of carbohydrates (Jansz *et al*., 1963; Lapierre *et al*.,1995).

Serum IGF-I concentrations are stimulated by GH (Stewart and Rotwein, 1996). Steers possessing the TT have greater presence of *GALR2*. A complementary relationship of elevated GH levels and IGF-1 could indicate that cattle possessing the TT genotype may experience increased release of GH leading to a subsequent increase in IGF-1 levels. This may explain why steers with the TT genotype may have naturally higher GH and IGF-1 levels. Additionally, all steers assigned to the 2× implant strategy exhibited increased concentrations of IGF-1. Further research is needed to determine why the differences in GH levels among the *GALR2c.-199T>G* genotype exist as well as the interaction effect of *GALR2c.-199T>G* genotype × implant strategy on IGF-1 levels.

In this study no differences were observed for gene expression within skeletal muscle. Based on previous work from the Duncombe (2016) group this was adverse to what we expected as it is hypothesized that GALR2 expression is elevated when a GG mutation is present at the *GALR2c.-199T>G* SNP through the intersertion of a Sp1 transcription factor binding site. Further research into *GALR2* expression differences among the genotypes (TT, TG, and GG) throughout different tissues in the body may be warranted. It is known that expression of *GALR2* and galanin protein is most heavily concentrated within the hypothalamic-pituitary axis and small intestine of the G.I. tract (Waters and Krause, 2000). Measuring protein concentration and *GALR2* expression within these tissues may be worth further investigation.

These findings help further develop the hypothesis that the *GALR2c.-199T>G* SNP may serve as a potential for marker-assisted management strategies as we see a divergence in optimal finishing times depending on genotype. There is also evidence to suggest that the TT genotypes

may reach an optimal finishing size with a shorter finishing period and with less feed intake than their GG counterparts. Additional research is needed to be conducted to further validate the benefits of varying finishing periods based on the *GALR2c.-199T>G* SNP. There may also be a benefit to investigating the effects of different implant strategies beyond those presented in this study. As stated previously, this study evaluated the effects of conservative vs. aggressive implant strategy of Revalor-S (Merk Animal Health, Summit, NJ); a combination implant that consists of 120 mg TBA and 24 mg estradiol. Subsequent research evaluating the effect of androgenic, estradiol, and combination implants on *GALR2c.-199T>G* genotype performance may further identify optimal management strategies for feedlot producers.

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CHAPTER 3. EFFECTS OF *GALR2* GENOTYPE AND DIFFERING IMPLANT STRATEGIES ON CARCASS CHARACTERISTICS OF CROSSBRED ANGUS FINISHING STEERS

Abstract

The objective of this study was to determine how the interaction of different implant strategies with the *galanin receptor 2* (*GALR2*) genotype would affect carcass characteristics and meat quality in crossbred Angus finishing steers. Angus crossbred steers (n = 93) were selected for this study based on their *GALR2c.-199T>G* genotype (GG, TT, and TG) and weaning weight with 19 GG, 36 TT, and 38 TG. Calves were blocked by initial body weight and fed a standard feedlot ration for 166 d or 202 d. Body weight data was collected every 28 d. Steers were randomly assigned to one of two implant strategies; 1) a single Revalor-S (Merk Animal Health, NJ) on d 77 (1×), or 2) a Revalor-S on d 0 and another on d 77 (2×). Data were analyzed as a 2 × 3 factorial design of implant strategy by *GALR2c.-199T>G* genotype using the mixed procedure in SAS, with repeated measures used for color, and significance was set at $P < 0.05$. Hot carcass weight and back fat were directly affected by implant strategy with steers receiving the aggressive strategy exhibited heavier hot carcass weights and increased back fat ($P < 0.05$). Cook loss and shear force were influenced by the interaction of genotype by implant strategy ($P < 0.05$). The GG1 × steers exhibited the greatest amount of cook loss while the GG 2 × steers exhibited a higher shear force value. The pH, water holding capacity, and color of the striploins were not affected by the genotype by implant strategy interaction or main effects of genotype and implant strategy. From this we concluded the interaction of *GALR2c.-199T>G* genotype × implant strategy does not have a negative effect on carcass characteristics or meat quality in crossbred Angus finishing steers.

Introduction

Grid pricing is determined using a price figured based on industry and/or slaughter abattoir averages that creates a base price. Premiums or discounts are then applied to the carcasses dependent on if the carcass meets standard specifications or excels beyond standard parameters or fails to meet the standard (Schroeder *et al*., 1997). Grid pricing allows for each individual animal to receive a price as opposed to "live weight" or "dressed weight" pricing where a price is determined by the lot average. Premiums are placed on carcasses meeting quality grades above the pre-determined base while cattle not meeting the quality grade average for that abattoir receive a discount.

Grid pricing is comprised of two main components: quality grade (**QG**) and yield grade (**YG**). A quality grade is a composite of evaluation factors that can influence the palatability of meat including tenderness, juiciness, and flavor. The following factors determine quality grades of beef: maturity, firmness, texture, color of lean, and the amount and distribution of marbling within lean muscle. Yield grades are an estimate on a scale of 1-5 to predict the amount of boneless, trimmed retail cuts from the high-value portions of the carcass (USDA, 1989).

