Use of Nutrition and Precision Technology to Improve Health, Performance, and Alleviate Stress of Beef Cattle

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Animal and Poultry Sciences

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April 28th, 2023
Blacksburg, Virginia

Keywords: beef cattle, trace minerals, yeast, monensin, condensed tannins, coccidiosis

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ABSTRACT

Concerns about beef production are growing among consumers, questioning the use of antibiotics, hormones, and metaphylactic treatments, as well as its environmental footprint. Therefore, beef production systems need to be more efficient to increase productivity while using less resources to become sustainable and reduce environmental impact. There is a need to develop and apply non-pharmaceutical alternatives to improve health, feed efficiency and performance of beef cattle. We investigated four different targeted strategies to enhance performance of beef cattle: 1) The effect of an injectable multi-mineral complex supplementation for grazing beef cows on overall mineral status, fertility, and subsequent offspring performance. Two doses of the trace mineral injection increased in pregnancy rate after artificial insemination, with a greater impact on cows with poor body condition score; 2) Inclusion of a yeast-derived product combining live yeast (probiotics) and cell wall components (prebiotics), on performance and physiological responses of beef steers during backgrounding and finishing phases. Including a yeast-derived product into a finishing diet containing monensin did not improve performance, physiological responses, and carcass quality of feedlot cattle. Nonetheless, inclusion of the yeast derived product as a substitute of monensin during the backgrounding and finishing phases decreased feed intake without affecting growth, with an overall improvement in feed efficiency; 3) Use of phytotherapy (condensed tannins) to reduce protozoa parasites load and prevent coccidiosis in peripartum beef heifers and their newborn calves. Daily supplementation of condensed tannins reduced coccidia load

in heifers and newborn calves, although this reduction was transient; and 4) following the smart farming approach, validate the use of an automated scale system for grazing or feedlot beef cattle, which was able to accurately measures body weight in grazing and feedlot systems for growing and mature beef cattle while reducing cattle handling, without disrupting feeding behavior, decreasing the probability of animal lesions, accidents and optimizing labor.

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GENERAL AUDIENCE ABSTRACT

Diet diversification is a response of a growing economy, growing population, and urbanization, which increases the demand of meat products, especially beef, in substitution of staple foods. However, concerns around beef production and its impact on the environment are becoming more relevant for consumers. The need to produce more with less resources requires a sustainable intensification process. Specifically, beef cattle production systems are less efficient when compared to poultry or swine in terms of time and amount of feed required. Beef production systems need to focus on environmental and socially conscious strategies to satisfy the demand, increase animal health, productivity, and profitability. One approach is based on nutritional strategies and applied technologies to enhance productivity, efficiency, welfare, and health. In this dissertation we present three different strategies to improve efficiency of beef production systems: 1) using injectable trace minerals supplementation to ensure adequate mineral nutrition in grazing cows, improving their pregnancy rate to artificial insemination, particularly when cows are in poor body condition; 2) use of yeast as natural additives to successfully substitute antibiotics as growth promotants in feedlot diets with an improvement in feed efficiency; 3) use of secondary plant compounds, such as tannins, to substitute prophylactic antibiotic utilization for coccidiosis in cows and newborn calves. We demonstrate three successful strategies that can improve health, feed efficiency and reproductive performance of beef cattle, all of which are important to achieve greater productivity and profitability in beef production systems.

To my dad, who believed in me since day one.

Acknowledgements

First, I would like to say thanks to my parents Daniel and Elizabeth, and my brother Bruno, for their continuous support during this journey. Being away from home for 5 years has not been easy, but their constant support made this an unforgettable experience. They were my anchor and my reason to not give up.

Mom and Bru: thanks for being there, for listening, and for understanding me even when I could not. Thanks for coming and staying with me, it was a dream come true to be able to share all the things that I love and enjoy here with both of you. You are the most precious thing I have in life. I love you both!

Dad: I love you, and I miss you every day. Thanks for believing in me, thanks for being proud of me, thanks for loving me. You were always my support, you never doubted me, and you encouraged me to take this opportunity and do what I love. My heart will be always with you, and I know you are with me, watching me from above.

Especially thanks to my husband Guille. You left everything behind and agreed to come with me to a foreign country so we could go through this adventure together. We built so many special memories together that will be with us for the rest of our lives. Thanks for always being by my side, for believing in me, and for loving me every day. I cannot be more grateful to have you in my life, I love you!

Thanks to my family. We have lost a lot these past years, but that taught me how much we care one for another, and that I can count on each one of you. I know as a fact that each of you has made what is in your power to help me thrive! Despite the distance you make me feel that you were by my side all the time, and I will remember that forever.

