THESIS

IMPACT OF GROWTH IMPLANTS AND TANNIN SUPPLEMENTATION ON ENTERIC METHANE EMISSIONS AND ESTIMATED NITROGEN EXCRETION IN GRAZING STOCKER STEERS

Submitted by

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ABSTRACT

IMPACT OF GROWTH IMPLANTS AND TANNIN SUPPLEMENTATION ON ENTERIC METHANE EMISSSIONS AND ESTIMATED NITROGEN EXCRETION IN GRAZING STOCKER STEERS

The objective of this experiment is to evaluate the effects of a growth-hormone implant (Revlor-G, Merck Animal Health., Rahway, NJ; 40 mg of trenbolone acetate and 8 mg of estradiol) and tannin supplementation (Silvafeed BX, Silva Team, San Michele Mondovi CN, Italy) on enteric methane (CH_4) emissions and estimated nitrogen (N) excretion in stocker cattle. Grazing stocker steers (n = 20; initial BW = 343 ± 14 kg) were trained for three weeks to use a portable automated head-chamber system (AHCS; C-Lock Inc., Rapid City, SD) and SmartFeed Pro automated feeder (C-Lock Inc., Rapid City, SD) for dietary supplementation. After the training period, steers were randomly assigned to one of four treatments: 1) no tannin and no implant (Control [CON]); 2) tannin supplement and no implant (Tannin [TAN]); 3) implant and no tannin (Implant [IMP]); and 4) tannin supplement and implant (Implant + Tannin [IMP + TAN]). The tannin was offered at 0.30% DM tannin intake through 0.5 kg/hd/d sweetfeed mix (Sweetfeed Mix, AgFinity., Eaton, CO). Treatment groups without tannin (Control and Implant) received the same sweetfeed mix ration at 0.5 kg/hd/d without the tannin supplementation. Daily forage intake was estimated using the NRC (1996) forage intake prediction equation. Total intake included the estimated forage, bait (alfalfa pellets from AHCS), and sweetfeed mix. Across the experiment, no animal consistently consumed all 0.5 kg/hd/d of the offered sweetfeed mix. On average, the CON cattle consumed 0.32 kg/hd/d, the TAN group

consumed 0.41 kg/hd/d, the IMP cattle consumed 0.44 kg/hd/d, and the IMP + TAN group consumed 0.36 kg/hd/d. Moreover, the lack of a tannin x implant interaction (two-way ANOVA; P=0.24) also suggested sweetfeed mix intake did not depend on either treatment level. In response, we evaluated the effect of tannin supplementation and a growth-promoting implant in a separate analysis and data were analyzed with treatment levels as follows: I1) NO-IMP: All animals that did not receive growth implant; I2) IMP: All animals that did receive growth implant; T1) NO-TAN: All animals that did not receive tannin supplement; T2) TAN: All animals that did receive tannin supplement. The sample size for the evaluation of the tannin effect included: NO-TAN (n = 9; 5 animals were implanted with growth promotant) and TAN (n = 9; 5 animals were implanted with growth promotant), while the growth implant effect included: NO-IMP (n = 8; 4 animals were supplemented tannin) and IMP (n = 10; 5 animals were supplemented tannin). Supplementation with tannin did not impact, animal performance metrics (initial body weight, final body weight, and ADG) across the entire study or within early or late study periods ($P \geq 0.33$). Steers supplemented with the NO-TAN supplement tended ($P \geq 0.10$) to have greater dry matter intake (DMI) and less CH_4 yield (MY) compared to cattle supplemented with TAN. There was no effect of tannin supplementation on enteric CH_4 production (g/d; P = 0.24) and EI (P = 0.23). N utilization as measured through blood urea nitrogen (BUN), urine N, fecal N, or fecal P was not different among TAN and NO-TAN animals ($P \geq 0.12$). Growth-promoting implants did not affect initial body weight (P = 0.86) or final body weight (P = 0.51). There was no effect of growth hormone implant on average daily gain (ADG) during the 90-d of the study (P = 0.80). However, IMP steers tended (P = 0.10) to have greater ADG during the first half of the study (d 0 to 45). Implanted steers also had greater forage (P = 0.05) and bait intake (P = 0.02), and numerically greater total DMI (P = 0.13) over

the 90-d study. For IMP steers, there was no effect ($P > 0.19$) of growth implant on methane (CH_4) production or emission intensity (EI; g CH_4/kg gain) during the 90-d study. However, IMP steers had decreased ($P = 0.03$) EI during the first period. Additionally, the IMP steers tended to have less CH_4 yield (MY; g CH_4/g DMI, $P = 0.09$) and BUN ($P = 0.08$) than NO-IMP steers. There was no growth-promoting implant effect ($P > 0.30$) on cattle urine and fecal N, creatinine, or fecal P. In summary, supplementing tannin in the diet of grazing stocker steers tended to reduce total estimated DMI but did not affect enteric CH_4 emissions compared to steers that received no tannin supplement. Implanting steers with Revalor-G tended to 1) increase total DMI in the 90 d study, 2) increase ADG in the early period (d 0 to 45) and 3) decrease CH_4 EI in the first 45 d post-implantation.

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CHAPTER 1 − REVIEW OF LITERATURE

INTRODUCTION

The World Commission on Environment and Development (1987) broadly defined sustainable development, as a development that refers to the use of resources, plants and animals, to produce food and fiber without damaging the natural resource base. This needs to be accomplished while also meeting the basic needs of producers and consumers long-term (Smit et al., 1996). Sustainable agriculture has gained much attention and agriculturists and scientists have devoted efforts into better defining sustainable development in agriculture. The goal of sustainable agriculture today - to meet society's food and material needs without compromising the ability of forthcoming generations to meet their own needs - is similar to the goal in 1987 (UC SAREP, 2021). Moreover, sustainable agriculture not only focuses on preserving natural resources and altering production practices but also encompasses a dedication to transforming public policies, economic institutions, and social values (UC SAREP, 2021). In 2013, the United Nations General Assembly set up a 30-member working group to develop a 2030 agenda for sustainable development that included 17 sustainable development goals (UNSDGs). Today, the Division of Sustainable Development and the United Nations Department of Economic and Social Affairs provides support for the UNSDGs and releases an annual progress report (*The 17 goals, sustainable development).* Furthermore, government agencies, scientists, producers, and consumers have all taken interest in finding more sustainable solutions for agriculture.

Greenhouse Gases

Greenhouse gases (GHG) are gas molecules in the atmosphere that warm the Earth by absorbing and slowing the rate at which the energy escapes to space (EPA, 2021). Most GHG are naturally occurring and would exist without humans on the Earth, just not in the large proportions of the atmosphere that they occupy today (EPA, 2021). The four most abundant

substances are: water vapor, carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (EPA, 2021). The three main gases that are emitted at the largest quantities are CO_2, CH_4, and N_2O (78.8%, 10.9%, and 7.1% respectively), mostly due to human activities (EPA, 2021). Not all GHGs are equal; they differ in their radiative efficiency and how long they stay in the atmosphere (EPA, 2021). Parts of the Earth's atmosphere act as a shielding blanket of just the right thickness, receiving appropriate solar energy to keep the global average temperature suitable for our kind of life (Darkwa et al., 2018). This 'blanket' is a collection of GHGs that capture heat (Darkwa et al., 2018). Greenhouse gases do this by trapping some of the Earth's outgoing energy, thus retaining heat in the atmosphere. When sunlight reaches the Earth's surface, some is absorbed and some reflects back to space as heat (Darkwa et al., 2018). Greenhouse gases in the atmosphere absorb that heat and then transmit some of the heat back toward the Earth and are crucial to keeping our planet at a suitable temperature for life (Darkwa et al., 2018). Natural factors, such as variations in solar radiation, volcanic activity, the Earth's orbit, the carbon cycle, and others, affect the Earth's radiative balance (EPA, 2021). However, human activities have been the primary cause of global warming the past 50 years. Nonetheless, without the natural greenhouse effect, the heat emitted by the Earth would simply pass outward from the Earth's surface into space and Earth would have an average temperature of about -20^{o}C. Agriculture contributes to the increase of anthropogenic CO_2, CH_4, and N_2O emissions through enteric fermentation, manure management, soil management, etc. (Figure 1; EPA, 2021).

All gases categorized as GHG have a global warming potential (GWP) value (Harvey, 1993). The GWP concept was developed to allow comparisons of the global warming impact of different gases and the value measures how effective each gas is at trapping heat in the atmosphere. The GWP of a GHG is defined as "the ratio of the accumulated radiative forcing

within a specific time horizon caused by emitting 1 kilogram of the gas, relative to that of the referenced gas CO_2" (IPCC 2013). Therefore, GWP-weighted emissions are provided in million metric ton of CO_2-equivalents (MMT CO_2 Eq.) (EPA, 2021). The GWP-weighted values allow us to gain a deeper understanding of how each gas contributes to the warming of the planet. Different metrics to quantify GHG emissions exist, with most using CO_2 as the base comparison gas for all other GHG (EPA, 2021). The most common metric is the standard 100-year GWP (GWP_{100}) (EPA, 2021), which evaluates the GWP of the GHG over 100 years (EPA, 2021). Carbon dioxide has a GWP of 1 because it is the gas being used as a reference (EPA, 2021). Methane has a GWP of 25, meaning 1 ton of CH_4 equals 25 tons of CO2 and thus captures more heat per molecule than CO_2 (EPA, 2021). Nitrous oxide has a GWP of 298.

New research has been evaluating GWP_{100}. GWP_{100} assumes that all GHG are stagnant in the atmosphere. Yet, CH_4 is a short-lived gas and breaks down in 10-12 years (EPA, 2021). While GWP_{100} does consider short-lived gases (i.e., CH_4), it does not account for their removal from the atmosphere. Therefore, previous CO_2-equivalents using GWP_{100} overestimate the effects of CH_4 on global warming temperatures (Allen et al., 2018). If short-lived gases, such as CH_4, stay at the same level in the atmosphere there should be no additional warming, but CO_2, a long-lived gas that takes hundreds of years to break down would continue to have a warming effect because the gas is building over time (Allen et. al., 2018). So, if CH_4 emissions decrease, a cooling effect will be included because CH_4, a more potent gas (GWP of 25), is being removed from the atmosphere (Allen et. al., 2018).

Cain et al. (2019) has suggested the new metric of GWP, denoted GWP* (GWP star), which represents a new approach to measuring carbon in the atmosphere, taking short-lived gas removal from the atmosphere into consideration (Cain et al., 2019; Allen et al., 2018; Liu et al.,

2021). This metric can be used to guide climate action aligned with temperature-based climate stabilization goals (Ridoutt et al., 2021). The new metric (GWP*) should be combined with GWP to work toward feasible strategies for combating climate change induced by short-lived climate pollutants (Liu et al., 2021). The GWP values allow policymakers to compare the impacts of emissions and reductions of different gases. However, policy currently does not take GWP* into consideration over GWP_{100}.

GHG and NH_3 emissions that derived from animal agriculture

In 2020, the agriculture sector was responsible for 11% of U.S. GHG emissions (CO_2-Eq) (EPA 2021). Of that 11%, N_2O was responsible for 57% of GHG emissions from the agriculture sector, CH_4 was responsible for 42%, and CO_2 was responsible for 1% (CO_2-Eq) (EPA 2021). These emissions come from multiple different agriculture activities. Primary GHG-emitting agricultural activities are soil management, enteric fermentation, and manure management, responsible for 53, 30, and 13%, respectively (EPA 2021). Other agriculture activities that contributed less than 3% to GHG emissions (CO_2-Eq) in 2020 were rice cultivation, urea fertilization, liming, and field burning of agricultural residues (EPA 2021). Ammonia (NH_3) emissions also need to be taken into consideration; in the U.S., 80% of total NH_3 emissions come from agriculture, and 58% of total U.S. NH_3 emissions come from animal manure.

In 2020, the livestock sector specifically accounted for 3.8% of U.S. GHG emissions (EPA 2021). The livestock industry alone accounted for 40% of the agriculture U.S. GHG emissions in 2020 (EPA 2021). The GHG emissions from the livestock sector are generated directly and indirectly from enteric fermentation and manure management, agriculture soil management, and converting forest land to agricultural land (EPA, 2021).

The livestock sector accounts for multiple farm animal species including cattle, swine, and poultry. Each species contributes to climate change differently. For example, in 2020, 72% (125.3 MMT CO_2 Eq) of total CH_4 emissions from enteric fermentation were from beef cattle (EPA, 2021). Dairy also contributes to livestock sector CH_4 emissions, yet only emitting 25% (43.6 MMT CO_2 Eq.) of CH_4 through enteric fermentation (EPA, 2021). Beef cattle continue to be the largest contributor to CH_4 emissions from enteric fermentation (EPA, 2021). However, the roles are reversed when evaluating CH_4 emissions derived from manure management. In 2020, beef cattle contributed only 3% of CH_4 emissions from manure management, whereas dairy cattle and swine contributed 53 and 38%, respectively (EPA, 2021). The N_2O emissions from manure management in 2020 are similar for beef and dairy cattle at 48% and 31%, respectively (EPA, 2021).

Nonetheless, with the world population projected to reach 9.7 billion in 2050 and 10.4 billion in 2100 (United Nations, 2022), the need for increased food production is at an all-time high. This increase also must be accomplished on less land while reducing environmental impact. The importance of research to reduce GHGs was only further encouraged when President Biden unveiled the U.S. Methane Emissions Reduction Action Plan to dramatically reduce U.S. CH_4 emissions (US EPA, 2022). This plan encompasses documenting baseline CH_4 emissions, cutting consumer costs, protecting workers and communities, creating job opportunities, and promoting U.S. innovation and manufacturing of critical new technologies.

BEEF CATTLE AND THEIR IMPACT ON CLIMATE CHANGE

Beef cattle are normally produced in three phases: cow-calf, stocker or backgrounder, and finishing. The cow-calf production stage is the longest, with cattle staying in this segment until 3-7 months of age (USDA-ERS, 2023) and weighing between 204 and 317 kg. Cattle then

transition into the stocker cattle phase for approximately 3 to 4 months (USDA-ERS., 2023) until they reach 204 to 408 kg. Cattle end their lifecycle in the finishing phase for 90 to 300 days (USDA-ERS., 2023), where cattle finish from 590 to 816 kg. In total, cattle spend ~80% of their lifetime in the cow-calf and stocker phase of production.

Cattle are ruminant animals, and CH_4 is produced as a normal digestive process. During the digestive process, microbes in the ruminant digestive system, ferment feed consumed by the animal. This fermentation process is also known as enteric fermentation and produces CH_4 as a byproduct, which the animal eructates. Rotz et al (2019) conducted a beef cattle production lifecycle assessment (LCA) with data from 150 representative beef cattle operations across seven different regions to quantify beef's impact on climate change. For individual beef cattle production systems, the total carbon footprint ranged from 17 to 40 kg CO_2e/kg carcass weight (CW) and the mean GHG emission intensity of all traditional beef cattle production systems in each region ranged from 20.2 to 28.9 kg CO_2e/kg CW (Rotz et al., 2019). The Rotz et al (2019) LCA indicated that the feed needed to produce 1 kg carcass CW of beef was about 22 kg of dry matter (DM).

Important emissions in beef cattle are NH_3, CH_4, and N_2O. Ammonia occurs in all phases of production and is emitted from the urine and feces. The NH_3 that comes from urine and feces, 35 to 43% is from the finishing phase, and about 44 to 50% is in the cow-calf phase (Rotz et al., 2015, 2019). In the U.S., 34% of NH_3 emissions originate from beef cattle production (EPA, 2014). On average, beef cattle produce 482 g CH_4 per kg of CW through all phases of production (Rotz et al., 2019), whereas N_2O is primarily produced through the nitrification and denitrification process following urine deposition or fertilizer application(Rotz et al., 2019). On average, 19.9 g of N_2O per kg of CW is produced in beef cattle production (Rotz et al., 2019).