The potential for superior profits when marketing on the gird as opposed to the "live weight" marketing can exist. However, it is pertinent that the cattle feeders are well versed in how grid pricing works and are aware of the quality of cattle they possess prior to marketing. All production decisions should be considered, including diet composition throughout the finishing phase, length of the finishing period and implant strategies (DiCostanzo and Dahlen, 2000).

Galanin receptor 2 (GALR2) is a neuropeptide receptor that is associated with feeding behavior, insulin release, and growth hormone secretion (Smith et al., 1997; Waters and Krause, 2000). A previous study in cattle examined the effect of a mutation in *GALR2* (*GALR2c*.-

199T>G) on carcass characteristics (Duncombe, 2016). The GG genotype was associated with greater rib-eye area, whereas the TT genotype was associated increased marbling score. This divergence between muscle and fat growth between the genotypes indicates the potential for a marker assisted management to be developed to aid producers in creating a more uniform pen finish among cattle. Utilizing different management practices based on genotype, such as conservative versus aggressive implant strategies, could improve carcass uniformity, and therefore profitability, for beef producers. Additionally, gaining insight into the biology and mechanisms of how GALR2 genotype affects muscle growth and fat deposition can point towards additional management strategies that could be implemented to improve beef production.

Materials and Methods

All animal procedures performed in this study were approved by the North Dakota State University Institute for Animal Care and Use Committee (Protocol #A18062). Procedures for this study were conducted at the North Dakota State University Central Grasslands Research Extension Center and North Dakota State University Beef Cattle Research Complex.

Experimental Design and Treatments

Cattle for this experiment were sourced from the North Dakota State University Central Grasslands Research Extension Center herd. Ninety-five steers were initially selected for the study, two steers were removed from the study for an inability to train to the feeding system. The distribution of the *GALR2c.-199T>G* genotype for the study was: 36 TT, 38 TG and 19 GG based on *GALR2c.-199T>G* genotype. Cattle were assigned to one of four pens based on initial body weight with an average of 254.8kg (± 80.44kg) and were allowed a two-week training period to adapt to the Insentec ® Feeders (Hokofarm Group B.V., Markness, The Netherlands).

Upon initiation of the study cattle were randomly assigned within *GALR2c.-199T>G* genotype to one of two growth promoting implant strategies; Revalor-S (120 mg trenbolone acetate and 24mg estradiol; Merk Animal Health, Summit, NJ) on d 77 of finishing (1×) or Revalor-S on d 0 and d 77 of finishing (2×). Forty-seven of the steers were assigned randomly to the aggressive (2×) strategy and 46 to the conservative (1×).

DNA Extraction and Genotyping

Bloods samples were collected via jugular venipuncture, using 10 ml EDTA vacutainer tubes (Fisher Scientific, Walmath, MA, U.S.), from all bull calves (n = 191) born at the North Dakota State University Central Grasslands Research and Extension Center cattle herd in 2018. The blood samples were centrifuged for 15 minutes at 3,000 × g. The buffy coat was collected for each sample and stored at -80ºC, until DNA extraction was performed. DNA extraction was performed using the Qiagen DNAeasy® Blood & Tissue Kit (Qiagen Germantown, MD).

Genotyping of all samples was performed using the KASP genotyping system (LCG Genomics, Beverly, MA), Aliquot ID 116365958 with target sequence CGCCTCGGCCGCCA[G/T]CGGGATCATCCCCCC. PCR conditions were 94º C for 15 minutes and 10 cycles at 94º C for 20 seconds, 68-62º C for 1 minute dropping 0.6º C per cycle, 94º C for 20 seconds, 62º C for one minute for 26 cycles, then 94º C for 20 seconds, 57º C for 1 minute for 3 cycles. Genotyping was performed on an initial population of 191 samples using the ABI 7500Fast real time PCR machine (Applied Biosystems, Waltham, MA, US) to identify the *GALR2c.-199T>G* genotype of each calf. Out of the 191 samples genotyped only 19 GG calves were identified, thus there was a lower number of steers for this genotype on study. Steers were then selected for the study at weaning based on *GALR2c.-199T>G* genotype, weaning weight, and date of birth to create the most uniform group.

Slaughter

Cattle were finished in two separate slaughter groups based on initial BW. Group one spent 166 d on feed and had an average final BW of 594.5 kg and group two spent 202 d on feed and had an average final BW of 598.9 kg. All cattle were slaughtered at a commercial abattoir (Tyson Fresh Meats., Dakota City, NE), and all carcass data were collected by trained personal. Hot carcass weight (**HCW**) was collected at slaughter while USDA quality grade (**QG**), yield grade (**YG**), back fat (**BF**), longissimus muscle area (**LMA**), and marbling score were collected 24hr post-mortem, calculated yield grade and kidney pelvic heart fat (**KPH**) percentage were also recorded. Marbling score, LMA, and BF were assessed using computer image analysis at the abattoir. Striploins were collected from the left side of each carcass and for further meat quality analysis.