Especially thanks to my grandparents Candi y Cacho, aunt Lori, and uncle Edgardo. I know you made the impossible to help me in every step. Thanks for always being there for me, thinking about me, loving me, and sharing my joy. To my aunt Silvia, uncle Ricardo, my cousins Giorgi y Anto, and the little ones, Martin, Franco, and Pauli, who filled my life with hope and happiness. Thanks for being family, for always being there rooting for me, and cheering me up when I needed it most.

To my dearest friends, Alice, and Fabi, who were always there to talk, to give me strength and support me when I needed it most. No matter the distance we were always there for each other. You are the best, and I am more than grateful for your friendship.

To the true friends that I found here who immediately accepted me for who I am. Alyshia and Sathya you were the first two people I met here, in a new class, new university, new country. Thanks for including me in your group and continuing our friendship during all these years. Caro, friend and neighbor, thanks for the beautiful friendship we started here, I am thankful LAIGSA brought us together, I hope life brings us back together again. Barb, I met you since your first day here, thanks for all the adventures and good moments we shared together, in Blacksburg, Texas, Oklahoma, and even New York. You are a wonderful person and friend, never change. I hope life brings us back together again. Mil, I feel like I have known you forever. Thanks for this beautiful friendship, for all the laughs and memorable moments we had together, I will keep those in my heart forever. We will be waiting for you back home. Nich, you have become part of our family. We shared everything for 5 years, grad school, celebrations, holidays, and even your wedding. Thanks for being such a good friend, I am sure life will bring us together many times more. Gabi, we met at a birthday party by chance and since

then we have built a special and wonderful friendship. We ended up having similar stories, families, interests, and background. I am truly grateful for finding you here, thanks for all the great moments we shared together, and thanks for always being there for me. You become family. I am sure life will bring us together again. You can count on me always. Uruguay will be waiting for you.

Thanks to Gustavo, Vero, and Cami. You opened the doors of your house and your heart and welcomed me as part of your family. Thank you so much for being there, for the talks, and for the great moments we shared together.

Thanks to Vero, my psychologist. You saved me from my most difficult moment. Thanks for helping me, for listening, and encouraging me to be better every day.

Thanks to Alvaro y Virginia, my mentors. Thanks for believing in me back when I was still an undergraduate student. You gave me the opportunity and the tools to start a career as an animal scientist, and to become a better person and a better professional.

Thanks to Dr Vitor Mercadante. You allowed me to make my dream come true. Thanks for accepting me as your student after a skype interview, even knowing that I was a nutrition enthusiast. Thanks for believing and trusting in me during all these years. And special thanks for allowing us to be part of your family, for listening to me when I needed it most, and for supporting me during the roughest personal moments. I will always be thankful to have such a great person as an advisor. You have a beautiful family, and I wish you all the happiness in the world. I hope you visit us in Uruguay!

Thanks to Dr David Gerrard, you gave me the immense opportunity to be here today, you changed my life, and I will be deeply grateful forever.

Thanks to my committee members, Dr John Currin, Dr Alan Ealy, Dr Samer El-Kadi, and Dr Robin White, for agreeing to be part of my committee and share all your knowledge with me. Thanks to Dr Darren Henry for all your help analyzing and discussing data. I learned so much from every meeting, thanks for your kindness.

Thanks to my lab mates and friends, Hannah, Trinity, and Claire. It was my pleasure to work with all of you. Thanks for all the good moments we shared, and all your help along the way. I will keep those memories with me forever. You become real friends in such a brief time. I hope life brings us together again.

Thanks to Chad Joines, Henry, Mead, Jake, and Jaelyn, for always being willing to help. Thanks to Dr Gabriel Pent and all the great people that work at the SVAREC. Thanks to Tracey Redifer. Without all of you, research projects would be impossible to accomplish.

Thanks to Dr Sally Johnson, Sasha Simpkins, and Pat Williams, for your support, and always being willing to help.

Thanks to Dr Cindy Wood, Dr Emma Helm, and Dr Katie Heiderscheit for giving me the opportunity of being a TA and finding a passion in there that I did not know I have it. I learned a lot from all of you, thanks for being so kind to me.

Accepting this opportunity was a challenge and an amazing experience at the same time. I will never forget all the things that I experienced, enjoyed, and learned here. Being from such a small town in a small country and being able to be here completing my program is more than a dream. I am coming back home being a completely different person, with a lot of learning to share. Thank everyone that made this possible.

I can say that I am proud, Stefi.