Previous life cycle assessments (LCAs) show that 70 to 80% of total GHG emissions from the US beef sector are contributed by grazing cattle, more specifically, cow-calf and stocker cattle, and are made up predominately of CH_4 (Beauchemin et al., 2010; Rotz et al. 2015, 2019). Specifically, grazing cattle contribute 89% of CH_4 emissions, 83% of N_2O emissions, and 64% of NH_3 emissions (Rotz et al., 2015, 2019). An LCA conducted by Beauchemin et al (2010) reported that grazing cattle contributed 86% of CH_4 emissions and ~ 20% of N_2O emissions in beef cattle production in western Canada. Other LCAs suggest the majority of CH_4 emissions are also attributed to grazing cattle (Pelletier et al., 2010; Asem-Hiablie et al., 2019). These large percentages are due to cattle intended for food production spending 80% of their lifecycle in a grazing system and cattle intended for breeding spending ~8-12 years maintained mainly on forage diets (USDA-ERS., 2023). Although there is value in cattle being able to convert non-usable land into a quality protein source, the length of beef production that cattle spend grazing or fed high forage diets is what contributes to the increase in CH_4 emissions from enteric fermentation.

Enteric CH_4 is a natural by-product of the anaerobic fermentation process in the rumen and hindgut of ruminants. Ruminants have four compartments in their stomach: the rumen, the reticulum, the omasum, and the abomasum. The rumen is a complex and diverse microbial ecosystem that operates primarily as an obligate anaerobic environment, facilitating the conversion of feedstuffs, including plants, into energy for the animal (Patra, 2012). This is done when ruminal bacteria, protozoa, and methanogens ferment these feedstuffs to short-chain volatile fatty acids, CO_2, H_2, and CH_4 (Li et al., 2018). The end product of their digestion is microbial cell protein and volatile fatty acids (VFA; acetate, butyrate, and propionate) that the host uses to meet its metabolic needs (Krehbiel, 2014; Thompson and Rowntree, 2020). This

process has allowed ruminant animals to thrive across biomes that other mammals cannot (Thompson and Rowntree, 2020). However, in addition to the VFA and protein production during fermentation, gaseous CO_2 and H_2 are produced. This product serves as the primary substrate for methanogens to produce CH_4. This process of cellular respiration by methanogenesis uses H_2 to make CH_4 and H_2O, which helps prevent H_2 from accumulating in the reticulo-rumen, which is crucial for healthy ruminal fermentation (Thompson and Rowntree, 2020).

Importance of grazing systems

The ability of ruminants to convert complex carbohydrates with high fiber content on untillable land into useable end products such as meat or milk is a unique advantage that other mammals do not have (Gerber et al., 2015; Carvalho et al., 2018; Thompson and Rowntree 2020). The U.S. land area total is ~2.3 billion acres (USDA-ERS., 2017). In the U.S., pasture and rangeland occupy 655 million acres, grazed forestland occupies another 130 million acres, and cropland pasture occupies a varied small amount (USDA-ERS., 2017). Cattle in grazing systems can be developed in harsh environments, such as dry lands and cold areas, and these systems are often mobile to use sparse and erratic resources (Gerber et al., 2015). This characteristic allows cattle to produce meat or milk in many versatile conditions. Managed grazing systems are the most extensive form of land use on the plant (Asner et al., 2004). For producers who manage livestock operations, prescribed grazing systems offer an effective way to reduce energy use, decrease costs, and improve animal health and productivity (USDA-ERS., 2017).

Furthermore, grazing systems on rangelands, pastures, and grasslands impact carbon sequestration (Follett and Reed, 2010). There is variability of how much and what kind of impact that grazing systems have on carbon sequestration (Follett and Reed, 2010). This variability is

attributed to differences in specific grazing management practices, such as the number of grazing animals per acre, fertilization, prior land use, and plant communities (Follett and Reed, 2010). However, an analysis of 115 grazing systems indicated that the soil carbon levels increase with improved management (i.e., fertilizing grazing environment, grazing intensity) (Conant et al., 2001). Managed grazing systems add value to the fight against climate change by positively impacting carbon sequestration.

DIFFERENT MEASUREMENT AND ESTIMATION METHODS OF ENTERIC CH_4

Although grazing systems provide a wide array of goods and services, grazing beef cattle still contribute to the U.S. GHG emissions. Methane emissions make up almost half of all GHG emissions in animal agriculture (EPA, 2021); therefore, a need to find solutions to reduce CH_4 emissions has proliferated. Enteric fermentation is responsible for the largest portion of CH_4 emissions (EPA, 2021); therefore, scientists are providing research on alternative strategies to reduce enteric CH_4 (The 17 Goals., 2013). However, scientists first need accurate CH_4 measurement systems to make progress toward reducing enteric CH_4 emissions in beef cattle. The previous gold standard for measuring enteric CH_4 from an animal was the respiration chamber and, similarly, the head-box method (e.g., Johnson and Johnson, 1995). Today, advanced tools such as the automated head chamber system (AHCS) allow scientists to measure enteric CH_4 emissions in the animal's natural environment.

Respiration chambers or open-circuit direct calorimetry chambers have been considered the 'gold standard' for many years because they are used to develop predictive models and equations to estimate ruminant livestock emissions for national GHG inventories (Hill et al., 2016). These methods allow for the measurement of everything that the animal emits, including the production of CH_4, gaseous exchanges (O_2 consumption and CO_2 production), other tracer

gases, and quantification of heat production. Enteric CH_4 production is quantified by multiplying airflow through the chamber by the difference in CH_4 concentration in and out of the chamber, measured by a gas analyzer (Hill et al., 2016). Additionally, total tract digestibility and total urine collections can be collected because the animal is confined in an enclosed area. For these reasons, an accurate assumption of emissions and digestibility from the animal can be collected. However, this measurement system comes with criticisms. The animal is often fed at maintenance, measurements are taken over short intervals, and their eating behavior is limited by stress and does not reflect a free-ranging pattern (Hill et al., 2016).

Tracer gases have also been used to measure CH_4 emissions in livestock. This method is based on constant release of sulfur hexafluoride (SF_6) tracer gas in the rumen. It has gained interest over the respiration chamber in grazing ruminants because it can be used when animals are grazing freely (Hill et al., 2016). However, to ensure that a gaseous tracer is suitable for the estimation of CH_4, the difference between background concentrations and those released must be as large as possible, which is a quality the SF_6 provides (Hill et al., 2016). Sulfur hexafluoride is a gas that is easily measurable and traceable at low concentrations, it is synthetic and not produced as part of any biological process, and its background concentration is naturally low (6 pmol/day) (Hill et al., 2016). However, this technique requires special analytical skills and equipment, and SF_6 is also a potent GHG categorized under fluorinated gases with a GWP of 22,800 (Vlaming et al., 2007).

The AHCS (GreenFeed, C-Lock, Inc., Rapid City, SD; Figure 2) was developed using spot measurements to estimate CH_4 production (Hristov et al., 2015; Gunter and Beck, 2018). The AHCS is a system used to measure CH_4, CO_2, and H_2 emissions and O_2 consumption from the breath of ruminant animals. Attached to the AHCS is a feed bin that drops small quantities of

baiting feed to individual animals to lure them. The animal can visit the AHCS multiple times daily. With each visit, the animal is allotted small quantities of bait at 30 second intervals to keep the animal occupied while measurement occurs.

When the animal approaches the AHCS, an arrangement of fans draws air over the animal's head and into an intake manifold, proceeding through an air collection pipe where the constant airflow rates are assessed (Hristov et al., 2015). Nondispersive near-infrared gas analyzers are employed to examine a subsample for concentrations of CO_2 and CH_4 (Hristov et al., 2015). These results are then compared with background gas concentrations before the animal entered the AHCS to determine gas emission rates. An average of all the spot estimates over the course of the sampling period is used to estimate each animal's daily gas production (Gunter and Beck, 2018). Several factors could affect emission estimates including the animal's visitation rate, length of sampling period, and airflow through the system (Gunter and Beck., 2018). To try to limit these factors, cattle require four weeks (up to 8 weeks of training may be necessary) to acclimate to the system (Gunter and Beck, 2018). In addition, the AHCS is less expensive than the respiration chamber method and requires less labor than the SF_6 method, and larger sample sizes are possible compared with other sampling techniques (Hristov et al., 2015).

METHANE AND NITROGEN MITIGATION STRATEGIES

There is a pressing matter of reducing CH_4 emissions in ruminant livestock; therefore, finding strategies to mitigate CH_4 is of the utmost importance. Different strategies have been suggested in ruminants and have been focused on obtaining economic and environmental benefits. Mitigation strategies such as increasing the level of grain in the diet, increasing rate of gain to lower emission intensity (EI; g CH_4/kg BW gain), inclusion of lipids, and the inclusion of ionophores have been discussed, and these strategies also have benefits for the producer because

they show increased efficiency (Beauchemin et al., 2008; Hristov et al., 2013) Furthermore, strategies like pasture management, leguminous forages containing tannins, and using legumes hold some promise in CH_4 mitigation (Beauchemin et al., 2008; Hristov et al., 2013; Thompson and Rowntree., 2020; Archimede et al., 2011) but further evaluation is needed. There are also numerous new strategies, including dietary supplementation with condensed and hydrolysable tannins, direct fed microbials (yeast products), the use of implants, and genetic selection of low CH_4-producing animals. However, these still require extensive research (Beauchemin et al., 2008, Hristov et al., 2013) Lastly, the thought of stacking previously stated technologies could have potential for further reduction but has not been explored.

Ionophores

Ionophores are antimicrobials that are typically used to moderate intake, control bloat, and improve efficacy in beef and dairy cattle production (McGuffey et al., 2001). Monensin is the most studied ionophore and allows cattle to gain faster, consume less feed, and require less feed to gain weight (Goodrich et al., 1984; Perry et al., 1976; Potter et al., 1976). Monensin has also been evaluated as a potential CH_4 mitigation strategy. A study by Thompson et al. (2019) showed that feeding an energy supplement with monensin to stocker steers grazing winter wheat increased supplement intake and reduced CH_4 emission intensity (g of CH_4/kg of BW gain; $P<0.03$). In a meta-analysis of 22 controlled studies (dairy and beef), Ranga Niroshan Appuhamy et al. (2013) found that monensin reduced the percentage of dietary gross energy lost as CH_4 from 5.97 to 5.43% and diets with greater neutral detergent fiber (NDF) contents (g/kg of DM) tended to enhance the monensin effect on CH_4 in beef steers. When adjusted for the NDF effect, monensin supplementation (32 mg/kg of DMI) reduced CH_4 emissions from beef steers fed a total mixed rations by 19±4 g/animal/d (Ranga Niroshan Appuhamy et al., 2013). Ranga

Niroshan Appuhamy et al. (2013) reported that for dairy cattle supplemented with monensin (avg 21 mg/kg of DMI) CH_4 emissions were reduced by 6±3 g/animal/d. The meta-analysis concluded that monensin had stronger antimethanogenic effect in beef steers than dairy cows mostly fed forage-based diets (Ranga Niroshan Appuhamy et al., 2013). In conclusion, ionophores have the potential to decrease CH_4 emission intensity by increasing weight of gain and decrease CH_4 yield by incorporating feedstuffs with greater NDF contents to enhance the monensin effect.

Dietary lipids

The addition of lipids (vegetable oil or animal fat) in the diet is one of the dietary options recognized to decrease enteric CH_4 emissions (Johnson and Johnson, 1995; Beauchemin et al., 2008; Thompson and Rowntree, 2020; Hristov et al., 2013), although results are not always conclusive or consistent. Fats are often utilized to increase the energy density of diets and also reduce CH_4 emissions by decreasing ruminal organic matter fermentation, the activity of methanogens and protozoal numbers, and for lipids rich in unsaturated fatty acids, through hydrogenation of fatty acids (Johnson and Johnson, 1995). Eugène et al. (2008) conducted a meta-analysis evaluating 25 diets across seven scientific publications to determine the effects of lipid supplementation on CH_4 production, milk production, and milk composition of lactating dairy cows. They reported that lipid supplementation decreased CH_4 production by 9%, but at the same time, found that lipid supplementation decreased DMI by 6.4% (Eugène et al., 2008). Another meta-analysis of 38 articles evaluating five groups of fat (tallows, calcium salts of palm fat, oilseeds, prilled fat, and other calcium salts) reported a consistent decrease in DMI; however, milk production was increased (Rabiee et al., 2012). Moate et al. (2011) reported that fat supplementation reduced CH_4 emissions. Similarly, a meta-analysis using data from 27 studies reported that a 10g/kg increase in dietary fat decreased CH_4 yield by 1g/kg DMI (Grainger and

Beauchemin, 2011). Reductions of CH_4 are possible with high levels of lipid supplementations, but it is recommended total fat should not exceed 6-7% for dietary DM; otherwise, a decrease in DMI may occur (Beauchemin et al., 2008).

Direct-Fed Microbials

Direct fed microbials (DFM) are dietary supplements that inhibit gastrointestinal infection. Direct fed microbials optimize microbial environments in the digestive tract. One of the more common DFMs used in ruminant nutrition are yeast-based products. The yeast culture *Saccharomyces cervisiae* is widely used in ruminant diets. Mutsvangwa et al. (1992) found that in bulls receiving a yeast culture composed of *S. cerevisiae* the mean in vitro gas production was lower ($P < 0.05$) and CH_4 production was significantly reduced after 12h ($P < 0.01$) but not 24h incubation. Another study by Sullivan and Martin (1999) failed to find a commercial yeast culture (XP Yeast, Diamond V) effect on CH_4 production over 24 h with ground corn as a substrate or over 48 h with alfalfa or Bermuda grass as substrates. Sullivan and Martin (1999) also investigated the effects of *S. cervisiae* culture and monensin on the mixed ruminal microorganism fermentation. They found that monensin altered the fermentation by decreasing concentrations of CH_4 and lactate and increasing concentrations of propionate, but there was no interaction between *S. cervisiae* culture and monensin. Newbold and Rode (2006) discuss using yeast products to mitigate CH_4 production, but more research is needed.

Inhibitors

Research in this area has targeted chemical compounds with a specific inhibitory effect on rumen archaea. Bromochloromethane, 2-bromoethane sulfonate, chloroform, and cyclodextrin are among the most successful compounds tested. Research by Knight et al. (2011) fed chloroform, a known methanogenesis inhibitor, in dry cows and found an immediate and

dramatic decrease in CH_4 emissions and methanogen numbers. However, CH_4 production gradually increased to about 62% of pre-treatment numbers by d 42, suggesting the rumen ecosystem adapted to the chloroform. Another study by Abecia et al. (2012) treated dairy goats with bromochloromethane (BMC). Bromochloromethane is a halogenated aliphatic hydrocarbon with potential antimethanogenic activity by reacting with cobalamin. Their results showed reduced CH_4 production by 33%. Tomkins et al. (2009) also used a BMC formulate to evaluate its effect on enteric methanogenesis in cattle fed grain-based diets. Through three experiments, they found that methane emissions were substantially reduced over the 90-day feedlot finishing period in Brahman (*Bos indicus*) cattle (Tomkins et al., 2009). Although this banned compound cannot be recommended as a CH_4 mitigation agent, alternative compounds with similar modes of action could be developed. The long-term effect of CH_4 inhibitors in beef production systems is still unknown and needs further study.

Feed Intake and Type of Feed

Feed intake is a critical component that affects CH_4 emissions. Johnson and Johnson (1995) stated that as the daily feed consumed increases, the dietary gross energy lost as CH_4 decreases by an average of 1.6% per level of intake. Restricting intake has been reported to reduce CH_4 emissions by Beauchemin and McGinn (2006), Molano and Clark (2008), and Basarab et al. (2013). Beauchemin and McGuinn (2006) reported that the reduction in CH_4 is proportional to the decline in intake.

The type of feed is also necessary to consider with regard to CH_4 emissions. Benchaar et al. (2001) used a modified version of a mechanistic and dynamic model of rumen digestion and found that, depending on the nature of the nutritional strategy, CH_4 production can be reduced by 10 to 40%. They stated that DMI and the proportion of concentrate in the diet reduced CH_4

production by 7 and 40%, respectively. Further, replacement of fibrous concentrate with starchy concentrate decreased CH_4 production by 22%. Feeding highly fermentable carbohydrates results in lower enteric CH_4 production per unit of DM consumed (Johnson and Johnson, 1995). These carbohydrates influence CH_4 production by shifting microbial populations to favor propionate production and increase ruminal rate of passage (Johnson and Johnson, 1995; Beauchemin et al., 2008;Thompson and Rowntree, 2020;). However, as previously stated, cattle (ruminants) can uniquely convert complex carbohydrates with high fiber content to usable end products. Thus, questions remain on how to utilize soil-plant-animal interrelationships best to meet productivity goals without impacting the climate (Tilman et al., 2011).