Meat Quality Analysis

Striploins (IMPS 180) were aged in vacuum packaged bags following slaughter and stored at 2.5°C for 14 d. Following aging striploins were defaced from the anterior side and 2.54 cm steaks were collected for color display and shear force, while 1.27 cm steaks were collected for ether extract values and western blot analysis respectively. Procedures described the AOAC (2010) were followed for intramuscular fat analysis via ether extract. The pH of the meat was measured at the medial end of the striploin and measured with a meat pH meter (Hanna Hi99163, Hanna Instruments, Woonsocket, RI). Additionally, a 50g meat cube was collected from both the lateral and medial sides of the striploin for drip loss analysis. Samples were weighed and stored in 8.5 cm × 4 cm whirl-pak sample bags (VWR Radnor, PA) and reweighed 24h later.

Color Display

Following the 14 d aging period Minolta color score was observed over a 10 d span. Steaks were wrapped with oxygen-permeable polyvinylchloride film and placed under display lighting conditions for 14 d stored at 2.5°C. Two measurements were taken of each steak each day for ten days with the Chroma meter CR-410 (Konica Minolta, Tokyo, Japan).

Shear Force and Cook Loss

Steaks collected for shear force and cook loss were sealed in vacuum packaging and frozen at -20°C until this analysis could take place. Steaks were allowed 12hr to thaw to room temperature prior to cooking. Steaks were first individually weighed and cooked to a medium degree of doneness at 71°C using a clamshell-style grill (Spectrum Brands, Madison, WI). Steaks were then allowed to rest for six minutes and then reweighed to calculate cook loss. Steaks were allowed to further cool to room temperature before shear force analysis was performed.

Upon cooling to room temperature, six 1.3 cm cores were taken from each steak coring parallel to the muscle fiber orientation. Cores were then sheared perpendicular to the muscle fibers using a Mecmesin BFG500N force gauge (Mecmesin, Slinfold, West Sussex, UK).

Statistical Analysis

The experiment was a completely randomized block design with a 2×3 factorial treatment of implant strategy (conservative vs. aggressive) by *GALR2* genotype (TT vs. TG vs. GG). Data were analyzed using the mixed procedure of SAS (version 9.4; SAS Inst., Cary, NC). Implant strategy, genotype, and their interaction were used as fixed effects of interest while initial body weight was used as the fixed effect of the block, individual animal serves as the experimental unit. When an interaction was present ($P \leq 0.05$), means were separated using the

LSMEANS procedure and $P \leq 0.05$ were considered significant. The repeated measures procedure was used for color analysis.

Results

Carcass Characteristics

Hot carcass weight and back fat thickness were not influenced by the interaction of *GALR2c.-199T>G* genotype × implant strategy (HCW $P = 0.61$; BF $P = 0.47$). Additionally, there was no effect of *GALR2c.-199T>G* genotype on HCW ($P = 0.47$) or BF ($P = 0.40$). Both HCW and BF were directly affected by implant strategy with steers receiving the steers assigned to the 2× strategy having heavier hot carcass weights ($P = 0.04$) and increased back fat ($P = 0.02$). Longissimus muscle area was not affected by the *GALR2c.-199T>G* genotype × implant strategy interaction ($P = 0.53$), the effect of *GALR2c.-199T>G* genotype ($P = 0.90$), or the effect of implant strategy ($P = 0.12$). Kidney pelvic and heart fat were not affected by the *GALR2c.-199T>G* genotype × implant strategy interaction ($P = 0.58$), the effect of *GALR2c.-199T>G* genotype ($P = 0.54$), or the effect of implant strategy ($P = 0.92$).

Yield grade was not affected by the *GALR2c.-199T>G* genotype × implant strategy interaction ($P = 0.91$) or the effect of *GALR2c.-199T>G* genotype ($P = 0.81$). There was however a tendency present for the effect of implant strategy ($P = 0.08$) for steers assigned to the 1× implant strategy to produce leaner carcasses. Finally, there was a tendency present for the *GALR2c.-199T>G* genotype × implant strategy interaction to influence marbling degree ($P = 0.08$) (Table 3.1). Steers assigned from the GG1× tended to produce carcasses with a higher degree of marbling.

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Table 3.1. Effects of *GALR2c.-199T>G* genotype and implant strategy interaction on carcass characteristics.