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List of abbreviations

ADG: average daily gain

AWW: adjusted weaning weight

BW: body weight

BCS: body condition score

CFU: colony forming units

CIDR: controlled internal drug release

CT: condensed tannins

Cp: ceruloplasmin

DM: dry matter

DMI: dry matter intake

DPP: days post-partum

DRT: days receiving treatment

FE: feed efficiency

FTAI: fixed time artificial insemination

HCW: hot carcass weight

Hp: haptoglobin

IGF-1: and insulin-like growth factor -1

LD50: lethal dose 50

OPG: oocysts per gram of feces

PSM: plant secondary metabolites

TMR: total mixed ration

VFA: volatile fatty acids

Chapter 1 : Introduction

Global population is expected to continue growing and reach 9.7 billion by 2050, which means an increase of almost 2 billion in the next 30 years according to the United Nations Department of Economic and Social Affairs (2022). Along with this, food consumption, measured in kcal/person/day, has increased 17% in the last four decades, and it is expected to increase 10.7% more by 2050. This predicted increase will be 14.5% for developing countries and only 3.8% for developed countries (Alexandratos & Bruinsma, 2012).

The composition of food has also changed, with an increase in the proportion of livestock products and oils, in substitution of staple food, especially in developing countries (Alexandratos & Bruinsma, 2012). Diet diversification is a response of a growing economy, growing population, and urbanization (Mottet et al., 2018). Greater income achieved in some large developing countries, like Brazil and China, caused a great impact on meat and dairy products demand (Alexandratos & Bruinsma, 2012; Mottet et al., 2017).

Since the 60's meat consumption has doubled its demand; meat consumption in developed countries is on average 80kg/person, while for developing countries is 28 kg/person. East Asia is expected to increase 60% its demand for a total of 71kg/person, while South Asia is expected an increase of more than 300%, growing from 4 to 18kg/person of meat. Meanwhile, developed countries are expected to have a demand of 91kg/person of meat (14% increase). Even though the expected demand of these products in kg/person, are not close to the developed countries demand, regions of south, central, and east Asia are the most populus regions of the world representing 55% of the global

population (United Nations Department of Economic and Social Affairs, 2022). Certainly, the tendency to a greater inclusion of meat in global population diets will have an impact on demand and production.

On the other hand, a growing part of the population is starting to show concerns around meat production and its impact on the environment, like water contamination, overgrazing, deforestation, and methane emissions (Sanchez-Sabate et al., 2019), as well as the impact on animal welfare, animal raising conditions, and health and safety of the final product (Biscarra-Bellio et al., 2023).

Natural resources, such as water and agricultural land are becoming limited, and livestock production systems need to produce more with less resources (Schneider et al., 2011). It is estimated that livestock production use 2.5 billion ha of land (Mottet et al., 2017), and this could be reduced through efficiency improvement. This intensification process need to be sustainable in the long term to satisfy the demand while minimizing the impact on the environment (Terry et al., 2021).

Beef cattle production system has a poorer feed efficiency when compared with other meat production systems, like poultry or swine (Mottet et al., 2017, 2018), in terms of time and amount of feed required to achieve finished weights. However, beef is considered a great source of protein, minerals and vitamins, that contributes to food security (Mottet et al., 2017, 2018; Terry et al., 2021), and does not compete with human food. Improving system efficiency is going to be essential to increase productivity and profitability, in an environmental and social conscious manner (Rotz et al., 2019).

Efficiency could be related to feed management and conversion, herd health and death losses, reproductive success, weight gain and finishing time, and environmental

footprint (Rotz et al., 2019; Terry et al., 2021). In addition, there is a tendency to increase natural and organic beef production, that does not allow the use of antimicrobials as growth promotants, hormonal implants, and metaphylactic treatments (Wileman et al., 2009). All of these technologies are widely used in the beef cattle industry and have been shown to improve efficiency, productivity and profitability (Wileman et al., 2009).

Use of applied technologies and nutrition to make beef production systems more efficient, productive, and profitable, while reducing environmental impacts, will be essential for the beef cattle industry in the future, where we can assure animal welfare and health while raising them.

Chapter 2 : Literature review

Beef cattle productive efficiency will depend on feed quality, proportion of non-productive breeding animals in the herd, and animal performance, measured through growth rate, health, and genetics (Mottet et al., 2018). Based on these, we propose three different ways to improve productive efficiency using targeted strategies: 1) Use of trace minerals to improve reproductive performance of grazing beef cows; 2) Inclusion of natural additives to improve stress response and feedlot performance of growing and finishing steers; and 3) Use of phytotherapy to reduce protozoa parasites infection in grazing heifers and its offspring.