Forage Type and Grazing Management

Improved grazing management, the type of forage being grazed, the stage of forage maturity, and supplementing low-quality forage with energy and protein have all been noted as potential strategies to reduce CH_4 production (Benchaar et al., 2001; Beauchemin et al., 2008; Hristov et al., 2013; Thompson and Rowntree, 2020). Improving pasture management and quality is considered a mitigation strategy by means of enhancing animal productivity (lowering CH_4 emissions per unit of animal product); however, it has yet to be demonstrated that improving pasture management of management-intensive improved pastures can reduce CH_4 emissions (Beauchemin et al., 2008).

Improving pasture quality can improve dietary digestibility and, in turn, results in less enteric CH_4 produced (Beauchemin et al., 2008; Archimede et al., 2011; Hristov et al., 2013; Thompson and Rowntree, 2020). Benchaar et al. (2001) found that using more digestible forage (less mature and processed forage) resulted in a 15% and 21% reduction in CH_4 production, respectively. In addition, CH_4 production was lower with legume than compared to grass forage

(28%) and silage compared to hay (20%). Beauchemin et al. (2008) stated that feeding forage legumes could lower CH_4 production in cattle compared to animals fed predominantly grasses. This reduction in CH_4 production could be explained by the presence of condensed tannins, lower fiber content, and/or a faster rate of passage in the rumen (Carulla et al., 2005; Beauchemin et al., 2007; Thompson and Rowntree, 2020). Chaves et al. (2006) reported that DMI was 20% higher and CH_4 production per unit of DMI was 39% lower when grazing grass compared to grazing alfalfa. The decreased DMI associated with grazing alfalfa could be because of the advanced maturity of the alfalfa. Archimède et al. (2011) conducted a meta-analysis (with 112 observations) examining CH_4 production in ruminants comparing the effects of C_4 (4 carbon, warm season, lower quality grasses) and C_3 (3 carbon, cool season/yearlong, higher quality grasses) grasses and warm and cold climate legumes. The analysis results indicated that ruminants fed C_4 grass produced 17% more CH_4 (per kg of organic matter intake) than those fed C_3 grass and 20% more than animals fed warm climate legumes. The C_3 grasses, or grasses that use C_3 photosynthetic pathway, are normally considered higher quality than C_4 grasses because they are typically lower in fiber, have a decreased amount of lignin production, and have greater protein contents (Thompson and Rowntree, 2020). The decreased fiber content in the diet resulted in lower enteric CH_4 production values (Barbehenn et al., 2004; Thompson and Rowntree, 2020). A reduction in CH_4 emissions is often related to greater nutrient quality and digestibility, two characteristics for which forage type and maturity might be indicators.

There has been much discussion on continuous or rotational grazing systems and their effect on long- and short-term effects on enteric CH_4 production, most of which is inconsistent (DeRamus et al., 2003; Alcock and Hegarty., 2006; Savian et al., 2018). DeRamus et al. (2003) evaluated the effects of management-intensive grazing compared with continuous grazing. They

found that the intensive grazing system significantly reduced the emission of CH_4 per unit of animal weight gain. The authors state the management-intensive grazing offers the potential for more efficient utilization of grazed forage crops than continuous grazing. However, Savian et al. (2014) reported that continuous stocking was the most efficient grazing management in reducing CH_4 emissions per unit animal production compared with a rotational system. Savian et al. (2018) found that in rotational grazing systems where the pre- and post-grazing sward target heights were 18 and 11 cm, there was a 64% reduction in CH_4 per area and a 170% reduction in CH_4 emission per unit of animal product when compared to traditional rotational grazing strategies. Improved grazing management, such as allowing forage to reach 18 cm in length, can impact soil carbon sequestration, offsetting animal emissions (Savian et al., 2018). Additionally, improved grazing management allows enough herbage mass to benefit animal performance, compared to other grazing strategies (i.e., decreased sward height) (Savian et al., 2018). Alcock and Hegarty (2006) used a model on an Australian lamb farm to stimulate the changes in annual CH_4, meat, wool, and gross margin ($/ha), resulting from sowing improved pasture and found that annual enteric CH_4 emission on sustainably managed pastures are greater if the pasture is improved.

Tannins

Tannins are a plant secondary compound and can be utilized as feed supplements or planted as tanniferous plants. Tannins have been studied for their use in alternative parasite management strategies (Niezen et al., 1995; Nguyen et al., 2005). Tannins have also been extensively studied for their potential to reduce CH_4 emissions (Jayanegara et al., 2015; Aboagye et al., 2018; Beauchemin et al., 2007) and improve N utilization; however, the results have been shown to be inconsistent. Tannins can be categorized as condensed tannins or hydrolysable

tannins based on their reactivity and structure. Condensed and hydrolysable tannins are the two major classes of tannins and come from a variety of different browse and warm climate forages. Condensed tannins are the most common type of tannin found in forage legumes and are not usually toxic to ruminants because they are not absorbed (Reed, 1995; Jayanegara et al., 2015). However, hydrolysable tannins have the potential to be toxic to ruminants when consumed in excessive amounts (Reed, 1995; Jayanegara et al., 2015).

Condensed Tannins

Condensed tannins come from quebracho wood, mimosa bark, grape seeds, pine barks, and spruce barks. Condensed tannins are flavonoid polymers (Reed, 1995). They have high binding capacity for dietary protein and can decrease the degradability of protein in the rumen and can be beneficial for animals fed diets with high concentrations of rumen degradable protein. Condensed tannins have a relatively higher molecular weight, bind to dietary protein, and their structure is more diverse than those of low molecular weight, which may explain the inconsistent effects of condensed tannins on CH_4 production (Aboagye and Beauchmin, 2019).

Hydrolysable Tannins

Hydrolysable tannins come from different vegetable plants such as chestnut wood, oak wood, tara pods, gallnuts, myrobalan, sumac, and Aleppo gallnuts. Hydrolysable tannins are gallic or ellagic acid polymers esterified to a core molecule (Reed, 1995). They have a relatively low molecular weight and affect rumen microbes by binding to them and influencing their function (Aboagye and Beauchmin, 2019). Moreover, these tannins can attach to proteins, which reduces the protein breakdown in the rumen (Aboagye and Beauchmin, 2019). This allows more protein to bypass rumen fermentation and be digested in the lower gastrointestinal tract (Min et al.. 2003; Carulla et al., 2005; Aboagye et al., 2018). This is a beneficial mechanism so that less

NH_3 (from rumen fermentation) is formed in the rumen, reducing the amount of excess N excreted in the urine. Aboagye et al. (2018) conducted a study evaluating hydrolysable tannins and condensed tannins effect on rumen NH_3 concentrations (an indicator for N excretion) and CH_4 emissions and reported that hydrolysable tannins (from chestnut) fed at 1.5% DM decreased urine N excretion without negatively affecting animal performance. The decrease in urine N excretion suggests that there was decreased protein degradation in the rumen, shifting the N excreted in the urine to N excreted in the feces. This is beneficial to the environment because decreasing urinary excretion of N would reduce the amount of volatilization of N in the form of NH_3.

Nitrogen Utilization

Tannins have a role in improving N utilization of ruminants. Approximately 10-40% of consumed N is retained as meat or milk by ruminants, with the remaining dietary N being excreted in feces and urine (Aboagye and Beauchemin, 2019). A solution to increase the percent of consumed N retained as meat or milk is important for ruminants so that 1) production is more efficient and 2) less N is excreted as urine with the potential to volatilize as NH_3 into the environment. Supplementing tannins to ruminants improves N utilization by decreasing rumen degradability of crude protein (CP) and sometimes CP digestibility in the total digestive tract, which would, in turn, shift N excretion from urine to feces and consequently reduce excretion of the more volatile form of N into the environment (Aboagye and Beauchemin, 2019). Carulla et al. (2005) found that condensed tannin supplementation decreased ruminal NH_3 concentration and urinary N excretion without affecting body N and energy retention.

Effect of Tannin Inclusion on CH_4 Production

Previous literature on tannins reducing CH_4 production has been variable. Multiple studies have concluded that including tannins in the diet shows promise for decreasing CH_4 emission from ruminants (Carulla et al., 2005; Animut et al., 2008; Bhatta et al., 2009). Beauchemin et al. (2007) concluded that feeding up to 20 g of condensed tannin (from quebracho trees) per kg of dietary DMI failed to reduce enteric CH_4 emissions. In addition, Jayanegara et al. (2012) conducted a meta-analysis of in vivo data from 30 experiments comprising 171 treatments and found that the reduction in CH_4 production (based on digestible organic matter intake) was highly variable when tannin concentrations were < 2.0 g /100 g of dietary dry matter. These low tannin levels may explain the contrasting literature reported.

Conversely, Carulla et al (2005) fed *Acacia mearnsii* tannin (condensed tannin) at 2.5% DM to six growing castrated male lambs fed three different basal haylage diets. Although Carulla et al. (2005) reported no interaction between basal diet and the addition of tannin supplement, they did find a 12% reduction in CH_4 production when lambs were supplemented with 2.5% condensed tannin. Therefore, an increased level of tannin supplementation shows promise.

Different tannins have different responses on rumen methanogenesis due to their distinct chemical structure (Aboagye and Beauchemin, 2019). Thus, considerable research has evaluated the effect of hydrolysable tannins compared to condensed tannins on enteric CH_4 production. Jayanegara et al. (2015) found that hydrolysable tannins decreased CH_4 and had a less adverse effect on digestibility than condensed tannins. However, Aboagye et al. (2018) found that steers fed a combination of condensed tannin and hydrolysable tannin (0.75% condensed and 0.75% hydrolysable) tended to decrease CH_4 yield. Similarly, Bhatta et al. (2009) found that tannin

sources containing both hydrolysable tannins and condensed tannins were more potent in suppressing methanogenesis.

Tannin's Impact on ADG and DMI

There has also been a controversial discussion on tannins increasing or decreasing ADG and DMI. Rivera-Mendez et al. (2017) results indicated that feeding a hydrolysable or condensed tannin (0.6% dietary DM) to feedlot Holstein steers did not affect ADG or DMI; however, a combination of 0.3% condensed tannin and 0.3% hydrolysable tannin mixture increased ADG and DMI. Aboagye et al. (2018) found no effect from feeding tannin (condensed, hydrolysable, and combination) on DMI or ADG.

Growth Implants

Growth implants have been utilized in the beef cattle industry since the 1950s, with over 30 commercially-available implants now marketed in the U.S. for beef cattle production. (Selk et al., 2006; Smith and Johnson, 2020). The purpose of these growth promotants is to enhance production efficiency, reduce the cost of production, and improve profitability for the producer (Tibbitts et al., 2017). Since the first commercial implant, significant research has evaluated ADG, carcass leanness, and feed efficiency (Reinhardt, 2007; Smith and Johnson, 2020). However, little work has examined how implanted cattle impact GHG emissions.

Types of implants

A large variety of implants are approved for use in the U.S. for beef cattle production. These growth implants are classified into low-, medium-, and high-potency implants or coated and non-coated implants (Johnson and Beckett 2014; Smith and Johnson., 2020; Beck et al., 2022). The active ingredient in these growth implants is estrogen, androgens and progestins. Estrogens mimic the naturally occurring hormone estrogen, the primary estrogenic compounds

used in implants are benzoate, estradiol 17-beta, and zeranol (Beck et al., 2022). The androgenic compound mimics the naturally occurring compound testosterone, the main androgenic compounds used in implants are testosterone propionate and trenbolone acetate (Beck et al., 2022). Lastly, progestins mimic the naturally occurring pregnancy hormone progesterone (Smith and Johnson, 2020). These anabolic compounds used in implants are utilized alone or in combination depending on implant type. The least potent anabolic implant formulations generally contain a low dose of estrogen alone or in combination with progestin. The moderate potency implants generally contain greater doses of estrogen alone or combined with a progestin or trenbolone acetate and estradiol 17-beta. The most potent implants generally contain trenbolone acetate alone or in combination with estradiol 17-beta or estradiol benzoate. Implants are also classified as non-coated or coated implants. When non-coated implants are administered, the anabolic compound is slowly released and is expected to release for 60-120 d, also known as the payout period (Smith and Johnson, 2020). The optimum payout period varies; however, combinations of estradiol + trenbolone acetate or estradiol 17-beta + trenbolone acetate are known to give large growth responses in steers (30 – 60%) during the first 28-35 d (Preston, 1999). The payout period can be various lengths depending on the anabolic compounds used in the implant formulation, the amount of pressure applied to the implants during the formation of the implant pellets, and various polymers that delay or slow the release of the anabolic compounds into the circulation of the animal (Smith and Johnson, 2020). Coated implant products are shown to extend the life of the implant payout period in excess of 200 d after implantation (Smith and Johnson, 2020). However, payouts for stocker implants generally range from 80-120 d (Beck et al., 2022).

Today, implants are a common practice for beef cattle producers. In the cow-calf production phase, 31% of large cow-calf operations implant their calves before weaning. In the stocker/backgrounder segment, Asem-Hiablie et al (2015) reported that stocker cattle operations indicated 77% of stocker calves are implanted (during a voluntary survey for ranchers and feedlots in Kansas, Oklahoma, and Texas). Whereas, Selk et al. (2006) reported that 90% of stocker producers implant their calves. In the feedlot segment, according to the 2011 USDA NAHMS Feedlot Survey (USDA, 2011), up to 94% of steers and heifers are implanted at least once during the finishing phase. Implant research trials have shown an improvement in average daily gains by 8 to 20% in stocker cattle (Kuhl 1996, Selk et al., 2006; Reinhardt and Thomson, 2016, Beck et al., 2022).

Revalor - G

Revalor-G (REV-G, Merck Animal Health, Madison, NJ) is a medium-potency implant. The product contains 40 mg of trenbolone acetate and 8 mg of estradiol 17-beta in a slow-release delivery system. Revalor-G is approved to be used in grazing steers and heifers. In a 151-d field study was conducted by Blasi and Kuhl (1998) comparing three anabolic implants for weight gain in grazing stocker heifers. Revalor-G significantly improved gain ($P < 0.05$) compared to the no implant-control; however, only during the first 32 d period heifers implanted with REV-G gain significantly faster ($P < 0.05$) than the no implant-control. Revalor-G implanted heifers gained rapidly early in the study but did not continue the same growth response for the entirety of the study. This outcome suggests that the REV-G implant demonstrated a classic “half-life” response over the 151 d (Blasi and Kuhl, 1998). In a similar 150-d field study conducted by Kuhl et al. (1997), heifers implanted with REV-G had an increased average daily gain ($P < 0.05$) in the

first 75 d and had a significantly higher ADG ($P < 0.05$) for the entirety of the study (Kuhl et al., 1997).

Factors Affecting Implants

Numerous variables may affect stocker cattle response to implants. These include growth rate, sex, weight, genetic gain potential, forage availability and quality, supplementation, and environmental conditions (Kuhl, 1996; Selk et al., 2006; Beck et al., 2022). Beck et al. (2022) reported that as ADG of no implanted-controls increases (due to pasture quality or other factors), the response to an implant also increases. The growth response to an implant is directly proportional to the nutrients available to cattle (Stewart, 2013; Reinhardt and Thomson, 2016). There is a greater growth response to implants with an increased plane of nutrition, but implantation will not negatively affect growth rates (Stewart, 2013; Reinhardt and Thomson, 2016).

Carbon Footprint and Ammonia Emissions

Increasing animal performance is proposed as one of the more successful mitigation strategies to decrease GHG and NH_3 emissions from cattle production per unit of product produced. Stackhouse et al. (2012) conducted a partial lifecycle assessment using the Integrated Farm System Model to estimate GHG and NH_3 emissions from representative beef production systems in California. Stackhouse et al. (2012) stated that a combined use of ionophores, growth implants, and BAA ($Beta_2$-adrenegic agonists; Zilmax) treatments decreased NH_3 emissions from the full cattle production system by 13%, and the C footprint of beef was decreased by 2.2 CO_2e/kg HCW using all growth-promoting technologies. However, specifically looking at the stocker phase, results showed the no-implant angus production system emitted 10% less GHG per animal than the implanted angus production system (Stackhouse et al., 2012). Similarly, the

implanted angus production system emitted more CH_4 than the no implant-control (Stackhouse et al., 2012). Yet, when calculating emission intensity (g CH_4/kg gain), the implanted angus stocker cattle had a 9% lower emission intensity.