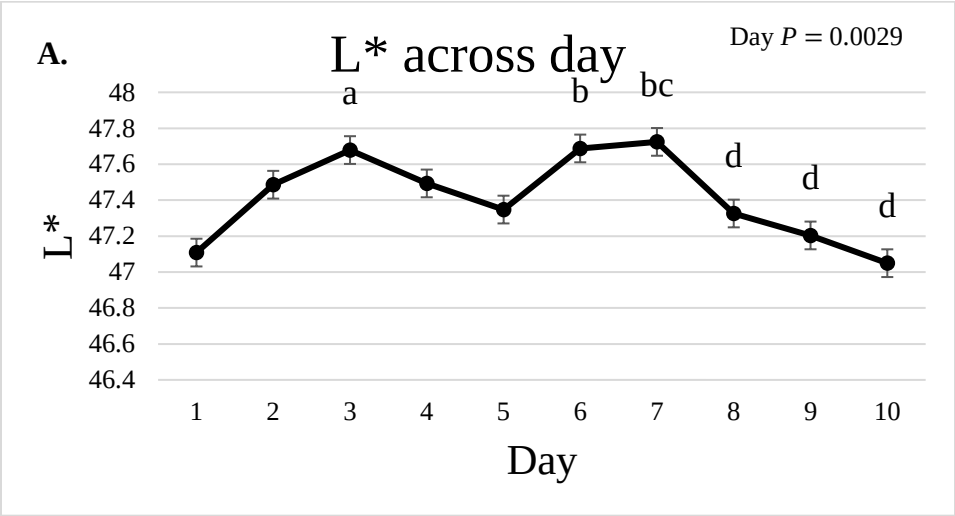
Item	Genotype			Implant		SEM		*P*-value			
	GG	TG	TT	1×	2×	Geno.	Imp.	Geno. × Imp.	Geno.	Imp.	Geno× Imp
HCW, kg	359	365	366	357[a]	370[b]	3.43	3.15	4.85	0.47	**0.04**	0.61
BF, cm	0.59	0.63	0.60	0.58 [a]	0.63 [b]	0.01	0.01	0.02	0.40	**0.02**	0.47
REA, cm^2	11.69	11.75	11.62	11.48	11.90	0.20	0.18	0.28	0.90	0.12	0.53
KPH, %	2.02	1.98	1.96	1.99	1.98	0.03	0.02	0.04	0.54	0.92	0.58
YG	3.47	3.57	3.55	3.43	3.63	0.08	0.07	0.12	0.81	0.08	0.91
MARB	492	409	423	439	419	13.48	12.30	18.89	0.18	0.23	0.12

Table 3.1 carcass characteristics collected at the commercial abattoir and their SEMs and *P*-values. All significant *P*-values are denoted in bold. SEM = standard error of the mean, Geno. = *GALR2c.-199T>G* Genotype, Imp. = Implant strategy HCW = hot carcass weight, BF = back fat, REA = ribeye area, KPH % = kidney, pelvic and heart fat percentage, YG = yield grade, MARB = marbling degree where 400 serves as choice and 0-10 = modest; 10-20 = small; 20-50. SEM = standard error of the mean. [ab] Values lacking a common superscript within a treatment differ.

Meat Quality Analysis

The pH values were not affected by the *GALR2c.-199T>G* genotype × implant strategy interaction ($P = 0.89$), the effect of *GALR2c.-199T>G* genotype ($P = 0.28$), or the effect of implant strategy ($P = 0.55$). Drip loss percentages were also not affected by the *GALR2c.-199T>G* genotype × implant strategy interaction ($P = 0.89$), the effect of *GALR2c.-199T>G* genotype ($P = 0.93$), or the effect of implant strategy ($P = 0.83$).

For L* values there was no effect of the *GALR2c.-199T>G* genotype × implant strategy interaction ($P = 0.54$), the effect of *GALR2c.-199T>G* genotype ($P = 0.93$), or the effect of implant strategy ($P = 0.67$). Similar to L* values, a* values saw no effect of the *GALR2c.-199T>G* genotype × implant strategy interaction ($P = 0.23$), the effect of *GALR2c.-199T>G* genotype ($P = 0.69$), or the effect of implant strategy ($P = 0.17$). When looking at yellowness, b* values were not influenced by the effect of the *GALR2c.-199T>G* genotype × implant strategy interaction ($P = 0.08$) or the effect of *GALR2c.-199T>G* genotype ($P = 0.21$). Yellowness however, was influenced by the effect of implant ($P = 0.05$). Color was affected by day for L* ($P = 0.0029$), a* ($P < 0.0001$), and b* ($P < 0.0001$) values (Figure 3.1).