Moreover, precision livestock farming, defined as the application of technology, and tools to be able to monitor and manage animals remotely, has as main objective to monitor animal health and welfare to improve productivity and reduce environmental impact (Berckmans, 2017; Tullo et al., 2019; Werkheiser, 2018). Most importantly, these technologies will allow to take action in real time, even in big farms, through a continuous and automated monitoring (Berckmans, 2017; Schillings et al., 2021).

There is a tendency of productive system intensification (Tullo et al., 2019), in order to produce more using less resources. In this scenario, an increase in farm size and animal density, make animal health, welfare, and environmental impact become ever more relevant to achieve economic and environmental sustainability (Tullo et al., 2019; Werkheiser, 2018). Precision livestock farming technologies have the potential to improve health and welfare on real time, decreasing negative affective experiences (Schillings et al., 2021).

One essential measure in beef cattle production systems, which can be used for diverse decision-making processes, is body weight. Evolution of body weight is used to detect the presence of health disorders, nutritional disorders associated with feed intake or feed quality, and to calculate growth rates and profitability of the production system based on weight gain curves (Segerkvist et al., 2020). Accordingly, it is essential to measure body weight regularly and accurately in precision livestock production system (Laca, 2009). However, the main problem of measuring body weight frequently is animal movement, which can be labor intense, interferes with animal environment (Charmley et al., 2006) and cause heavy breathing, loss of grazing time, reduction in appetite and feed intake, which collectively negatively impact animal performance. Based on these, we propose a fourth strategy on precision livestock farming: 4) validate the use of an automated scale for grazing or feedlot beef cattle.

Minerals

Minerals are inorganic compounds required in smaller quantities, essential for immunity, health, maintenance, growth, lactation and reproduction (Arthington & Ranches, 2021; Kegley et al., 2016; Olson, 2007; Smith & Akinbamijo, 2000). Beef cattle requires at least seventeen different minerals that are classified in macro and microminerals based on the required amounts of each one. Required macrominerals are: calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), chlorine (Cl), and sulfur (S) (NRC, 2000). Calcium and phosphorus are the most abundant minerals in the body, both required for bone formation, and the concentration in the diet should be regulated to maintain a 1-2:1 Ca-P ratio, and no more than 7:1 to avoid negative impact on growth and performance and calcium absorption (Dowe et al., 1957; Wise et al., 1963). Calcium deficiencies can cause rickets in young animals and osteomalacia in adult animals (NRC, 2000). Phosphorus deficiencies are associated with impaired growth and fertility, reduction in appetite and weakened bones (Karn, 2001). However, an excess in phosphorus supplementation will have a negative impact on the environment through phosphorus excretion in feces, pollution, and water eutrophication, without extra benefits in animal performance (Erickson et al., 2002; Prados et al., 2017). In general, well managed pastures will have enough content of calcium and phosphorus to cover the requirements, without the need for an extra supplementation (NRC, 2000). Cereal grains are characterized by low Ca concentration, and relatively high in P content, for which highly inclusion of grains in the diet will require Ca supplementation.

Magnesium has a role in the nervous system and carbohydrate metabolism; to keep Mg homeostasis cattle require a constant supply of Mg, because only 20% is

absorbed, and the rest eliminated in feces (Kemp et al., 1961; Martens et al., 2018). Deficiencies in Mg cause problem in calves like anorexia, excitability and convulsions, and hypomagnesemia in lactating and pregnant beef cows that could cause mortality if not treated (Doncel et al., 2021; NRC, 2000). Deficiencies in Mg are associated with a reduced availability from the diet (Martens et al., 2018); an excess of nitrogen (N), K, and P in fast-growing or lush grasses (Doncel et al., 2021) would interfere and decrease Mg ruminal absorption (Kemp et al., 1961).

Potassium is the major cation in intracellular fluid, and together with Na (major cation in extracellular fluid) and chlorine (major anion in extracellular fluid) are needed to regulate osmotic pressure, acid-base balance and fluid balance in the tissues (Henry, 1995). A deficiency of potassium in grazing cattle is mostly unlikely to happen, however diets based on cereal grains tend to be deficient in this mineral. The use of meals from oilseeds is a good source to supplement potassium. Deficiencies on this mineral can cause a reduction in feed intake and weight gain (NRC, 2000). Sodium and chlorine are generally supplemented in the form of common salt (NaCl) (Henry, 1995). Salt can be used to regulate feed intake in cattle, with a high tolerance when water supply is assured (NRC, 2000).