Nitrogen

The increased intake and production levels associated with implanting cattle (NRC,1996 Wileman et al., 2009; Stackhouse et al., 2012; Smith and Johnson, 2020) mean there is a surplus of nutrients excreted in feces and urine (Hristov et al., 2013). The overall range in N utilization efficiency is 15 - 40% (Hristov et al., 2013). Decreased protein in the diet results in fecal N containing larger proportion of N intake than urine N, but when there is an increase of protein in the diet, fecal N decreases and urinary N excretion increases (Hristov et al., 2013). Increased N in the urine is associated with a greater susceptibility to N volatilized to NH_3 compared to N in the feces (Hristov et al., 2013). This is because most of the N in cattle urine is urea, which hydrolyses upon excretion and becomes susceptible to NH_3 volatilization (Hristov et al., 2013). However, studies suggest that steers implanted with coated trenbolone acetate and estradiol-17-beta have shown a decrease ($P < 0.05$) in serum urea N compared to the no implant-control (Bryant et al., 2010; Parr et al., 2014).

CONCLUSION

Many factors influence CH_4 emissions in cattle, including ionophores, dietary lipids, direct-fed microbials, inhibitors, the type and amount of feed intake, forage type, and grazing management. A magnitude of reviews have been published on these potential strategies to mitigate GHG emissions (Waghorn and Herarty., 2011; Knaap et al., 2014; Arndt et al., 2020; Thompson and Rowntree, 2020); however, little work has been done examining the effect on growth implants affect on CH_4 emissions and stacking this technology with tannin

supplementation. That being said, Johnson and Johnson (1995) suggest that modest reductions in CH_4 emissions are possible with current technologies, such as growth promotants, while maintaining or enhancing productivity.

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CHAPTER 2 − IMPACT OF GROWTH IMPLANTS AND TANNIN SUPPLEMENTATION ON ENTERIC METHANE EMISSSIONS AND ESTIMATED NITROGEN EXCRETION IN GRAZING STOCKER STEERS

INTRODUCTION

A growing concern exists regarding the contribution of livestock production to anthropogenic greenhouse gas (GHG) emissions, primarily driven by enteric methane (CH_4) and manure nitrous oxide (N_2O). In 2020, the agriculture sector was responsible for 11% of total U.S. GHG emissions, and 40% of that is attributed to the livestock sector (EPA 2021). Therefore, the livestock sector is responsible for 3.8% of total US emissions (EPA 2021). Agriculture contributes 42% of CH_4 and 57% of N_2O emissions from the combined sources of soil management, manure management, and enteric fermentation which is responsible for 30% of agriculture GHG emissions (EPA 2021). Beef cattle are the largest contributor to enteric CH_4 emissions in the agriculture sector (EPA, 2021). Methane is a potent GHG with a global warming potential (GWP) of 25 times that of CO_2 (EPA 2021). Therefore, mitigation of enteric CH_4 is important to explore.

In the U.S., grazing cattle, more specifically, cow-calf and stocker segments contribute 70 to 80% of total GHG emissions from the beef sector (Rotz et al. 2015, 2019). Grazing cattle contribute 89% of CH_4 emissions, 83% of N_2O emissions, and 64% of NH_3 emissions (Rotz et al., 2019). These results are similar to those reported by Buauchman et al. (2010) and Franzluebbers (2020). Grazing systems supply 34% of global beef production and support most of the breeding herd. The ability of ruminants to convert complex carbohydrates with high fiber content on untillable land into useable end products such as meat or milk is a unique advantage that other mammals do not have, and it is crucial for overall food security (Gerber et al., 2015; Carvalho et al., 2018; Thompson and Rowntree, 2020).

Therefore, it is necessary to formulate strategies that can effectively mitigate CH_4, N_2O, and NH_3 emissions without compromising animal performance and the net return for beef cattle producers. Many potential mitigation strategies have been reviewed (Berndt and Tomkins, 2013; Gerber et al., 2013; Hristov et al., 2013; Thompson and Rowntree, 2020), including the use of tannins and cattle management strategies. Tannins are a diverse group of plant secondary compounds that interact with ruminal N and fiber fermentation (Min et al., 2003; Carulla et al., 2005). Hydrolysable and condensed tannins have the ability to reduce enteric CH_4 emissions due to their ability to bind proteins and carbohydrates within the rumen (McSweeney et al., 2001; Min et al., 2003). This binding action inhibits microbial attachment, reducing ruminal fiber fermentation while increasing the availability of bypass protein (Min et al., 2003). Although literature on tannins reducing CH_4 production has been variable, studies have concluded that the inclusion of tannins in the diet shows promise for decreasing CH_4 emissions in ruminants (Min et al., 2003, Carulla et al., 2005; Bhatta el al., 2009). According to Min et al. (2003), the consumption of condensed tannins at levels between 20 and 45 g/kg of DM may result in a decline in protein degradation; however, including more than 55 g/kg of DM may lead to a decrease in voluntary feed intake.

Management strategies, such as using anabolic growth implants, are a common practice for beef cattle producers. Over 75% of cattle in the stocker/background segment are implanted due to the benefit of increased ADG (Kuhl, 1996; Selk et al., 2006; Asem-Hiablie et al., 2015; Reinhardt and Thompson, 2016; Beck et al., 2022). Revalor-G (REV-G, Merck Animal Health, Madison, NJ) is a medium-potency growth implant that contains 40 mg of trenbolone acetate and 8 mg of estradiol 17-beta in a slow-release delivery system. Revalor-G has been shown to significantly ($P < 0.05$) improve ADG (Kuhl et al., 1997; Blasi and Kuhl., 1998). As ADG

increases, emissions intensity (g CH_4/ kg gain) can decrease (McAuliffe et al., 2018). Therefore, if the growth implant increases ADG, there is potential for a decrease in emission intensity (Stackhouse et al., 2012). However, limited work has been done examining implant response to CH_4 production. Considering the large contribution of the grazing sector to enteric CH_4 emissions, the primary objective of this experiment was to understand how implanting with REV-G and supplementing with a blend of chestnut and quebracho tannins (Silvafeed BX) will impact enteric CH_4 and N utilization in growing stocker steers.

MATERIALS AND METHODS

The experimental procedure was approved by the Institutional Animal Care and Use Committee at Colorado State University (CSU; IACUC #3356). All cattle under the care of this study were maintained and managed following all guidelines outlined in the protocol and this document and with the utmost care and humane handling.

Location and Pasture

The experiment was conducted between June 19, 2022, and September 18, 2022 (90 d) on an 82-ha pivot-irrigated pasture located at the CSU Agriculture, Research, Development, and Education Center (ARDEC; Figure 3, 40o 65'N, 105 o 00W, 1557.528 m asl). The local climate is a mid-latitude dry, cold, semiarid steppe (Kottek et al., 2006). During the experiment, there was a total precipitation of 87.5 mm, and the mean air temperature was 21.7^{o} C. The pivot-irrigated pasture contained 31 different grazing sections ranging from 2.19 to 3.08 hectares. The sections were predominantly cool season grasses including *Dactylis glomerata* (orchard grass) with *Festuca arundinacea* (tall fescue), *Festuca pratensis* (meadow fescue), *Lolium perenne L.* (perennial ryegrass), *Bromus biebersteinnii Roem* (meadow brome), *Bromis inermis L.* (smooth brome) and *Medicago sativa* (alfalfa). Steers were rotated on 2 to 4 d intervals depending on

forage availability in a graze and follow grazing management plan with the resident cow-herd at a stocking density of 7.33 animal units/ha. Animals were offered *ad libitum* access to drinking water and commercially available free-choice salt block. Further description of the study site is described by Shawver et al. (2021).

Forage Measurements

Forage samples were taken every two weeks for the duration of the study using the quadrat method (Thompson et al., 2021). The sections where forage was collected is shown in Figure 3, and nutritive content of forage is shown in Table 1. Prior to rotating animals, pregrazed samples were collected by randomly placing six 0.25-m^2 quadrats and clipping them to a 5-cm stubble height in each experimental section. All clippings were weighed wet, dried in a 65^{o}C oven for 3 d, and weighed again to calculate dry matter (DM) content. Dry matter content was then used to calculate forage productivity. Samples were then ground to pass through a 1 mm screen (Thomas A. Wiley Laboratory Mill, Swedesboro, NJ) and composited by weight prior to analysis. Composite samples were then analyzed for crude protein (CP), neutral detergent fiber (aNDF), acid detergent fiber (ADF), lignin, total digestible nutrients (TDN), net energy for maintenance (NEm), net energy for growth (NEg), and neutral detergent fiber digestibility (NDFD) at a commercial laboratory (Table 1, DairyOne; Ithaca, NY).

Animals and Treatment

Thirty crossbred Angus *Bos taurus* steers (BW = 358 ± 43 kg) all originating from the John E. Rouse-CSU Beef Improvement Center located nine miles east of Riverside and twenty-six miles southeast of Saratoga, Wyoming along the North Platte River. Steers were acclimated to a portable SmartFeed Pro self-feeder (Figure 2; Smartfeed Pro, C-Lock Inc., Rapid City, SD) and portable, automated head-chamber system (Figure 3; AHCS; Greenfeed, C-Lock Inc., Rapid

City, SD) for three weeks. Twenty steers (initial BW = 343 ± 14 kg) were selected for the experiment based on the training acclimation rate to both the AHCS and Smartfeed Pro to be used in the experiment. Steer body weights (BW) were collected every 30 d using a Silencer hydraulic scale at the ARDEC working facility. An additional weight measurement occurred halfway through the 90-d study at d 45.

At the beginning of the study, steers were injected with a 7-way clostridial vaccine (Vision 7; Merck Animal Health), vaccinated with a modified live, 5-way vaccine for infectious bovine rhinotracheitis, bovine respiratory parainfluenza-3, bovine virus diarrhea types 1 and 2, Mannheimia Heamolytica and Pasteurella multocida (Vista Once; Merck Animal Health), dewormed (Safeguard; Merck Animal Health), treated with a pour-on topical insecticide (Cyonara Plus; Control Solutions Inc.) and then randomly assigned to one of the four treatments. The experimental design was a 2 by 2 factorial with tannin supplement (Silvafeed BX, Silva Team., San Michele Mondovi CN, Italy) and a growth-hormone implant (Revalor-G, Merck Animal Health, Rahway, NJ). The treatments were as follows:

1) No tannin and no implant (Control [CON])
2) Tannin supplement and no implant (Tannin [TAN])
3) Implant and no tannin (Implant [IMP])
4) Tannin and implant (Implant + Tannin (IMP + TAN])

The tannin supplement was fed at the rate of 0.30% DM tannin intake (as recommended by the manufacturer) through 0.5 kg/hd/d using sweetfeed mix (Table 2; Sweet Mix, Agfinity., Eaton, CO). Treatment groups without tannin (Control and Implant) received the same sweetfeed ration at 0.5 kg/hd/d without the tannin supplementation (Table 2). Each growth implant

administered contained 40 mg of tenbolone acetate and 8 mg of estradiol (Revalor-G, Merck Animal Health., Rahway, NJ).

Acclimation

The SmartFeed Pro allows cattle to be supplemented on pasture and has a specially designed door that allows for controlled individual animal intake. The AHCS system allows for measurement of CH_4 and CO_2 emission and O_2 consumption. Acclimation for the AHCS was done according to Gunter and Beck (2018). During the acclimation period, the AHCS was initially introduced with no panels or windbreaks around the machine. Panels were then added at a wide distance and slowly placed closer together until only one animal could fit in the ally leading up to the AHCS animal inlet. The AHCS uses bait to attract and occupy the animal while gas flux measurements are taken. In the current study, the bait was alfalfa (*Medicago sativa*) pellets. During the acclimation period, the animals were kept in a 0.09-hectare feedlot pen and offered a forage-based diet. At the beginning of acclimation, the SmartFeed Pro had the automatic head gates locked open to allow ad libitum access for each animal. Two weeks into acclimation, the automatic head gates on the automated feeder were raised to allow acclimation to the feeder's doors. After 30 d, all steers were moved onto the pivot-irrigated pasture to allow for acclimation to the pasture and electric fence for ten days. While on pasture, animals were assigned access to one of two feeders of the SmartFeed Pro. After acclimation, 20 steers were selected based on the acclimation rate to both the AHCS and SmartFeed Pro to be used in the experiment.

Forage Intake

Forage intake was originally planned to be estimated using the double marker method described by Kartchner (1980). The external marker was titanium dioxide (TiO_2), and the

internal marker was indigestible ADF (iADF). Over 14 d of the experiment (August 12, 2022, to August 25, 2022), all experimental animals were provided TiO_2-containing pellets daily through the AHCS as described by Beck et al. (2021). The pellets were formulated to contain 1% of TiO_2 and were administered by dropping (approximately 34 g per drop) the pellet at each first daily visit (max of 6 drops) per individual visitor. To ensure daily intake of TiO_2, daily drops were accounted for each animal. During the last 5 d (August 21, 2022, to August 25, 2022) of this 14d TiO_2 feeding period, fecal samples were taken twice daily (0600 and 1800) in a squeeze chute via rectal grab. However, because visitation to the AHCS is voluntary, animals did not always visit the AHCS every day. Therefore, TiO_2 intake was variable and inconsistent. Intake is an important measurement for comparison of CH_4 yeild (MY; g CH_4 kg total DMI). Therefore, forage intake was also estimated using two different intake prediction equations.

Equation 1

Nutrient Requirements of Beef Cattle equation for all-forage diets (NRC, 1996, pg. 94, Eq: CP__ADF)

$$\text{DMI kg/kg } SBW^{0.75} = 0.002774 \times \text{CP percent in the forage} - 0.000864 \times \text{ADF percent in the forage} + 0.09826$$

In Equation 1, percentage of CP and ADF in the forage are expressed on a dry matter basis (Table 3). Kilograms of $SBW^{0.75}$ (metabolic BW based on shrunk BW) was converted to DMI (kg/d).

Equation 2

Estimating Forage Intake from the Growth of Beef Cattle (Minson and McDonald 1987)

$$\text{DMI kg DM/d} = (1.185 + 0.00454L \times 0.0000026L^2 + 0.315G)^2$$

In Equation 2, L represents liveweight gain and G represents growth rate of cattle (kg/d).

After comparison of measured predicted intake and both predicted intake equations for the study animals, the NRC (1996) equation was used to predict forage intake in this experiment because it considered forage quality and BW. Further, this equation reflected similar forage intake values as seen in the literature for steers of this body size unlike TiO_2 results, which were substantially greater than the NRC (1996) equation results (ANOVA $P < 0.01$, Table 3). Total DMI is presented as the sum of estimated forage intake, bait (alfala pellet) intake, and sweetfeed mix intake.

Gas Measurement

Due to operating challenges with the AHCS system during our acclimation period, the AHCS started collecting data on d 9 (June 29, 2022). The AHCS recorded individual CH_4 and CO_2 emissions and O_2 consumption each time an animal visited using the cattle's radio frequency identification (RFID) during the 81 d of data collection from the AHCS. A more advanced AHCS system was introduced on day 62 that also collected H_2 emissions. Animals were allowed a maximum of six visits each day, with a maximum of six drops each visit (Gunter and Bradford, 2017; Gunter and Beck, 2018). On average, a single drop of the alfalfa pellet bait weighed 34 g and there were 30 s intervals between drops. This was set to encourage the animal to remain in the AHCS for a minimum of three minutes (min; Velazco et al., 2016). All visits that recorded less than three min and greater than eight min were removed from analysis. The minimum time between visits was four h to ensure distribution over 24 h periods to capture diurnal variation in CH_4 production (Della Rosa et al., 2021). The alfalfa pellet bait feed (Table 2; 9.5-mm-diameter pellets, ~34 g/dispense) was provided by AgFinity (AgFinity; Eaton, CO). Pellets were sampled monthly and analyzed for nutritive value by a commercial laboratory (DairyOne; Ithaca, NY). During each visit the animal would enter a narrow panel system to

ensure that only one animal was visiting the machine. Once the animal inserted its head into the AHCS, the system would scan and register the animal's RFID and begin dropping the pelleted bait feed. The process of CH_4 and CO_2 measurements by the AHCS used are further described by Hristrov et al. (2015). The AHCS was auto-calibrated weekly and CO_2 recoveries were completed before, during, and after the study with recoveries of 100±5%. Spot measurements were averaged over the course of the 81 d of gas collection to determine average daily CH_4 production for each animal.