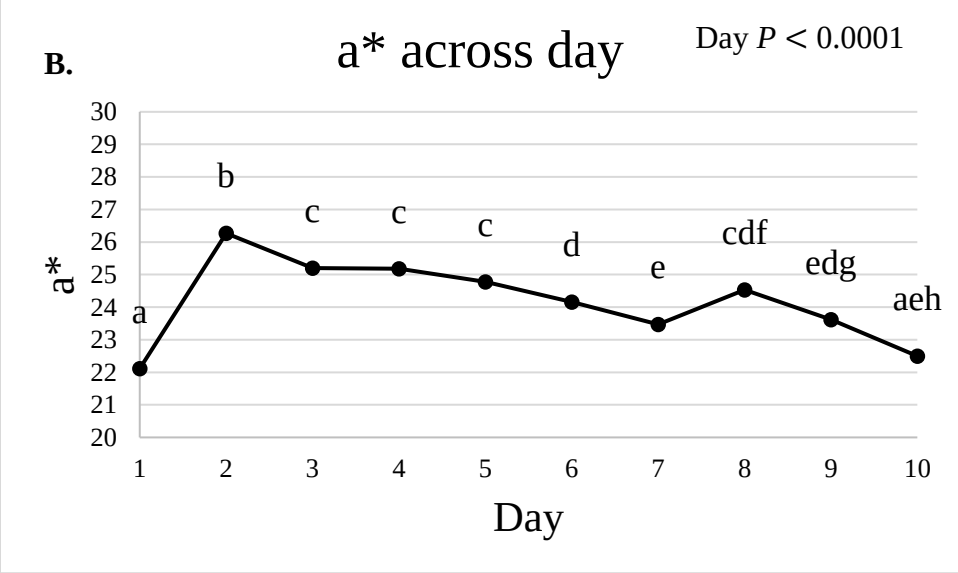


Figure 3.1. Minolta color values across a 10 d shelf life period of ribeye steaks. Error bars depict the SEM. Days exhibiting a different value posse a different superscript. A. L* values across d, $P = 0.0029$. B. a* values across d, $P < 0.0001$ C. b* values across d, $P < 0.0001$.

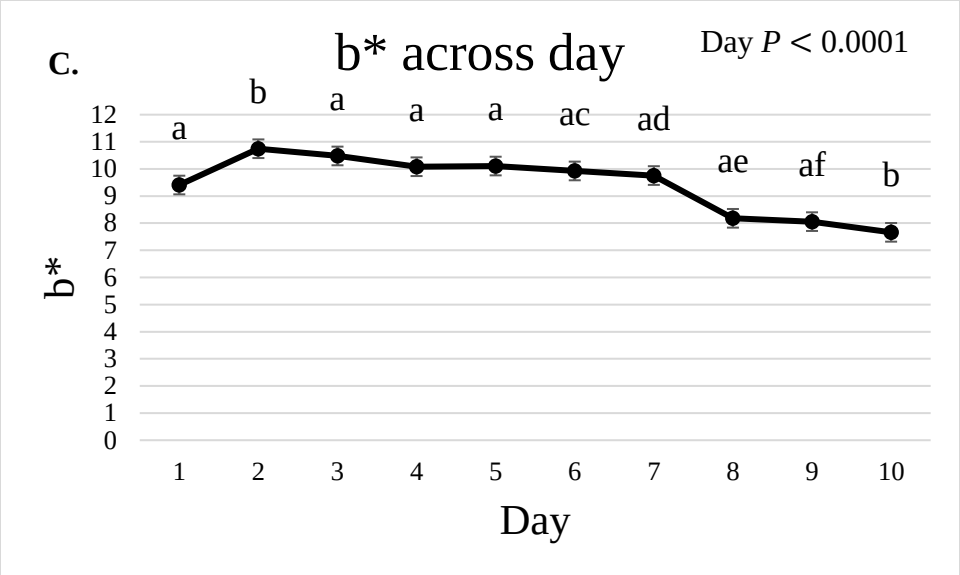


Figure 3.1. Minolta color values across a 10 d shelf life period of ribeye steaks (continued). Error bars depict the SEM. Days exhibiting a different value posse a different superscript. A. L* values across d, P = 0.0029. B. a* values across d, P < 0.0001 C. b* values across d, P < 0.0001.

Cook loss was influenced by the interaction of *GALR2c.-199T>G* genotype × implant strategy with the GG2× steers having greater cook loss in comparison to all other steers ($P = 0.04$) (Figure 3.2). Similarly, when evaluating shear force, there was an influence of the *GALR2c.-199T>G* genotype × implant strategy interaction ($P = 0.0045$) with the GG2× steers having the highest shear force values (Figure 3.3). A summary of meat quality attributes measured are in Table 3.2.

Table 3.2. Effects of *GALR2c.-199T>G* genotype and implant strategy interaction on meat quality attributes.

Item	Genotype			Implant		SEM		*P*-value			
	GG	TG	TT	1×	2×	Geno.	Imp.	Geno. × Imp.	Geno.	Imp.	Geno× Imp
Drip loss %	0.70	0.72	0.66	0.71	0.67	0.12	0.10	0.16	0.93	0.83	0.89
pH	5.49	5.55	5.51	5.51	5.53	0.02	0.01	0.03	0.28	0.55	0.89
Shear Force[1], kg	2.00	1.69	1.77	1.58	2.06	0.06	0.05	0.08	**0.01**	**<0.0001**	**0.0045**
Cook loss %	0.43	0.45	0.47	0.41	0.49	0.24	0.21	0.33	0.66	**0.01**	**0.04**
Color[2]											
L*	47.29	47.50	47.42	47.31	47.50	0.33	0.31	0.45	0.93	0.67	0.54
a*	23.92	24.44	24.17	24.50	23.85	0.36	0.33	0.49	0.69	0.17	0.23
b*	9.06	9.85	9.40	9.79**a**	9.08**b**	0.27	0.24	0.37	0.21	**0.05**	0.08

Table 3.2 meat quality attributes measured in the meat lab from striploins collected at a commercial abattoir and their SEMs and *P*-values. All significant *P*-values are denoted in bold. SEM = standard error of the mean, Geno. = *GALR2c.-199T>G* Genotype, Imp. = Implant strategy, SEM = standard error of the mean. [1]Shear Force values are representative of a six core average per steak. [2]Color values are representative of a 10 d average. Values lacking a common superscript within a treatment differ.