Sulfur, is actually required for rumen bacteria growth and metabolism, in particular cellulolytic bacteria (Spears et al., 1976), and to synthesize sulfur-containing amino acids (Drewnoski et al., 2014). An excess of sulfur will impair performance, feed intake, dietary net energy, carcass merit, and trace mineral absorption (Drewnoski et al., 2014; Zinn et al., 1997). Lately, sulfur excess become more common due to greater contents of this mineral in distillers grains and water sources (Drewnoski et al., 2014).

Essential microminerals, or also called trace minerals, are chromium, cobalt, copper, iodine, iron, manganese, molybdenum, nickel, selenium, and zinc (NRC, 2000). Among these, only selenium (Se), copper (Cu), zinc (Zn), manganese (Mn), iodine (I), and cobalt (Co) have practical relevance. In general, commonly used feedstuff will supply mineral requirements, however in grazing systems mineral availability depend on forage species, soil fertilization, and weather impact over it (Greene, 2016; Mcbride & Mathews, 2011).

Grasslands are the foundation of cow-calf operations. At least, 85% of cows total feed is based on pastures or native grass (Greene, 2000). The nutritional balance of grazing cattle depends on the interaction between soil, forage, weather, and animals. Minerals are the nutritional component with the highest variability in forage in comparison with energy or protein content. Minerals content in forage will depend on soil fertility, chemical fertilizers and forage type and growth (Greene, 2016). Weather will affect directly forage quantity, quality, type, and variability in production levels between years (Reeves & Bagne, 2016). In the US availability of trace minerals in the soil varies within regions, with reported deficiencies of Co, Cu, Se, and Zn in forages (Arthington & Ranches, 2021). When deficiencies in forage are known, animals should be supplemented to avoid deficiencies (Swecker, 2014).

Trace minerals deficiencies have been frequently reported in livestock grazing systems (McDowell, 1996; Olson, 2007), as the result of an inadequate intake, or the presence of antagonists that impair absorption or metabolism (Olson et al., 1999). Evaluate status from diet evaluation requires to know exactly amount and feed type

consumed per each animal, which is practically impossible in grazing conditions (Herdt & Hoff, 2011).

Requirements of trace minerals are in mg/kg or µg/kg and it variates during productive cycle (Greene, 2016), making even more difficult to define requirements and supply through feedstuff. Trace mineral status in beef cattle is not commonly assessed (Van Emon et al., 2020) and will be affected by chemical interaction and antagonists present in the gastrointestinal tract, and the intestinal absorption. And it will variate with breed, physiological state, health status, nutrition, and environment (Palomares, 2022).

Trace mineral deficiencies can be classified as marginal, subclinical, or clinical. Marginal deficiencies are not evident, but could increase animal susceptibility to secondary diseases through a reduction of immune function (Palomares, 2022). Subclinical deficiency would be identified through a decrease in growth and reproductive efficiency in the herd; while a clinical deficiency will show a specific symptom related with a specific mineral deficiency (Palomares, 2022). Physiological functions get affected progressively, because different mechanisms or processes requires different amount of minerals, which makes it even more difficult to identify trace minerals deficiencies in the herd, causing economic losses before deficiencies are diagnosed (Kincaid, 2000). When mineral intake is not enough to cover the requirements, reserve pool is the first one to be depleted, releasing required minerals into the bloodstream; after this, if the restriction continues, transport pool will be depleted (Underwood & Suttle, 1999). Once both pools are depleted, clinical signs of functional deficiencies will appear (Herdt & Hoff, 2011; Underwood & Suttle, 1999).

Trace mineral status could be assessed using blood measurements or liver biopsies when storage pool or transport forms are known. Measuring mineral concentration in blood, plasma, or serum, is less invasive, but it is not always representative of restriction periods. Minerals concentration in plasma could be maintained through homeostatic control, during periods of inadequate intake (Herdt & Hoff, 2011) until reserves are completed depleted (Kincaid, 2000). Serum concentrations are preferred to avoid anticoagulant addition (Herdt & Hoff, 2011).

To make a better diagnosis, is always recommended to measure mineral status in the storage pool, if this is accessible (Herdt & Hoff, 2011). The most accurate way to determine mineral status is through liver biopsy (Olson, 2007). Minerals will be stored in the liver, incorporated as enzymes, and released when needed (Counotte et al., 2019; Palomares, 2022). However, in some cases, with selenium and zinc, their reservoir is better represented in muscle, but biopsies of this tissue are not practical to take (Kincaid, 2000; Palomares, 2022). Herdt & Hoff (2011) defined the following adequate trace mineral concentration ranges in liver (μg/g DM) for growing and adult bovines: Cobalt 0.1-0.4, copper 50-600, iron 140-1000, manganese 5-15, molybdenum 1-4, selenium 0.7-2.5, and zinc 90-400. Trace minerals concentration under recommended ranges could be associated with deficiencies and the need for external supplementation.