CH_4 emissions were also estimated using two different prediction equations to compare our measured results to estimated results (ANOVA $P < 0.01$, Table 4). The IPCC (2019) equation for animals consuming a >75% forage diet used DMI and an estimated CH_4 yield value as factors to calculate the predicted CH_4. We calculated animal DMI value based on the NRC (1996) predicted intake value for this equation. The IPCC (2019) equation was then converted to g of CH_4 per animal per day.

Tier 2 approach: Methane emission factors for enteric fermentation from a livestock category (IPCC, 2019)

Equation 3

IPCC (2019) equation for animals consuming a >75% forage diet

$$EF = DMI \times (MY/1000) \times 365$$

In equation 3, emission factor (EF), represents kg CH_4 per head per year, DMI represents kg DMI per day, 365 represents the days in a year, and 1000 represents the conversion from g of CH_4 to kg of CH_4.

Thompson et al (2019) used a regression model to predict CH_4 production in which stocker cattle were grazing wheat. Thompson et al (2019) models were selected using the backward stepwise

procedure to minimize Mallows cp. DMI of forage was estimated using the NRC (1996) equation.

Equation 4

Predictive Methane Equation (Thompson et al, 2019)

$CH_4 = 98.33 + 0.17 \times IBW + 5.22 \times FI - 11.31 \times SEX$

Where, IBW represents the initial body weight, FI represents DMI of the forage, and SEX is 1 for heifers and 0 for steers.

N Utilization

Blood Urea Nitrogen

Blood samples were collected after a 12-hour fasting period. Blood was drawn from the jugular vein on d 0, 45, and 90. Blood was collected in EDTA tubes (BD Vacutainer EDTA blood tube; Fisher Scientific, Pittsburgh, PA) and were centrifuged for 10 min at 210 x g at 4°C. Serum was removed and stored at -80°C. After study completion, serum samples were sent to the CSU Diagnostic Laboratory (Fort Collins, CO) for blood urea nitrogen (BUN) analysis.

Urine Samples

Urine was collected manually on d 0, 45, and 90. Once collected, each urine sample was placed in 50 mL tube with 10 mL of HCL and frozen at 4 °C. After study completion, all samples were sent to Ward Laboratories, Kearney, NE for urine N analysis and CSU Veterinary Clinical Pathology Lab, Fort Collins, CO for urine creatine analysis.

Fecal Samples

Fecal samples were collected via rectal grab on d 0, 45, and 90. Samples were placed in quart-sized Ziploc bags and transported to CSU ARDEC laboratory facilities. Samples were weighed wet, dried in a 65°C oven for 3 d, and weighed again to calculate DM content. Dried

fecal samples were ground to pass through a 1-mm screen (Thomas A. Wiley Laboratory Mill, Swedesboro, NJ) and placed in Whirl PAK bags to prevent sample contamination until further analysis. Samples were sent to Ward Laboratories, Kearney, NE for fecal N and P analysis.

Statistical Analysis

One steer from the TAN treatment gained significantly less (~50%) than all other animals during the 90 d of the study. Therefore, that individual was excluded from analysis. One steer from the CON treatment group had significant supplement refusal during the 90 d of the study, this that individual was also removed from analysis. Data were analyzed as a completely randomized design. Each animal was considered the experimental unit (n = 18). Treatment was included in the one-way analysis of variance (ANOVA) model as a fixed effect.

To determine when treatments were most effective within the study, we split the 90-d study into two 45-d periods. Treatment effects were also evaluated across the whole 90-d study. For analysis considering emissions data (enteric CH_4 and CO_2 production, EI, MY), individuals with ≥ 10 good (animals that remained in AHCS system for 3-8 minutes; Arthur et al., 2017; Beck et al., 2018) visits were selected for final analysis within the period (early = d 0 to d 45; late = d 45 to d 90; all = d 0 to 90). This step resulted in excising two individuals from each period for emissions-related analyses (treatment evaluation n = 16). Dependent (response) variables were ADG, DMI, daily CH_4 production (g of CH_4/hd/d), CH_4 MY (g CH_4 /kg total DMI), and CH_4 EI (g CH_4/ kg BW gain). For urine measurements, response variables were urine N and urine creatine. For blood measurements, the response variable was BUN. We used analysis of variance for treatment means comparisons ($\alpha = 0.05$). For fecal measurements, the response variables were fecal N, and fecal P. Average daily gain was determined via the slope coefficient of a linear regression model as a function of gain and day. The effect of treatment was

determined significant at $P \leq 0.05$, and tendency was determined at $P \leq 0.10$. R software was used for all analyses (R Core Team, 2021, v. 4.1.2).

Compared to the predicted forage intake equations (NRC., 1996; Minson and McDonald., 1987), the forage intake estimation results from the current experiment using the double marker method described by Kartchner (1980) seemed unreasonable (Table 3). The mean estimated DMI using the double marker method was 14.94 kg/d, and the mean estimated DMI from NRC (1996) and Minson and McDonald (1987) were 9.24 and 8.71, respectively. The double marker method results were significantly greater ($P < 0.01$) than the other estimated intake equations. Therefore, forage DMI was estimated using the NRC (1996) intake equation for all forage diets. This estimation was used for estimated forage DMI, total DMI, and MY.

RESULTS

There was no interaction between the inclusion of a growth hormone implant and tannin supplement ($P = 0.24$). Therefore, the main effect of each independent variable of interest (growth implant and tannin supplement independent effects) is reported in separate analyses and data were analyzed with treatment levels as follows:

I1) NO-IMP: All animals that did not receive growth implant

I2) IMP: All animals that did receive growth implant

T1) NO-TAN: All animals that did not receive tannin supplement

T2) TAN: All animals that did receive tannin supplement

Animal Growth Performance

Initial BW of the cattle ranged from 343 to 346 kg and there were no differences detected ($P > 0.37$) between treatments indicating an equal body weight distribution among treatments (Table 5). Despite a difference of 5 kg, final BW was also not different between treatments ($P >$

0.42). Neither tannin supplement (P = 0.76) or growth implant (P = 0.80) affected ADG during the 90 d of the study (Table 5). However, ADG tended to be greater for IMP steers (0.92 kg/d) in the early period (d 0 to d 45) when compared to the NO-IMP steers (0.83 kg/d; P = 0.10; Table 5).

Total DMI ranged from 9.04 to 9.76 kg/d. Total DMI tended to be greater for NO-TAN (9.22 kg/d) steers compared to TAN steers (9.04 kg/d; P = 0.08) for the 90 d of the study; however, there were no differences in the early (P = 0.12) and late (P = 0.66) period (Table 5). Total DMI tended to be greater in IMP steers (9.20 kg/d) than NO-IMP (9.05 kg/d; P = 0.13) steers over the 90 d study. In the early period, total DMI was similar between IMP steers (8.81kg/d) and NO-IMP steers (8.71 kg/d; P = 0.32), while in the late period (d 45 to d 90) IMP (9.58 kg/d) steers did not consume more than the NO-IMP steers (9.40 kg/d; P = 0.29).

Estimated forage intake was similar among the TAN steers (8.53 kg/d) and NO-TAN steers (8.57 kg/d; P = 0.41) over the 90 d of the study. Similarly, tannin supplement did not affect estimated forage intake in the early period (P = 0.60) or the late period (P = 0.54; Table 5). Estimated forage intake was greater in IMP steers (8.58 kg/d) than the NO-IMP steers (8.52 kg/d) over the 90 d of the study (P = 0.05) (Table 5). In the early and late periods there were no differences in estimated forage intake (P = 0.12 and P = 0.19), respectively.

Daily sweetfeed mix intake ranged from 0.20 to 0.51 kg DMI/d and treatments did not meet the target intake of 0.5 kg/d, therefore did not receive the full dose of tannin supplement every d (Table 5). The inclusion of tannin as a supplement nor growth implants affected sweetfeed mix intake (P = 0.20 and P = 0.68), respectively.

Alfalfa pellet (bait from AHCS) intake was greater for NO-TAN steers (0.27 kg/d) than TAN steers (0.21 kg/d; P = 0.02) for the 90 d of the study. Similarly, in the early period, NO-

TAN steers had greater alfalfa pellet intake (0.27 kg/d) than the TAN steers (0.21 kg/d; P = 0.03); however, there was no difference in the late period (P = 0.15; Table 5). Alfalfa pellet intake was greater in IMP steers (0.28 kg/d) than NO-IMP steers (0.21 kg/d; P=0.02) over the 90 d of the study (Table 5). In the early period, growth implants did not affect alfalfa pellet intake (P = 0.23); however, in the late period, IMP steers consumed more bait alfalfa pellets (0.29 kg/d) than NO-IMP steers (0.20 kg/d; P = 0.04; Table 5).

Gaseous Emissions

Neither the inclusion of tannin as a supplement nor growth implants had an effect on CH_4 production (g/hd/d) for the 90 d of the study (P = 0.24 and P = 0.15), respectively. Similarly, neither tannin inclusion or growth implants had an effect on CH_4 production in the early period (P = 0.37 and P=0.24), or the late period (P=0.68 and P=0.43; Table 7), respectively.

Methane yield (MY; g CH_4/kg DMI) tended to be lower in NO-TAN steers (23.4) compared to TAN animals (24.1; P = 0.10) for 90 d of the study (Table 7). In the early and late period, the inclusion of tannin as a supplement did not affect MY (P = 0.26 and P = 0.79), respectively. Methane yield tended to be lower in IMP steers (23.2) compared to NO-IMP steers (24.4; P=0.09) throughout the 90 d of the study. In the early and late periods, growth implants had no effect on MY (P=0.20 and P=0.25), respectively (Table 7).

The inclusion of tannin as a supplement had no effect on CH_4 emission intensity (EI; g CH_4/kg BW gain; P = 0.23), CO_2 production (P = 0.84), O_2 consumption (P = 0.83), or H_2 production (P = 0.82; Table 7). Growth implants had no effect on EI (0.19), CO_2 production (P = 0.94), O_2 consumption (P = 0.88), or H_2 production (P = 0.98; Table 7). In the early period, EI was lower in IMP steers (252) compared to NO-IMP steers (288; P = 0.03; Figure 6).

BUN, Nitrogen, Creatinine, and Phosphorus

Neither the inclusion of tannin as a supplement nor growth implants affected urinary N (ppmN) ($P = 0.49$ and $P = 0.30$), creatinine (mg/dL) ($P = 0.45$ and $P = 0.46$), fecal N (% N) ($P = 0.74$ and $P = 0.98$), or fecal phosphorus (P; $\%P_2O_5$) ($P = 0.77$ and $P = 0.95$), respectively (Table 6). Tannin also did not affect blood urea nitrogen (BUN; mg/dL; $P = 0.12$) however, growth implants tended ($P = 0.08$) to increase BUN (Table 6).

DISCUSSION

Animal Performance

After comparing the measured intake results using the double marker method to the NRC (1996) and Minson and McDonald (1987) DMI prediction equations, we concluded that the double marker method measured values were unreasonable because they were significantly greater than the other two predicted intake equations ($P < 0.001$; Table 3). Thompson et al (2019) used titanium dioxide (TiO_2) to estimate forage intake, with results ranging from 5.00 to 8.93 kg DM/d while the current study ranged from 9.04 to 17.31 kg DM/d. However, Thompson et al (2019) bolused cattle with TiO_2 and the current study followed methods described in Beck et al (2021) using the AHCS to dose our steers. As previously discussed, the steer's first visitation of the day (during the dosing period) was the TiO_2 pellet. Therefore, the TiO_2 dose from the AHCS could occur at varying times throughout the day compared to a bolus or handfed technique (Beck et al., 2021). The animal's first visitation to the AHCS allowed them a maximum of six drops of the TiO_2 pellet. Depending on visit duration, steers could have a varying inclusion of TiO_2 dose compared to the bolus or handfed technique. Based on this, more research needs to be done on dosing levels and time of dosing with the AHCS measurement system in grazing animals as a delivery mechanism of TiO_2 to measure forage intake.

The current study used the equation presented in the NRC (1996) for feed intake by beef cattle with special consideration for all-forage diets. This equation incorporated the percent of CP and ADF in the forage and shrunk BW. All cattle grazed together, therefore BW was the key driver in estimating forage intake.

Effect of tannin supplementation

Previous research has reported that tannins have varying effects on BW, ADG, and DMI. However, there is a strong relationship between DMI and BW gain in cattle (Min et al, 2022). Therefore, because there was only a 0.18 kg/d difference in total DMI between TAN and NO-TAN steers, it makes sense there was also no difference in ADG (Table 5). The lack of effect of tannin supplementation on ADG agree with results reported in Beauchemin et al (2007) when they supplemented steers fed a high forage diet with up to 2% quebracho tannin extract and found no significant difference in ADG. Similarly, Aboagye et al (2018) who supplemented steers with a combination (50:50) of hydrolysable tannin and condensed tannin up to 1.5% DM yielded no difference in ADG. Ebert et al (2017) reported similar results to Beauchmin et al (2007), Aboagye et al (2018), and the current study when supplementing condensed tannin manufactured by Silvafeed® at 0, 0.5, and 1% DM to beef cattle, yielding no difference in ADG.

There was no difference in initial BW, final BW, or ADG between NO-TAN and TAN steers; therefore, with the NRC (1996) predicted forage intake equation that was used, all steers consumed similar amounts of forage during the 90-d study. As expected, forage intake was similar between NO-TAN and TAN steers and ranged from 8.34 to 8.92 kg DM/d for the 90 d of the study. All steers had the same opportunity to graze the same forage, therefore we assume the nutrient content of the forage they were consuming was similar. There are individual animal factors that can affect forage intake such as body composition, sex, age, physiological state and

frame size (NRC, 1996). Although we did not measure all of these variables, all cattle were steers and were sourced from the same herd at the same ranch, therefore we can assume genetics and environment are similar between cattle. Cattle also search for forage they want to eat first such as new growth or green plant material and this pattern continues until almost no green is left (Lyons and Machen., 2012). The current study used the quadrat method described by Thompson et al (2021) to collect forage samples; however, this method isn't selective to what cattle 'want' to eat. Therefore, there may be some variation in CP and ADF content in the forage of individual animal intake, which would alter the output of the NRC (1996) predicted forage intake equation. The present study did not find tannin supplementation fed at 0.30 % DM to alter forage DMI. Similar results to the current study were reported in Beauchmin et al (2007) when quebracho tannin extract supplement was included in a basal diet that consisted of ~70% forage fed to beef steers and heifers at 0%, 1% and 2% of dietary DM, yielding no effect on DMI. Aboagye et al (2018) found similar results to the present study observing no effect on DMI, when a combination (50:50) of hydrolysable chestnut tannin and condensed quebracho tannin was fed at 1.5% dietary DM to beef steers fed a high forage diet made up of alfalfa silage and barley silage. However, Piñeiro-Vázquez et al (2018) found a reduction in DMI at 4% tannin inclusion rates when compared to no inclusion in *Bos taurus x Bos indicus* crossbred heifers fed a low-quality fresh chopped *Pennisetum purpureum* (Taiwan grass) diet supplemented with quebracho tannin extract at 0, 1, 2, 3, and 4% of DMI. These results suggest that tannin can be added at low (0.30% DM) or high (up to 3% DM) levels to a forage-based diet and not affect forage DMI; however, fed at levels of 4% DM or more may start to negatively affect DMI in high forage diets. However, conflicting results were reported when Norris et al (2020) conducted a study evaluating condensed tannin supplemented at 0, 1.5, 3, and 4.5% DM to steers fed a 56.5%

roughage diet and reported that supplementing quebraho condensed tannin at 4.5% DM increased DMI compared to steers that received no tannin supplement. Diet composition and quality may contribute to the variable results reported in the literature.