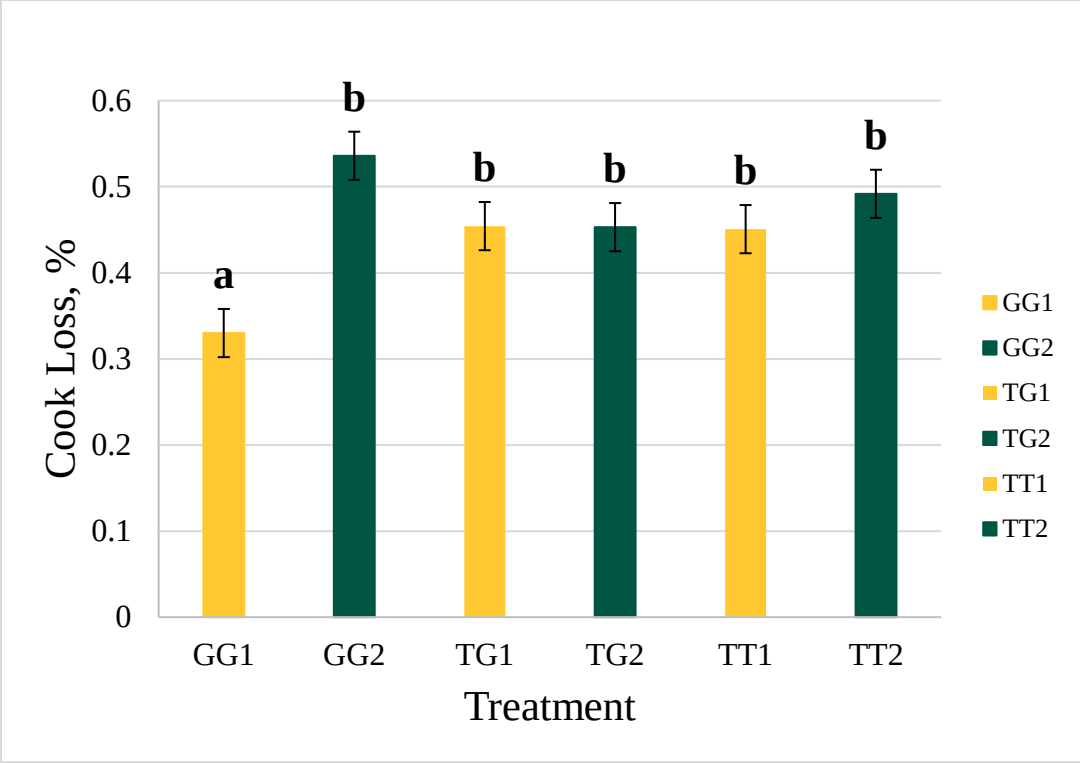


Figure 3.2. Effect of *GALR2c.-199T>G* genotype × implant strategy on cook loss percentage. Where initial weights were collected prior to cooking and final weights after steaks were cooked to 71 °C using a clam style grill and allowed to rest 7 minutes prior to re-weighing. Where GG, TG, and TT represent *GALR2c.-199T>G* genotype and 1 is the 1× implant of Revlor-S on d 77 and 2 is the 2× implant of Revalor-S on d 0 and d77 of the finishing period. Error bars depict the SEM. Bars not sharing a common superscript are significantly different (*GALR2c.-199T>G* genotype × implant strategy $P = 0.04$; Genotype $P = 0.66$; Implant $P = 0.01$). Geno. = *GALR2c.-199T>G* genotype, Imp. = Implant strategy.