Supplementation methods could be divided into indirect or direct methods. Indirect supplementation involves soil fertilization and pasture species diversity, which are an expensive and non-effective method; while direct supplementation includes mineral blocks (licking), ruminal boluses, mineral inclusion in the water, and injections (McDowell, 1996; Palomares, 2022).

The most common method of supplementation is the free-choice using a salt-based mixture (Arthington & Swenson, 2004). However, this method does not allow to target individual supplementation, and ends up with a high intake variability (Van Emon et al., 2020). Furthermore, the presence of mineral antagonists, iron (Fe), molybdenum (Mo), and sulfur, in forages would decrease even more the absorption and availability of these minerals when fed orally (Arthington & Ranches, 2021; Pogge et al., 2012). Copper and selenium concentrations in the liver are significantly decrease when Fe and Mo are present in the diet (Genther & Hansen, 2014).

Free-range supplementation does not allow to control individual intake and covers the necessary supply for each trace mineral. Voluntary mineral intake will variate during different seasons, depending on forage availability and dry matter content, type of supplement and palatability, and mineral content in the water (Arthington & Swenson, 2004). Also, there is a risk of toxicity, caused by excess of Cu, Se, and I (Swecker, 2014), that could negatively influence reproductive performance (Stokes et al., 2019).

Supplementation of injectable trace minerals allowed us to individually supply Cu, Mn, Se, and Zn based on animal weigh, in a controlled quantity that will be rapidly absorbed (Palomares, 2022; Pogge et al., 2012). After injection minerals are up taken into blood stream and used by cells as needed, or stored in the liver, binding storage proteins for long term use or excreted (Suttle, 2010). This method allows bypassing the rumen and avoid the formation of insoluble complexes and negative interactions that can occur during digestion and absorption, increasing trace mineral bioavailability (Pogge et al., 2012). Specifically, after injection, trace mineral levels will increase its concentration in bloodstream within 8-10h, increasing enzymatic activity of superoxide dismutase and

glutathione peroxidase and liver storage after 24 h post injection (Genther & Hansen, 2014; Pogge et al., 2012). These effect should persist for approximately 90 days, with a peak in enzymatic function 15 days after injection (Genther & Hansen, 2014; Machado et al., 2013; Pogge et al., 2012). These four trace minerals are associated with reproductive performance and health and immune response in different ways (Palomares, 2022), affecting the maternal side and the fetal growth and development, as neonatal health (Van Emon et al., 2020).

Copper is needed for reproductive success with a positive effect on oocyte development through early embryogenesis and pregnancy success. Presence of delayed estrus, embryonic death, or infertility, could be a symptom of Cu deficiency (Arthington et al., 1995), or Mo-induced Cu deficiency (Underwood & Suttle, 1999). Copper deficiency is mainly caused by antagonists (Van Emon et al., 2020). Manganese deficiency may inhibit synthesis of cholesterol, downregulate steroidogenesis during pregnancy (Underwood and Suttle, 1999), and negatively affect progesterone and estrogen synthesis (Van Emon et al., 2020). Due to this role Mn deficiency is associated with low pregnancy rate and abortion (Milatovic and Gupta, 2018). Selenium appears to have a role in protect the oocyte from the oxidative stress during folliculogenesis, and directly promote folliculogenesis, having as target large follicles and ovarian tissue (Van Emon et al., 2020). Zinc is related with a viable pregnancy, acting directly on the cumulus-oocyte complex, and allowing a normal luteal activity. It also may limit uterine oxidative stress, returning to estrus earlier in time (Van Emon et al., 2020). According to the European Commission regulations, trace minerals can also be classified as nutritional additives to provide specific nutrient supply for optimal growth (Pandey et al., 2019).

Feed additives

Feed additives are defined by the European Commission as products used to improve feed quality, animal performance, health, or final product quality. Feed additives can be classified based on function and origin (Pandey et al., 2019). This classification defines eight categories: additives to enhance feed intake (antioxidants, flavoring), to improve feed acceptability and quality, to enhance digestibility and absorption, to alter metabolism (hormones and implants), to improve health status, enzymes, growth promoters, and phytogenic additives.