In the current study total DMI includes forage intake, alfalfa pellet intake, and the sweetfeed mix intake. The NO-TAN steers tended to visit the AHCS more frequently which resulted in an increased amount of alfalfa pellet bait feed intake. Therefore, because alfalfa pellet bait intake (from the AHCS) was greater in NO-TAN steers, the overall, or total DMI tended to be greater for steers not receiving the tannin supplement. The reduction in alfalfa pellet intake in the TAN steers may be due to 1) animals feeling sustained because there is more fiber content relative to previous studies (Beauchmin et al 2007 and Aboagye et al 2018) 2) tannins formed complexes with fiber components limiting microbial access and reducing fiber degradation or 3) a combination of both. The average NDF content in the current study was 54.3% DM which is higher than reported in Beauchmin et al (2007) and Aboagye et al (2018) at 45.1 and 43.8% DM, respectively. Tannins have the ability to form complexes with fiber components which may limit microbial access to these substrates. This complex can reduce fiber degradation in the rumen which may limit the energy source for ruminants. In a pasture with higher NDF content, the additional impact of tannins on fiber digestion may further reduce overall digestibility, which would reduce intake. Furthermore, it has been reported that condensed tannin or hydrolysable tannins at > 50 g/kg of DM would result in a reduction in DMI. Scientific reviews have suggested that the reduced intake could be due to a reduction of palatability of diets when tannin is supplemented, decreased rate of digestion in the rumen and the development of toxicity (Frutos et al., 2004; Patra and Saxena, 2011). Although there was a tendency, the NO-TAN supplemented animals only consumed 0.18 kg/d more total DMI than the TAN animals.

Effect of growth implant

Forage intake ranged from 8.39 to 8.93 kg/d in the IMP steers and 8.34 to 8.77 kg/d in the NO-IMP steers. Forage intake was higher for IMP animals than the NO-IMP steers for the 90 d of the study (P = 0.05). Similarly, growth implants appeared to positively affect total DMI (estimated forage plus alfalfa pellet plus sweetfeed mix), although the outcome was not statistically different for the treatments (P=0.13). These results are similar to those reported in Wileman et al (2009), Rumsey et al (1999), Parr et al (2011), and Song and Choi (2001). Wileman et al (2009) conducted a meta-analysis of conventional versus nonconventional beef production and concluded that growth implanted steers increased DMI by 0.53 kg/d (P<0.01) relative to nonimplanted controls. Similar results were observed by Parr et al (2011) when crossbred cattle on a finishing diet were implanted with Revalor-S (120 mg of trenbolone acetate (TBA) and 24 mg of estradiol 17β [E_2]), Revalor-IS followed by Revalor-S (cumulatively 200 mg of TBA and 40 mg of E_2; reimplanted at 68 to 74 d), or Revalor-XS (200 mg of TBA and 40 mg of E_2) (Merck Animal Health, Madison, NJ), yielding a significant increase in DMI in all growth implant treatment groups compared to the no implant controls. Growth implants stimulate DMI (Smith and Johnson., 2020), therefore in the current study, the finding of growth implants to numerically increase total DMI compared to the NO-IMP animals is expected.

The addition of a growth implant did not affect final BW or ADG over the full 90-d of the study, which conflicts with other studies evaluating implant response in grazing beef cattle. Parr et al (2011b) implanted cattle on a finishing diet with three different types of implants and reported a significant increase in final BW and ADG in all implant treatment groups compared to the non-implanted animals. Similarly, Parr et al (2011a) reported a significant increase in final BW and ADG in all implant treatment groups compared to the non-implanted steers when

crossbred steers on a finishing diet were implanted with Revalor-S or Revalor-XS. Although the active ingredient is the same in these implants and Revalor-G used in the current study, the amount of each ingredient is differing. Revalor-S (120 mg of TBA + 24 mg of E_2), Revalor-XS (200 mg of TBA + 40 mg of E_2), and a combination of Revalor-IS and Revalor-S (cumulatively 200 mg of TBA and 40 mg of E_2) all have increased TBA and E_2 compared to Revalor-G, which could be the cause in the increased final BW and ADG. The results of the current study are also conflicting with other studies evaluating implant response in grazing cattle. McMurphy et al (2011) implanted steers grazing summer pasture with Ralgro (36 mg of zeranol) or Component TE-G with Tylan (40 mg of TBA and 8 mg of E_2; 29 mg of tylosin tartrate) and reported a significant increase in final BW and a 8.1% improvement in ADG ($P = 0.01$) during the first 95-d regardless of implant type employed in the trial. Component TE-G contains the same amount of active ingredients as Revalor-G; however, the current study product lacked inclusion of Tylan. Additionally, Beck et al (2014) implanted steers grazing wheat pastures in the fall months with Component TE-G (40 mg of TBA and 8 mg of E_2) and reported that the addition of the growth implant increased ADG by 0.14 kg/d ($P < 0.01$). Beck et al (2014) also evaluated the stacked technology of monensin (Elanco Animal Health, Greenfield, IN) supplement via mineral (1.78 g/kg) or pressed block (0.33 g/kg) with or without the addition of the growth implant and found no interaction between supplement and growth promoting implants ($P > 0.71$). The addition of growth implants increases ADG by 6-16% in grazing cattle compared to the no implant-control (Blasi and Kuhl., 1998; Shockey et al., 1996; Parr et al., 2011a, b; McMurphy et al., 2011; Beck et al., 2014; Tibbitts et al., 2017). Additionally, the lack of effect of implant status on ADG is contrary to previous studies specifically evaluating the implant Revalor-G in grazing beef cattle, where a 6 - 8.5% increase in ADG was observed (Blasi and Kuhl., 1998; Kuhl et al., 1997;

Shockey et al., 1996; Tibbitts et al., 2017). While the current study lacked a statistically significant contrast in ADG between IMP cattle and NO-IMP cattle, there was a 6% increase in ADG in IMP cattle. Additionally, in the early period, implanted cattle tended to increase ADG by 10% (P = 0.10; Figure 5). The initial implant response found in Revalor-G is consistent with those reported in Ball et al (2020), Blasi and Kuhl (1998), and Blasi et al (1998). Blasi and Kuhl (1998) conducted a 151-d field study comparing three anabolic implants in stocker heifers grazing center pivot-irrigated pastures of winter rye. They found that only during the first 32-d period after implantation Revalor-G implanted heifers gained significantly faster than the no-implant control. Beck et al (2014) used a similar implant (Component TE-G, 40 mg trenbolone acetate and 8 mg estradiol) on grazing steers and reported that implanted steers had an 11% increase in ADG compared to the no implant-control. Similarly, McMurphy et al (2011), also investigating Component TE-G, found an 8% increase in ADG in implanted steers compared to the no implant-control in steers grazing summer pasture. In the current study, the implanted steers had a numerically lower ADG (1.06 vs 1.08; P = 0.80) compared to the no implant-control for the full 90-d of the study. This outcome could be due to an initial implant response leveling off or to varied forage quality in the latter half of the study.

Gaseous Emissions

The mean daily CH_4 emissions for all animals observed in this experiment was 216 g/d. The current study mean is 7% higher than reported in Beck et al. (2019) where steers grazed warm-season pasture and supplemented whole cotton seed or supplement containing soybean and weighed 269 kg and 20% higher than reported in Thompson et al. (2019) where steers and heifers weighed 262 and 240 kg, respectively, and grazed wheat forage with or without a monensin supplement. However, the current study mean is 4% lower than reported by Beck et al.

(2018) where steers weighed 316 kg and grazed warm-season pasture with or without whole cottonseed supplementation. The current study evaluated the measured CH_4 emissions from the AHCS compared to the IPCC Tier 2 model (IPCC, 2019) predicted CH_4 equation and the CH_4 predicted equation presented in Thompson et al. (2019) and found that our measured CH_4 emissions from the AHCS were significantly higher (Table 4). However, both of these predicted CH_4 equations use DMI in the equation. The current study used an estimated DMI for the predicted equations, which may explain the variation in CH_4 emission outcomes.

Effect of tannin supplementation

Tannin supplemented at approximately 0.30% DM did not alter CH_4 emissions (Table 7). The results of the current study agree with those found in Beauchemin et al. (2007), who fed the tannin supplement at up to 2% of DM to spayed angus heifers fed a forage-based diet (70%) and reported no effect on CH_4 emissions. Although Beauchemin et al. (2007) found no effect of quebracho tannin extract supplement on CH_4 production, protein binding was evident because there was less ruminal NH_3 concentration. Conversely, Carulla et al. (2005) fed *Acacia mearnsii* tannin (condensed tannin) at 2.5% DM to six growing castrated male lambs fed three different basal haylage diets. Although Carulla et al. (2005) reported no interaction between basal diet and the addition of tannin supplement, he did find a 12% reduction in CH_4 production when lambs were supplemented with 2.5% condensed tannin. The current study used a mixture of quebracho tannin (condensed) and chestnut tannin (hydrolysable). Tannins from different plants vary in their ability to bind to carbohydrates and proteins (McAllister et al., 2005). Therefore, it is possible that tannin from quebracho tree bark is a less effective tannin at reducing CH_4 production compared to other tannin sources such at *A. mearnsii* tannin used by Carulla et al. (2005). To further support this theory, Aboagye et al (2018) found no effect of feeding a mixture

of chestnut tannin (hydrolysable) and quebracho tannin (condensed) at 1.5% DM on CH_4 production. Various other studies have indicated than feeding condensed tannin-containing forages to ruminants reduces CH_4 emissions (Waghorn et al., 2002; Pinares-Patino et al. 2003; Crulla et al., 2005; Puchala et al., 2005). The tannin-containing forages in those studies varied, and the percent of tannin included in the diet varied. However, in most of these studies, there were changes in forage quality, such as lower NDF, which could be associated with a reduction in CH_4. For example, Puchala et al. (2005) grazed 24 angora goats on *Sericea lespedeza*, a forage containing 17.7% (DM) condensed tannin, and crabgrass/tall fescue, a forage containing 0.5% (DM) condensed tannin, and reported CH_4 emissions were 30% lower for goats grazing sericea lespedeza than for goats grazing crabgrass/tall fescue. However, the NDF content (% of DM) of *S. lespedeza* was 28% lower than crabgrass/tall fescue. Therefore, because lower-fiber diets are associated with lower CH_4 emissions (Johnson and Johnson, 1995), the reduction in CH_4 could be due to the change in nutrient composition. In the current study, the NDF value of the grazed forage was, on average, 54.4 % DM and was greater than the 45.1 % DM reported by Beauchmin et al. (2007) when feeding heifers a 70% forage diet. Similarly, Aboagye et al. (2018) fed steers an alfalfa and barley mix with an NDF value of 43.8% DM. The reduction of NDF in the forage could partially explain the lack of effect of tannin supplementation on CH_4 production. Fiber in the diet tends to be less digestible because of the added structural carbohydrates (cellulose, hemicellulose, and lignin), which are more resistant to enzymatic breakdown in the digestive system and require microbial breakdown in the rumen for breakdown (Varga and Kolver., 1997). The fibrous components in higher fiber diets take longer to break down in the rumen, increasing the amount of time available for microbial fermentation and CH_4 production. Other studies also utilize a higher dose of tannin supplement compared to our current study. For example, Crulla et

al. (2005) reported that in sheep supplementing the diet with 2.5% condensed tannins decreased CH_4 production by approximately 12%. Similarly, Puchala et al. (2005) reported that goats grazing condensed tannin at 17.5% reduced CH_4 emissions by 30% compared to goats fed condensed tannin at 0.5%. Additionally, Piñeiro-Vazquez et al. (2017) observed the greatest reductions in CH_4 production at 3% and 4% tannin inclusion rates compared to 0% and 1% tannin inclusion rates in crossbred heifers supplemented with quebracho tannin extract at 0, 1, 2, 3, and 4% of DM. However, Pineiro-Vazquez et al. (2018) found a reduction in DMI at the 4% inclusion rate which correlates to a reduction in CH_4 production. In the current study, the absence of an impact from the supplementation of blended chestnut and quebracho tannins on CH_4 production might be attributed to the dose level we employed (e.g., 0.30% DM vs.>2.0% DM), which was prescribed by the manufacturer, the type of tannin that was used, and the amount of fiber in the diet. Additionally, tannin-supplemented animals did not consistently receive the complete does of tannin supplement daily. This may be due to supplement intake being completely voluntary through the Smartfeed Pro. This is further explained when McClain et al. (2020) offered supplements through the Smartfeed Pro to 59 yearling commercial heifers grazing dryland pastures and found that supplement intake appeared to be influenced by section move dates, which seemed to be related to forage quantity/quality of each section. Previous studies (Crulla et al., 2005; Beauchmin et al., 2007; Aboagye et al. 2018) hand fed (hay or fresh-cut forage) the forage diet with the tannin included in TMR, whereas the current study fed the tannin supplement through a sweetfeed mix that animals had to consume voluntarily. As tannin supplementation did not impact ADG, total DMI or CH_4 production, there was no influence on EI or MY.

Effect of growth implant

Enteric Ch4 production in grazing environments can be directly influenced by animal performance due to the increase in DMI, which in turn, increases ruminal fermentation and methanogenesis (Gerber et al., 2013; Thompson and Rowntree, 2020; Min et al., 2022). In the present study, there was no difference in initial BW, final BW, or whole study ADG but there was an increase in estimated forage intake in IMP cattle compared to NO-IMP for the 90 d study (Table 5). Mean CH_4 production ranged from 208 to 289 g CH_4/d for all cattle; however, the addition of growth implants did not affect CH_4 production (P = 0.44). These results are conflicting with those reported by Stackhouse et al (2012) who conducted a partial lifecycle assessment using the Integrated Farm System Model and suggested that implanted angus cattle emit 11% more CH_4 (kg CO_{2e}/animal) than the angus natural-control in the stocker segment alone. This may be due to growth-implanted cattle having an increased DMI. Thus, there is still considerable uncertainty about the effect of implantation on CH_4 production in grazing steers.

Methane yield for IMP (23.2 g CH_4/kg of total DMI) and NO-IMP (24.4 g CH_4/kg of total DMI) cattle are comparable to the literature (Grainger et al., 2010; Min et al., 2022; Hristov et al., 2013) where values ranged from 19.4 - 21.5 g CH_4/kg DMI. In the present study, IMP cattle had numerically greater DMI (P = 0.13), no difference in CH_4 production (P = 0.15), and a tendency (P = 0.09) for MY to be less (Table 7) than NO-IMP cattle. Min et al (2022) conducted a meta-analysis to evaluate how DMI and ADG were related to CH_4 emissions from cattle, and across multiple different mitigation strategies, they concluded there is a strong linear relationship between DMI and CH_4 production. As daily DMI increases, the CH_4 production also increases.

Although previous literature shows that increasing animal performance influences CH_4 production, it also decreases EI based on g CH_4 emitted per kg of gain (Stackhouse et al., 2012).

Therefore, because there was a 10% increase in ADG in the IMP cattle in the early period and no difference in CH_4 production, we see a decrease in EI by 12.5% in implanted cattle in the early period ($P = 0.03$; Figure 6). Increasing animal performance is proposed as one of the more successful mitigation strategies to decrease GHG emissions from cattle production per unit of product produced (Johnson and Johnson., 1995). Growth implants are a productivity-enhancing technology that improves the growth and feed efficiency of beef cattle and lower cost of production (Aboagye et al., 2022). By improving growth, beef cattle meet their market-ready endpoint on fewer days on feed than cattle grown without growth promoting technologies. Aboagye et al. (2022) conducted a LCA within the feedlot phase of beef production using data reported in Ribeiro et al (2020) and a farm-scale model and reported that steers and heifers that received a growth implant (steers: Component TE-S with Tylan; heifers: Revalor-200, 200 mg TBG and 20 mg E_2) had an increased final BW and ADG compared to non-implanted cattle; however, they also had an increase in absolute GHG emissions (kg CO_2e). However, Aboagye and others (2022) only considered emissions during the finishing phase. Basarab et al. (2012) considered emissions throughout the beef production cycle. They reported that harvest of non-implanted cattle at the same BW as implanted cattle resulted in an increase of 12 to 17 days on feed, and the longer feeding duration resulted in a 10.5 to 15.8% increase in the carbon footprint. Although the current study did not measure beyond the backgrounding stage of production, the increase in ADG in the early period shows cattle implanted with growth-promoting technologies have the opportunity to reach their harvest endpoint on fewer days on feed.