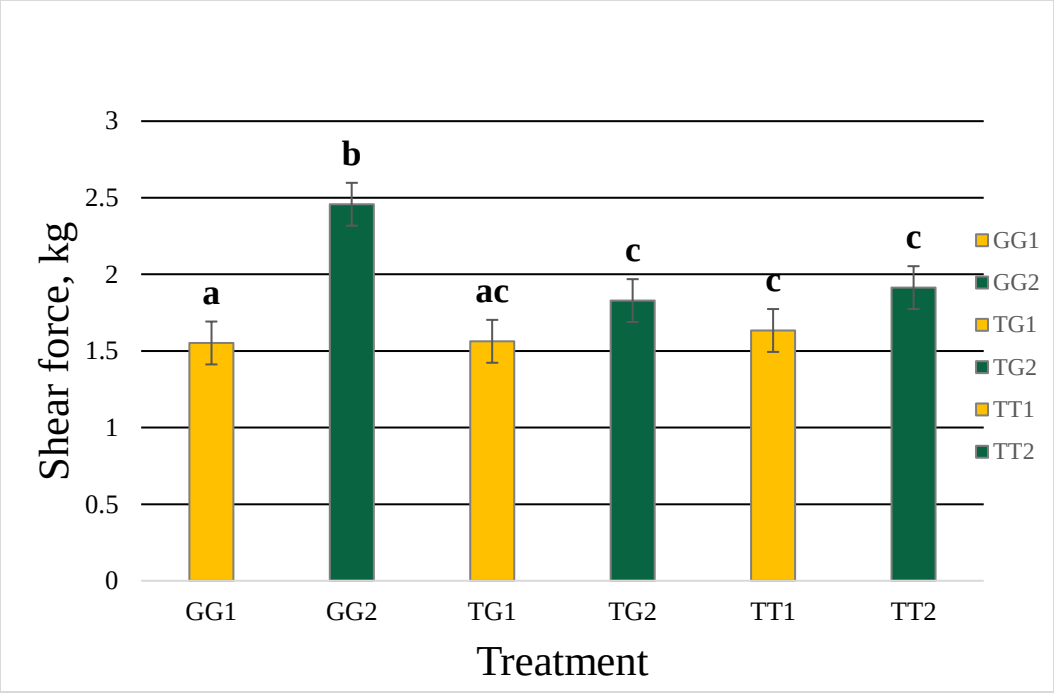


Figure 3.3. Effect of *GALR2c.-199T>G* genotype × implant strategy on Warner-Bratzler shear force measurement. Values are representative of a six core average. Where GG, TG, and TT represent *GALR2c.-199T>G* genotype and 1 is the 1× implant of Revlor-S on d 77 and 2 is the 2× implant of Revalor-S on d 0 and d77 of the finishing period. Error bars depict the SEM. Bars not sharing a common superscript are significantly different (*GALR2c.-199T>G* genotype × implant strategy $P = 0.0045$; Genotype $P = 0.01$; Implant $P < 0.0001$). Geno. = *GALR2c.-199T>G* genotype, Imp. = Implant strategy.

Discussion

When considering the profitability of beef, it is important to understand the role meat quality plays. Meat quality can have a two-fold effect of profitability both at the packing plant and at the retail case. At the packing plant, cattle can qualify for various branded beef programs and capture increased profits (Schroeder *et al*., 1997). While meat quality attributes, such as color and marbling, are indicators of an ensured superior eating experience for consumers based on previous purchases (Shackelford *et al*., 2001).

Although there was no effect of *GALR2c.-199T>G* genotype there was an effect of implant strategy on carcass characteristics. In contrast, a previous study by (Duncombe, 2016) reported the *GALR2c.-199T>G* genotype was found to elicit a significant effect on marbling and

back fat distribution for cattle possessing the TT genotype. Furthermore, the *GALR2c.-199T>G* genotype was also found to influence overall protein accretion with the GG genotype exhibiting increased LMA and heavier HCW over the TT cohorts. The difference in results could be attributed to a difference in sample size where the study by Duncombe (2016) had n = 2000 and this study has n = 91 additionally, environment during the feeding period varied in these studies. The effect of implant strategy led to an increase in HCW and decrease in back fat that are complementary to the previous work suggesting the *GALR2c.-199T>G* genotype does not have a negative influence on the added benefits of implants (Duncombe, 2016). Further work studying the effect of *GALR2c.-199T>G* genotype on estrogenic implants may be important to reaffirm this finding as this study focused on a combination implant as they are the most widely used by North American feedlots. Further research is needed to confirm the influence of the *GALR2c.-199T>G* genotype and implant strategies on carcass characteristics to determine if the *GALR2c.-199T>G* SNP is viable genetic marker for marker-assisted management or selection in feedlot cattle.

An evaluation of the effect of the *GALR2c.-199T>G* genotype × implant strategy on the meat quality attributes of pH and drip loss and genetic expression of *GALR2* in beef cattle is novel research to our knowledge. With no differences in ultimate pH, the lack of effect for drip loss aligns with the knowledge that rate and extent of pH decline are principle determinates of water holding capacity (Aberle et al., 1975).

For color values there was an effect of implant strategy on b* values or yellowness, with steers assigned to the 1× implant strategy exhibiting greater yellowness. This may indicate that implant strategy may alter the valance state of the iron within myoglobin. More work is needed to determine the influence implants have on color. Additionally color, was affected by day, the

greatest change was for a* values indicating a loss in redness overtime. Meat color is the considered the single most important quality trait of meat when determining point-of-sale for consumers (USDA FSIS, 2014). Continued exposure to lighting and contact of myoglobin and oxymyoglobin with oxygen leads to the formation of metmyoglobin resulting in the cherry red color being replaced for a browner color. Thus, seeing the most notable effect of d on a* values follows this pattern.

There was a significant effect of *GALR2c.-199T>G* genotype × implant strategy on shear force with the biggest difference in shear force and cook loss values being with the steaks from steers of the GG group with GG2× exhibiting greater cool loss and increased toughness in comparison to steaks from all other treatments. Anabolic growth promotants have been found to negatively affect eating satisfaction as supported by "tough" beef that is objectively determined by Warner-Bratzler shear force (Samber *et al*., 1996; Foutz *et al*., 1997; Morgan *et al*., 1997). A study conducted by Roeber et al. (2000) evaluated seven different implant strategies on tenderness and found a significant difference of implant on Warner-Bratzler shear force value. The difference was mainly driven by the control, no implant, to an implant strategy. In the current study, there was a significant interaction of *GALR2c.-199T>G* genotype × implant strategy on shear force. It may be worth further investigation of different implant strategies beyond Revalor-S on shear force values to determine any negative influences, especially within steers of the GG genotype.