Growth promoters: Ionophores, probiotics, and prebiotics

Additives classified as growth promoters are included in the diet with the aim to improve growth and feed utilization efficiency and enhance system productivity and profitability (Callaway et al., 2003; Russell & Strobel, 1989; Vohra et al., 2016). These additives can be divided in two big groups: medicated, or also called antibiotic growth promoters, and non-medicated additives, that includes prebiotics and probiotics (Pandey et al., 2019).

Antibiotic growth promoters had been widely used in beef cattle production systems to improve productivity and health (Bretschneider et al., 2008). Ionophores are the most common antibiotic growth promoter used in ruminant production to improve rate gain and feed efficiency throughout altering microbial ecology of the rumen (Callaway et al., 2003). Ionophores are approved by the Food and Drug Administration to be used in food-producing animals, and are classified as non-medical important based on its use to control coccidiosis in livestock species, not use in human medicine, and nonexistent cross-resistance concern (FDA, 2022; McEwen & Fedorka-Cray, 2002).

Ionophores used as feed additives are carboxylic polyether antibiotic, naturally produced by *Streptomyces spp*. bacteria, and highly lipophilic molecules (Callaway et al., 2003; Marques & Cooke, 2021). Their function is based on ion concentration gradient disruption, directly affecting protozoa and gram-positive bacteria metabolism (Constable et al., 2017). Gram-positive bacteria cells are surrounded by a porous peptidoglycan layer that allow the lipophilic ionophores to pass through and insert itself into the cell membrane; while gram-negative bacteria seems to be not affected by ionophores due to a lipopolysaccharide layer, outer membrane, and periplasmic space surrounding the cell (Callaway et al., 2003; Russell & Strobel, 1989; Rutkowski & Brzezinski, 2013). Normally, ruminal bacteria maintain a high concentration of K^+ inside the cell, and lower intracellular concentration of Na^+ (Russell, 1987), to keep a neutral intracellular pH while surrounded by the acidic ruminal environment (Simjee et al., 2012). Ionophores, adhere to cell membrane and act as a metal/proton antiporter, causing an influx of Na^+, efflux of K^+, and pH acidification, entering in a futile ion cycle (Russell & Houlihan, 2003; Russell & Strobel, 1989). In order to pump Na^+ and H^+ outside the cell, activation of ATPase systems deplete energy reserves and compromise cell viability and growth (Russell, 1987; Russell & Houlihan, 2003; Russell & Strobel, 1989). This targeted effect decreases gram-positive bacteria population relative to gram-negative bacteria, and cause certain defaunation, decreasing protozoa and fungi population that produces H_2 (Azzaz et al., 2015). This effect allows to reduce methane production, acetic acid, lactic acid, and butyric acid (Marques & Cooke, 2021), and increase propionate synthesis, resulting in more efficient use of feed energy and a reduction of acidosis risk (Azzaz et al., 2015). There is a shift in volatile fatty acids molar ratio toward propionate (Ensley, 2020), that

will increase hepatic glucogenic flux and glucose supply to the animal, providing more energy from feed (Duffield et al., 2012).

Ionophores also have an impact on ruminal nitrogen metabolism, decreasing ruminal protein degradation. This effect will reduce ruminal ammonia N and increase dietary peptides and amino acids that arrive to the duodenum (Azzaz et al., 2015; Marques & Cooke, 2021). Inclusion of ionophores in cattle diets as growth promoters started in the 70's and become a widely adopted and common practice (Bretschneider et al., 2008; Ensley, 2020), with more than 90% of large feedlots (>1000 heads) incorporating an ionophore in their diets as part of the feeding routine (Sneeringer et al., 2015), with very well-known positive results in performance and feed efficiency (Duffield et al., 2012). Ionophores commercialization in the United States represented 38% of whole antibiotic sales to be used in food-producing animals, which equals to more than 4 million kg of ionophores sold in 2021 (FDA, 2022).

There are several commercial products available, but only three are approved to be used in beef cattle: monensin, lasalocid, and laidlomycin (Russell & Houlihan, 2003). Monensin, produced by *Streptomyces cinnamonensis*, was first discovered in 1967 by Agtarap and collaborators (1967), and it became the most extensively studied and used ionophore in beef cattle, and was the first ionophoric antibiotic approved by the Food and Drug Administration (Łowicki & Huczyński, 2013). Monensin, is generally provided orally as a sodium salt mixed with the ration (Duffield et al., 2012).

Monensin positively affects cattle performance by enhancing feed efficiency through a decrease in feed intake, either maintaining the same level of weight gain or improving it (Duffield et al., 2012). Also, it reduces proliferation of parasites (Gram

positive bacteria *Micrococcus, Bacillus and Staphylococcus*) with antimicrobial properties by changes in pH and sodium/potassium balance in the cell. Monensin changes intestinal microflora and improves feed metabolism, which ensures better use of feed and increases the amount of assimilable digestible protein and fast growth (Łowicki & Huczyński, 2013).