BUN, Nitrogen, Creatinine, and Phosphorus

Lavery and Ferris (2021) explain that timing of sampling can influence BUN concentrations because BUN levels can fluctuate throughout the day, with the highest levels

normally detected 4 to 6 h after feeding. The current study collected blood samples after a 12 hour fast and the only difference found was IMP cattle tended to have increased BUN ($P = 0.08$). Blood samples for BUN analysis may have been collected at a time when BUN concentration was low due to no feed intake. Future grazing studies should take this methodological problem into consideration and, if possible, potentially collect multiple samples throughout the day to to estimate N excretion more accurately.

Effect of growth implant

In the current study, the inclusion of a growth implant had no effect on urinary N, creatinine, fecal N, or fecal P. However, there was a tendency ($P=0.08$) for IMP steers to have an increased BUN compared to the NO-IMP animals for the 90 d experiment. Differing results than the current study were reported when Bryant et al. (2010) implanted heifers with 200 mg of TBA, 20 mg of E_2, and supplemented 250 mg of ractopamine-yielding a decreased serum urea nitrogen (SUN) compared to the control. Parr et al (2014) reported similar results in British x Continental steers implanted with Revalor-S (120 mg TBA and 24 mg of E_2) or Revalor-XS (200 mg of TBA and 40 mg of E_2) - no matter the implant type employed, implanting decreased SUN from d 2 through 131 ($P < 0.05$). The mixed outcomes in the literature could be due to the variation in active ingredients of the Revalor product and sampling methods. Future research should include how active ingredients in growth implants impacts urea N concentrations in the blood.

Effect of tannin supplementation

Tannins have the affinity to bind to protein in the rumen, increasing bypass protein into the small intestine and decreasing ruminal degradation of protein, therefore it can be assumed that tannins in the diet could decrease the amount of N excreted in the urine and increase the

amount of N excreted in the feces (Carulla et al., 2005; Aboagye et al., 2018). However, in the present study the inclusion of tannin supplemented at 0.30% DM had no effect on BUN, urinary N, creatinine, or fecal N. Stewart at al (2019) fed bird's-foot trefoil, sanfoin (condensed tannin-containing legumes), and small burnet (hydrolysable tannin-containing forb) at 2.5%, 0.6%, and 4.5% of DM, respectively to beef cows and heifers and reported that all tannin-containing diets reduced BUN compared to animals not receiving that tannin-containing diet. BUN comparable to plasma urea N (PUN) because urea readily diffuses in and out of the blood cells (Larvery and Feris, 2021). Aboagye et al. (2018) reported that supplementing a 50:50 mixture of chestnut tannin (hydrolysable) and quebracho tannin (condensed) at 0.25% DM to steers fed a forage-based diet significantly reduced PUN compared to the controls; however, feeding the same mixture at 1.5% DM did not affect PUN. Evidence has shown that feeding levels as low at 0.25% of DM can decrease BUN and PUN levels; therefore, the lack of effect found in the current study may be attributed to the timing of blood samples being collected.

CONCLUSION

Including tannin in the diet did not affect animal performance or N utilization; however, tannin decreased the intake of alfalfa pellets (from the AHCS) and, in turn, tended to reduce total DMI. Tannin supplementation also did not reduce CH_4 emissions compared to steers without tannin supplementation. The lack of effect of tannin inclusion on CH_4 production may have resulted from steers not consuming the full dose of tannin daily. The type of tannin used and the level of tannin supplementation is varied between studies; thus, the results from tannin supplementation to reduce CH_4 emissions are variable. Further investigation is needed to determine the most beneficial type of tannin to be used and a range in which tannins can be supplemented to mitigate emissions from grazing stocker steers. Investigators should consider

the difficulty of supplementing cattle in a grazing study and account for the variation of daily intake on an individual animal basis. Growth implants numerically increased total DMI and tended to increase ADG in the early period. Revalor-G did not affect N utilization or CH4 production; however, it decreased EI in the early period and tended to decrease MY during the 90-d experiment. The inclusion of growth implants in grazing stocker steers shows promise in increasing ADG while decreasing CH_4 EI. However, more work needs to directly examine the effect growth implants have on CH_4 emissions in a grazing environment. Further investigation is required to determine how different growth implant active ingredients and dose of growth implant might be used to alter CH_4 production, CH_4 EI, and CH_4 MY in a grazing environment where beef cattle reside for the majority of their life. Investigators should consider the difficulty of accurately determining DMI in a grazing environment and how DMI is important in measuring CH_4 MY.

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Table 1. Nutritive content (% DM basis) of forage grazed by growing steers during the June 2022 to September 2022 grazing season.

	Day[1]						
Analyzed Nutrient Composition	0	14	28	42	56	70	84
DM, % as fed	36.8	34.6	27.8	38.9	31.2	39.9	31.7
CP, % DM	16.3	18.0	20.0	8.4	25.8	12.0	12.5
ADF, % DM	29.1	31.7	35.9	44.5	29.1	39.8	35.8
NDF, % DM	50.8	52.7	49.7	65.1	46.1	61.0	55.7
TDN, % DM	70	69	68	56	68	65	69
Lignin, % DM	3.2	3.6	4.8	6.4	5.3	4.4	3.1
NEm. Mcal/kg	0.70	0.68	0.67	0.49	0.68	0.62	0.68
NEg, Mcal/kg	0.43	0.42	0.41	0.24	0.41	0.36	0.41
NDFD, % of NDF	79	77	68	51	82	62	74

[1] Forage samples collected every two weeks (June 19, 2022 to September 18, 2022)

Table 2. Ingredient and nutrient content of alfalfa pellet, titanium dioxide pellet, and sweetfeed mix.

Item	Alfalfa Pellet[1]	Titanium Dioxide Pellet[2]	Sweetfeed Mix
Formulation, % DM			
Wheat Middlings Shorts	-	93.55	-
Calcium Carbonate Limestone	-	5	-
Titanium Dioxide	-	1	-
Alltech All-Bind	-	0.25	-
BulletProof Bunk Stabilizer	-	0.2	-
Nutritive Value, %DM			
CP	21.4	17.9	11.17
ADF	34.4	10.4	4.69
Lignin	6.7	2.2	1.27
aNDF	42.6	22.7	9.01
NEm	0.63	0.91	0.94
NEg	0.37	0.61	0.64
NDFD, % of NDF	48	43	54
DM, % as offered	91.4	96.2	94.4

[1]Supplier (Agfinity; Eaton, CO) did not supply ingredient list; alfalfa pellet was used as AHCS bait

[2]Titanium dioxide pellet used as AHCS bait during 14 d of the study for the double marker method

[3]Supplier (Agfinity; Eaton, CO) did not supply ingredient list; sweetfeed mix was used as base feed mixed with tannin supplement and was fed out of the Smartfeed Pro.

Table 3. Double marker measured forage dry matter intake (DMI) and estimated forage DMI for 18 steers in this experiment. Mean DMI for the 18 study steers are presented.

	Double Marker Method	NRC 1996[1]	Minson and McDonald 1987[2]	P-Value
DMI (kg/d)	14.94[a]	9.24[b]	8.71[b]	<0.001

[1]DMI was estimated using the NRC 1996 intake equation (special considerations for all-forage diets) + sweetfeed mix + AHCS bait.
[2]DMI was estimated using Minson and McDonald (1987) + sweetfeed mix + AHCS bait.

Table 4. AHCS-measured emissions in this study and estimated CH_4 emissions for 15 steers in this experiment. Mean CH_4 emissions for the 15 steers are presented.

	AHCS Measured Emissions	IPCC Tier 2[1]	Thompson et al. (2019)[2]	P-Value
CH_4, g/d	216[a]	194[b]	205[b]	<0.01

Table 5. Effects of growth implant and tannin on growth performance of stocker steers grazing pivot-irrigated pasture at ARDEC, Fort Collins, Colorado.

	Growth Implant[1]				Tannin[2]				
Item	NO-IMP	IMP	SEM	*P*	NO-TAN	TAN	SEM	*P*	*TAN x IMP*
n, animals	8	10			9	9			
Initial BW, kg (d 0)	344	345	2.43	0.86	346	343	2.34	0.37	0.41
Final BW, kg (d 90)	428	433	4.47	0.51	433	428	4.54	0.42	0.53
ADG, kg/d (linear model slope)[3]	0.91	0.95	0.03	0.80	0.94	0.92	0.03	0.76	0.93
Early[4]	0.83	0.92	0.04	0.10	0.87	0.89	0.05	0.66	0.79
Late[5]	1.08	1.06	0.07	0.88	1.11	1.02	0.07	0.33	0.51
Intake, kg DM/d									
Total DMI, kg/d[3,6]	9.05	9.20	0.06	0.13	9.22	9.04	0.05	0.08	0.99
Early[4]	8.71	8.81	0.08	0.32	8.83	8.69	0.09	0.12	0.90
Late[5]	9.40	9.58	0.09	0.29	9.51	9.47	0.09	0.66	0.77
Forage[7]	8.52	8.58	0.04	0.05	8.57	8.53	0.04	0.41	0.43
Early[4]	8.21	8.17	0.05	0.12	8.21	8.17	0.05	0.60	0.44
Late[5]	8.88	8.94	0.06	0.19	8.94	8.89	0.06	0.54	0.19
Sweetfeed mix[3]	0.32	0.35	0.05	0.68	0.37	0.30	0.05	0.20	0.24
Early[4]	0.32	0.34	0.05	0.80	0.35	0.30	0.08	0.50	0.74
Late[5]	0.32	0.34	0.05	0.66	0.35	0.34	0.07	0.80	0.40
Alfalfa pellet[3]	0.21	0.28	0.03	0.02	0.27	0.21	0.02	0.02	0.72
Early[4]	0.21	0.26	0.04	0.23	0.27	0.21	0.06	0.03	0.62
Late[5]	0.20	0.29	0.03	0.04	0.28	0.23	0.04	0.15	0.28

[1]NO-IMP = no implant given; IMP = implanted with Revalor-G (Merck Animal Health; Madison, NJ)
[2] NO-TAN = no tannin supplement; TAN = tannin supplemented at 0.30% DMI
[3] Whole study period = d 0 to d 90
[4]Early = d 0 to d 45
[5] Late = d 45 to d 90
[6]Total DMI = estimated forage intake + sweetfeed mix intake + AHCS bait (alfalfa pellet) intake.
[7] Forage intake was estimated using NRC (1996) special considerations for all-forage diets intake equation.

Table 6. Effect of tannin supplementation and growth implants on blood metabolites, creatinine and N and P metabolism of stocker steers

Item	Growth Implant[1]				Tannin[2]				
	NO-IMP	IMP	SEM	*P*	NO-TAN	TAN	SEM	*P*	*TAN x IMP P*
n, animals	8	10			9	9			
Blood urea nitrogen, mg/dL[3]	12.1	13.0	0.47	0.08	12.3	13.0	0.48	0.12	0.79
Early[4]	13.4	13.7	0.74	0.73	13.5	14.2	0.41	0.48	0.40
Late[5]	9.5	10.8	0.63	0.14	9.9	10.6	0.75	0.46	0.74
Urinary N, ppmN	1063	1496	255	0.30	1445	1162	275	0.49	0.24
Creatinine, mg/dL	12.3	17.4	3.8	0.46	17.4	12.9	4.01	0.45	0.41
Fecal N, % N	1.96	1.94	0.06	0.98	1.94	1.96	0.05	0.74	0.55
Fecal P, % P_2O_5	1.09	1.09	0.06	0.95	1.08	1.10	0.07	0.77	0.65

[1] NO-IMP = no implant; IMP = implanted with Revalor-G (Merck Animal Health; Madison, NJ)
[2] NO-TAN = no tannin supplement; TAN = tannin supplemented at 0.30% DMI
[3] Whole study period = d 0 to d 90
[4] Early = d 0 to d 45
[5] Late = d 45 to d 90

Table 7. Effects of growth implants and tannin on emission measurements of stocker steers grazing pivot-irrigated pasture at ARDEC, Fort Collins, Colorado.

	Growth Implant[1]				Tannin[2]				
Item	NO-IMP	IMP	SEM	*P*	NO-TAN	TAN	SEM	*P*	*TAN x IMP P*
n, animals[3]	7	9			7	9			
CH_4, g/d[4]	220	211	5.68	0.15	214	217	4.21	0.24	0.33
Early[5]	245	233	10.2	0.24	234	244	7.63	0.37	0.82
Late[6]	194	190	5.71	0.41	193	191	3.93	0.68	0.49
CO_2, g/d[3]	7651	7662	139	0.83	7668	7645	100	0.84	0.26
Early[5]	8036	7970	209	0.76	7939	8067	210	0.55	0.31
Late[6]	7264	7357	189	0.63	7394	7227	187	0.39	0.61
EI, g CH_4/kg gain[3]	234	218	9.71	0.19	221	231	13.2	0.23	0.21
Early[5]	288	252	11.6	0.03	262	273	17.4	0.55	0.60
Late[6]	183	185	20.7	0.93	179	189	20.8	0.63	0.42
MY, g CH_4/kg total DMI[3]	24.4	23.2	0.69	0.09	23.4	24.1	0.51	0.10	0.25
Early[5]	28.2	26.5	1.27	0.20	26.5	28.1	1.31	0.26	0.79
Late[6]	20.6	19.9	0.60	0.25	20.3	20.2	0.64	0.79	0.44
O_2 consumption[3]	5602	5621	86	0.88	5647	5576	82.6	0.83	0.25
Early[5]	5944	5896	195	0.81	5871	5969	196	0.63	0.44
Late[6]	5257	5350	127	0.48	5419	5188	115	0.07	0.85
H_2 (g d^{-1})[3]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Early[5]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Late[6]	1.02	1.02	0.10	0.98	1.00	1.03	0.14	0.82	0.37

[1] NO-IMP = no implant; IMP = Implanted with Revalor-G (Merck Animal Health; Madison, NJ)

[2] NO-TAN = no tannin supplement; TAN = tannin supplement (0.30% DMI)

[3]Only animals with ≥ 10 "good" (define) visits were selected for emissions-related analysis within period.