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CHAPTER 4. GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

General Conclusions

With this study the overall objective was to examine the potential of the *GALR2c.-199T>G* genotype as a marker-assisted management strategy when implementing two differing implant strategies (1× vs. 2×) on crossbred Angus finishing steers.

From the feeding intake and behavior data it is evident that the *GALR2c.-199T>G* genotype had an effect on overall DMI during the finishing phase with the GG and TG steers consuming more feed. This is supported by the GH data as well with the GG steers having the lowest GH levels throughout the finishing phase. In addition, *GALR2c.-199T>G* genotype × implant strategy had an effect on IGF-1 levels with all steers assigned to the 2× implant strategy having elevated levels. Steers of the GG and TG genotype assigned to the 2× implant strategy have similar levels while of the steers assigned to the 1× implant strategy TT and TG steers have similar levels and TT 2× implant strategy steers have higher overall IGF-1 levels. Based on work done by Duncombe (2016) steers possessing the TT genotype have a greater presence of *GALR2*, with that in mind this data may suggest that a greater presence of *GALR2* increases the release of GH. While there is no effect of genotype on G: F there is an implant effect indicating the *GALR2c.-199T>G* genotype does not have a negative influence on the benefits of implants. Previous work by Duncombe (2016) show that cattle with the GG genotype possess a greater propensity for protein accretion with heavier HCW our data supports that this may be driven through increased DMI.

The interaction of *GALR2c.-199T>G* genotype × implant strategy had no effect on carcass characteristics leading to the conclusion that the *GALR2c.-199T>G* genotype does not negatively influence the effectiveness of implant strategies as there was an effect of implant on

HCW with the steers on the aggressive implant strategy yielding heavier HCW. The findings of this study are in opposition to that of Duncombe (2016) where there was an effect of *GALR2c.-199T>G* genotype on marbling score, REA, and HCW. These differences in findings could be due to a difference in sample size. In the Duncombe (2016) study there was an n = 2000 in comparison to this study with an n = 93. The larger sample size of the previous study may have allowed for some effects of the *GALR2c.-199T>G* genotype to be discerned that were not evident in the current study. When evaluating the influence of carcass quality the Duncombe (2016) study found increased marbling degree with cattle possessing the TT and TG genotypes. However, in this study we found a tendency for increased marbling degree for steers exhibiting the GG genotype.

For the meat quality evaluation there was a difference in color affected by d. This effect was within an expected range of change over the 10 days of the study for other shelf life studies. While there was no difference in drip loss there was a difference in cook loss with the GG genotype × aggressive implant steers having greater cook loss in comparison to all other steers. This difference in cook loss may help explain the interaction effect on shear force. The interaction of *GALR2c.-199T>G* genotype × implant strategy had an influence on shear force with GG steers with the aggressive implant strategy exhibited a greater shear force value than all other interactions. However, it should be noted that while there was an increased shear force value that all shear force values for each interaction effect fell below the perceived threshold of tenderness of 4 kg of force and thus no negative influence in tenderness is expected from the interaction of the *GALR2c.-199T>G* genotype × implant strategy. Because the binding of galanin to GALR2 leads to increased influx of calcium (Lang *et al*., 2007) within the body and cattle possessing the GG genotype may have a greater difference in shear force values due to the

activation of the calpain and calpastatin system that is largely responsible for the breakdown of proteins to determine tenderness.

It is thought that cattle possessing the GG genotype may exhibit increased galanin concentrations and increased *GALR2* expression. In this study, we investigated the differences in *GALR2* expression among genotypes within skeletal muscle. While we found no differences in *GALR2* expression based on genotype it may be worth investigation into gene expression levels and galanin protein concentration among other tissues. Galanin and *GALR2* expression is known to be most heavily concentrated in the hypo-pituitary axis within the brain as well as in the small intestine of the GI tract (Waters and Krause, 2000).

From this we conclude that the *GALR2c.-199T>G* SNP may serve as a good potential candidate to become a maker-assisted management strategy based on the ability to determine appetite. The *GALR2c.-199T>G* SNP had no adverse effects on production efficiencies, carcass characteristics or meat quality regardless of implant strategy used in finishing cattle. Further research is needed to validate the ability of the *GALR2c.-199T>G* SNP to predict carcass characteristics and meat quality in finishing cattle.

Future Directions

To date all the work with the *GALR2c.-199T>G* SNP has been done in *Bos tarus* and while *Bos tarus* make up a large majority of cattle in feedlots today it may be worth investigating if the *GALR2c.-199T>G* SNP exists in *Bos indicus* cattle as well to know if this is a SNP has the same effects on carcass characteristics and production efficiencies. Additionally, this study focused on the interaction of the *GALR2c.-199T>G* genotype with a combination implant (Revalor S, 120 mg trenbolone acetate and 24mg estradiol; Merk Animal Health). Investigating the interaction of the *GALR2c.-199T>G* genotype with estrogenic and androgenic implants

would be beneficial to demonstrating the role of the *GALR2c.-199T>G* SNP as a candidate to become a marker-assisted management strategy for finishing cattle.

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