[4]Whole study period = d 0 to d 90

[5]Early = d 0 to d 45

[6]Late = d 45 to d 90

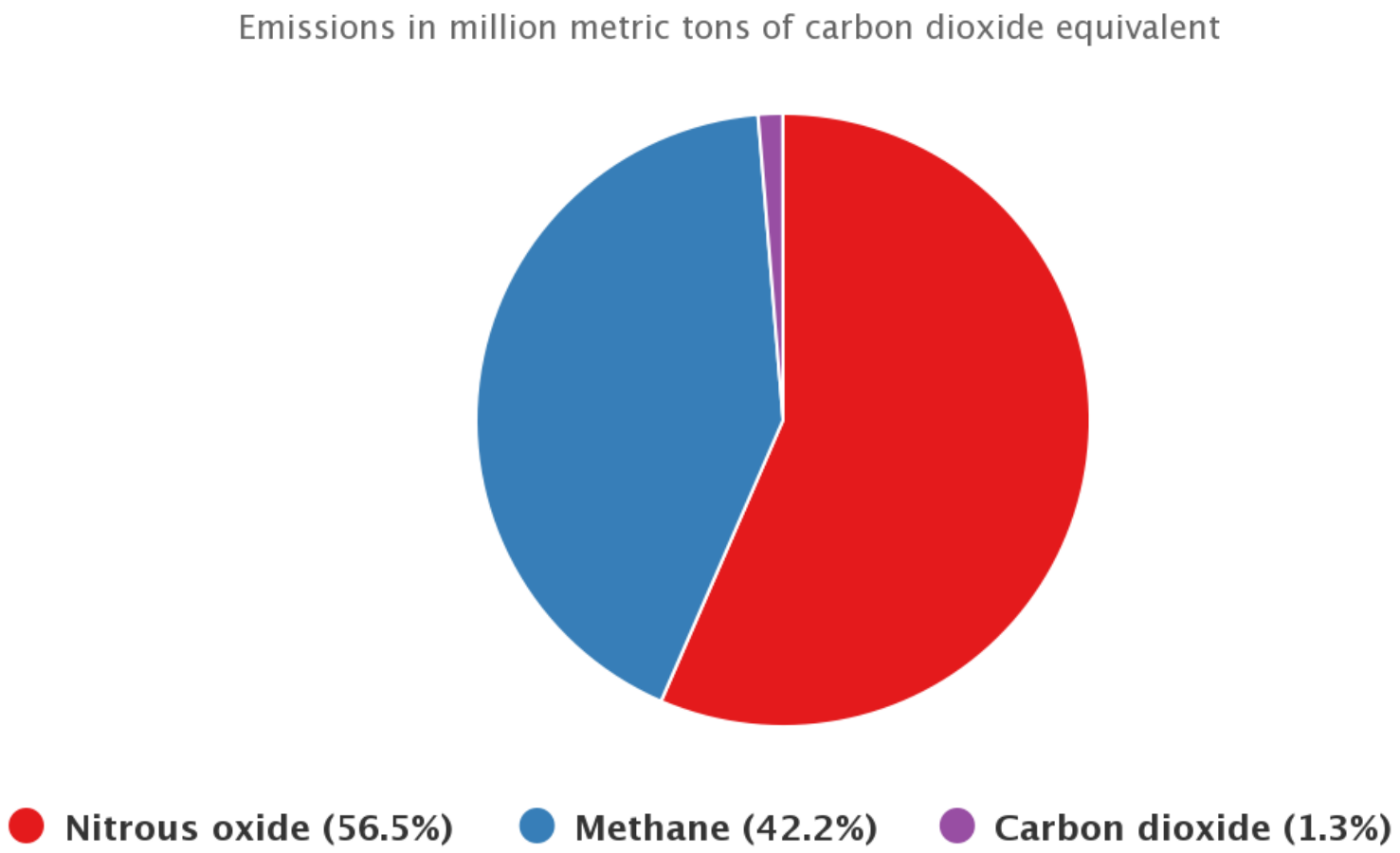

Figure 1. EPA U.S. Greenhouse Gas Emissions from Agricultural Activities by gas in 2020 (altered from EPA, 2021)

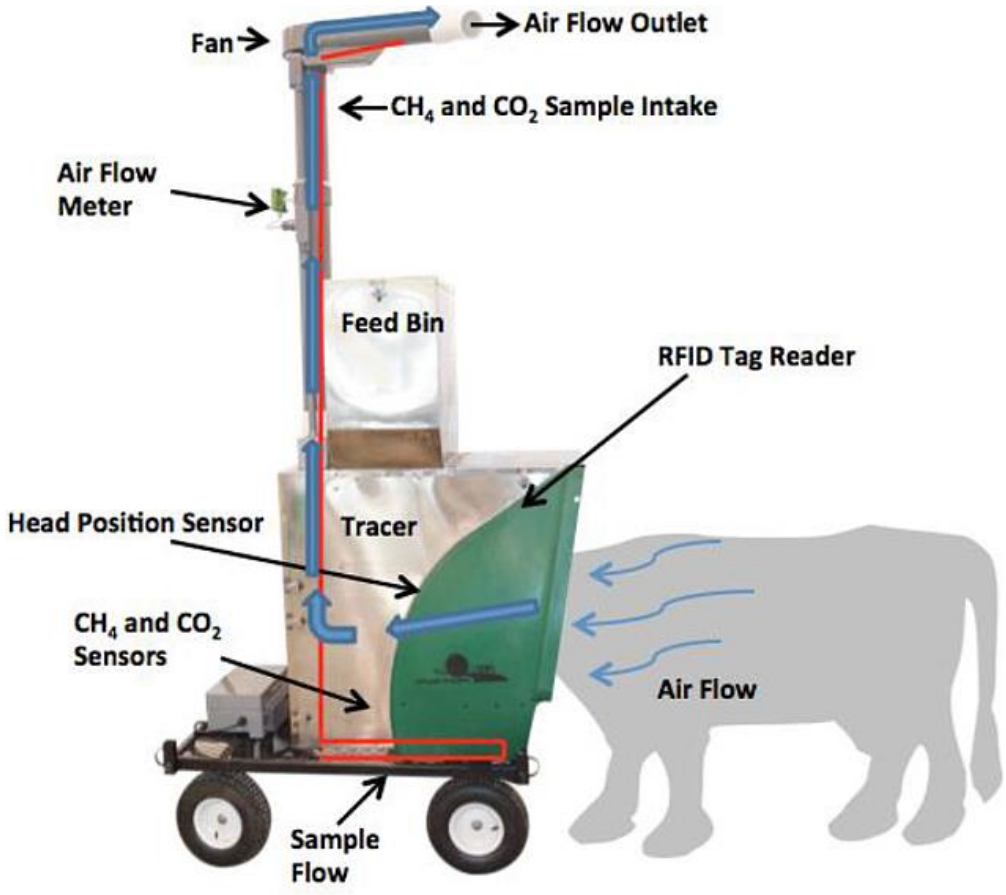

Figure 2. Components of the Automated Head-Chamber System (AHCS) used for measuring enteric emissions production (Altered from Hristov et al., 2015).

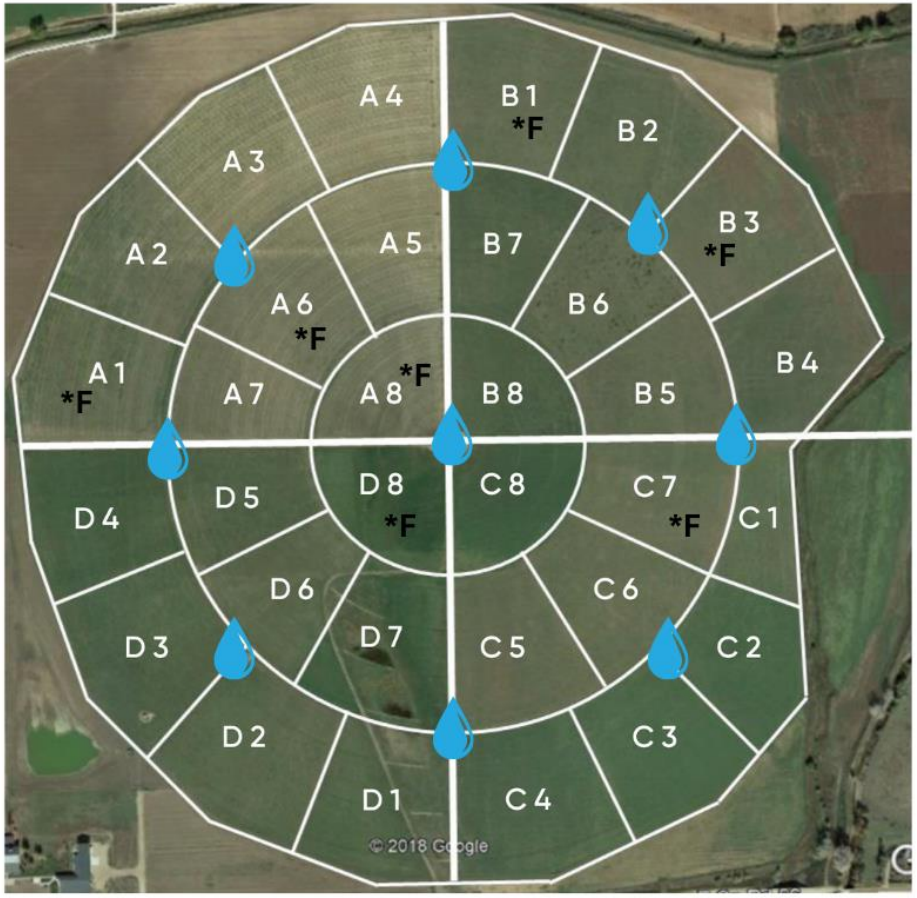

Figure 3. Sections within the pivot-irrigated pasture at the CSU Agricultural Research, Development, and Education Center (ARDEC) Fort Collins, Colorado (Altered from Shawver et al. 2021).

*F = sections of the pivot-irrigated pasture where forage was collected.
White lines represent section electric fencing perimeter.

Figure 4. Smartfeed Pro Trailer used to deliver sweetfeed mix supplementation with and without tannin inclusion.

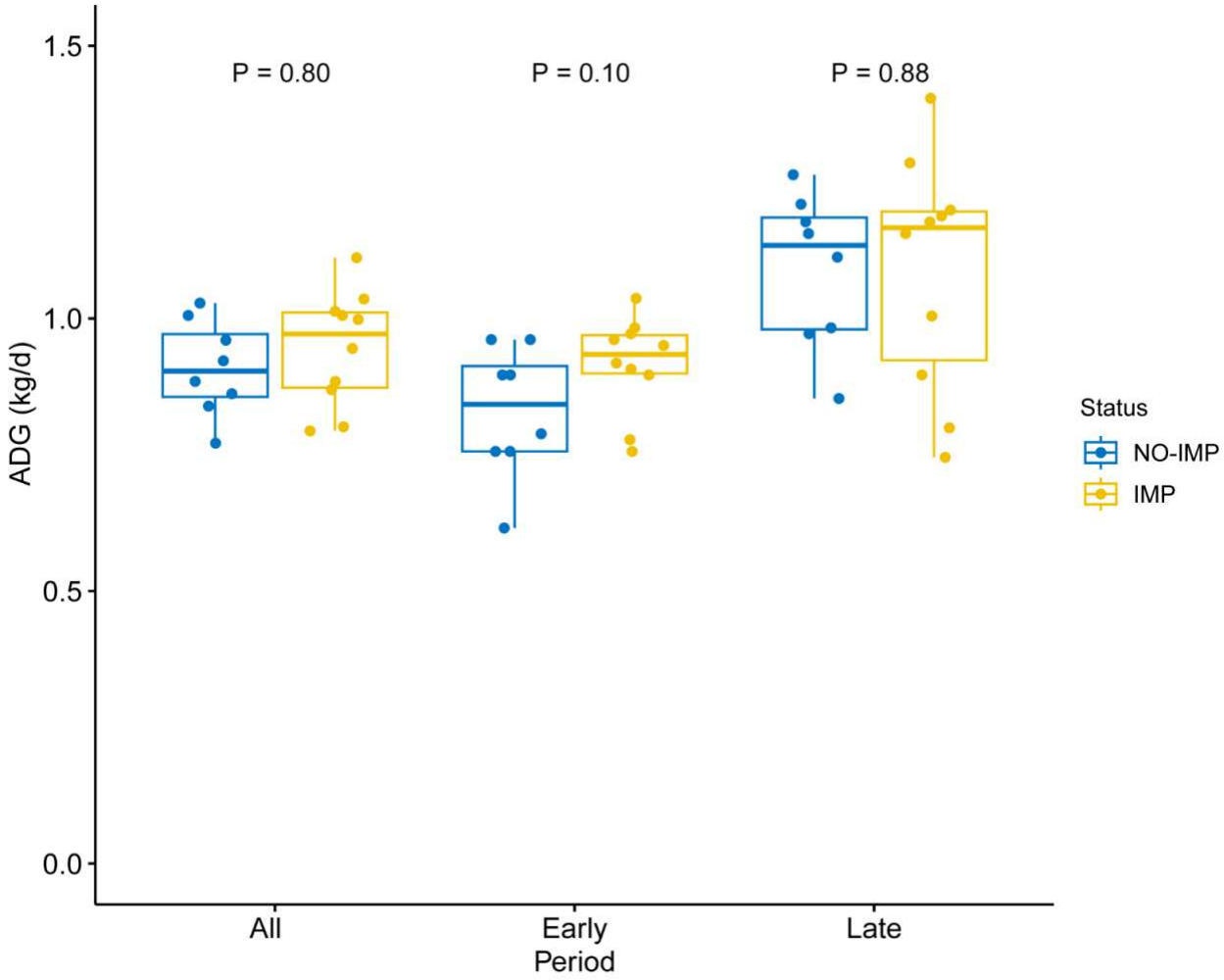

Figure 5. Average daily gain (ADG; kg/d) of steers with implant (IMP) and without implant (NO-IMP) for the whole 90 d study, the early period (d 0 to d45), and the late period (d 45 to d 90).

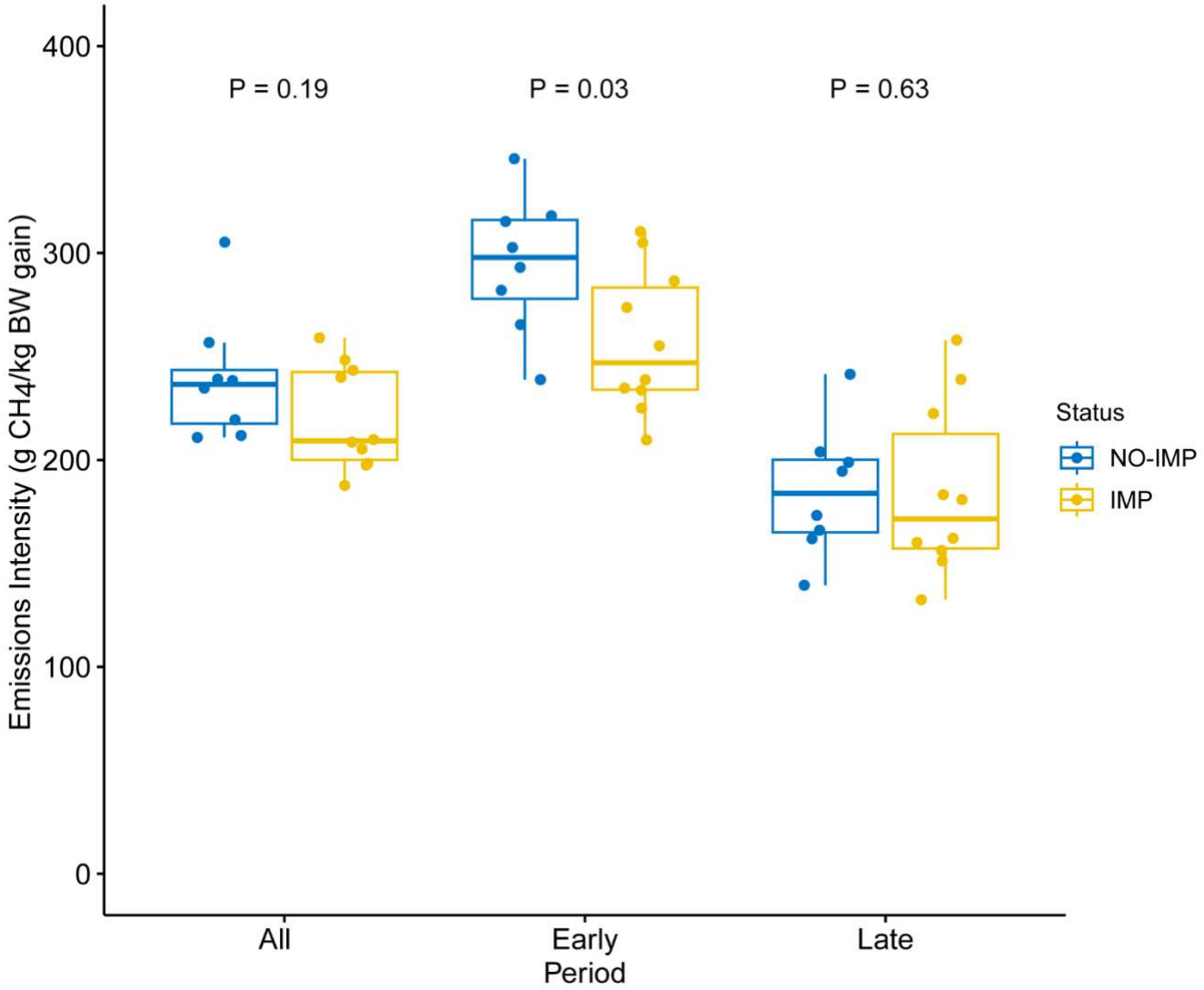

Figure 6. Emission intensity (g CH_4 / kg gain) of steers with implant (IMP) and without implant (NO-IMP) for the whole 90 d study, the early period (d 0 to d 45), and the late period (d 45 to d 90).