At ruminal fermentation level, monensin reduces methane production, reduces protein deamination and excreted ammonia in urine, decreases lactic acid concentration, increases ruminal pH and enhances propionate concentration and energy availability (Callaway et al., 2003; Russell & Strobel, 1989). Greater ruminal pH enhances cellulose digestion, increasing feed digestibility and feed energy utilization (Russell & Strobel, 1989) and reduces ruminal acidosis risk (Callaway et al., 2003). In summary, monensin increases efficiency of energy metabolism in the rumen, improves nitrogen metabolism in the rumen, decreases methane production and reduces the risk of lactic acidosis and bloat (Al-Dobaib & Mousa, 2009).

Consistency of monensin beneficial effects will depend on dose, diet, animal type, and delivery method (Marques & Cooke, 2021). In modern feedlot systems, it is expected that the use of monensin improve feed efficiency in 2.5 to 3.5% depending on diet energy and dose (Duffield et al., 2012). The benefit of ionophores supplementation in grazing systems depend on forage quality and dosage, showing a quadratic response in average daily gain (ADG) and feed efficiency with increasing dose of monensin or lasalocid (Bretschneider et al., 2008). The problem with the use of low dosage, wide spectrum antibiotics as growth promoters is that they can potentially contaminate animal products and cause future drug resistance in humans due to a continuous exposure to small doses

of antibiotics present as residues in meat (5%) or milk (2.7%) (Al-Dobaib & Mousa, 2009). However, risk of cross-resistance to antibiotics caused by monensin exposure seems to be theoretical until now (Simjee et al., 2012).

Monensin is not used in human medicine as an antibiotic against bacterial infection, and most concern foodborne bacteria like *Salmonella, Escherichia coli, Campylobacter* are gram-negative bacteria, intrinsically resistant to monensin (Russell & Houlihan, 2003; Simjee et al., 2012) even when exposed to 10 times greater than normal ruminal ionophore concentrations (Edrington et al., 2003). However, some gram-negative bacteria seem to be susceptible or need an adaptation period before being able to grow in the presence of ionophores in the rumen, like *Prevotella bryantii* (Callaway & Russell, 1999); and some gram-positive bacteria, like *Clostridium aminophilum*, seem to be resistant (Houlihan & Russell, 2003).

Other gram-positive bacteria, associated with human infection and foodborne illness, are *Enterococcus spp*. and *Clostridium perfringens*. Monensin adaptation in *Enterococcus faecium, Enterococcus faecalis* and *Clostridium perfringens* was detected after continue exposure to this ionophore, that allowed growth after a lag time (Simjee et al., 2012). However this resistance is reverted after the exposure finishes, showing that there were not genes related with resistance that could be transferred and create cross-resistance to other antibiotics (Simjee et al., 2012). Apparently same happened with *Clostridium aminophilum*, that is able to develop resistant mechanisms that could be reverted (Rychlik & Russell, 2002), and that when resistance is present seems to be ionophore specific (Houlihan & Russell, 2003; Rychlik & Russell, 2002).

Ionophore resistance appears to be based on physiological selection instead of gene mutation (Russell & Houlihan, 2003). The risk of developing a therapeutic antibiotic resistance, spread between bacterium, and transfer to humans, seems low to null. Despite the lack of evidence that ionophores could create resistant strains of zoonotic pathogens, controversy around use of antimicrobials as growth promoters in food-producing animal triggered/caused the European Union to ban the usage of antibiotics as growth promoters, based on the principle of precaution, including monensin for use in beef cattle since 2006 (EC Regulation No. 1831/2003) (Millet & Maertens, 2011; Turnidge, 2003). As mentioned before, in the United States it is still allowed to use monensin and other ionophores as growth promoters, under the principle of proof, before taking any action (FDA, 2022; Turnidge, 2003). In the other hand, a risk of ionophore toxicosis also exists. However, cattle is the least susceptible specie when compared to others, with an acute oral LD50 of 26.4mg/kg BW (Ensley, 2020; Łowicki & Huczyński, 2013).

Due to the controversy around antibiotic growth promoters, European Union ban, and social pressure to ensure safety in animal products for human consumption, emerged the possibility to remove antibiotic growth promotants from animal agriculture (Jouany & Morgavi, 2007; Placha et al., 2022). However, the Animal Health Institute of America has estimated a requirement of an extra 23 million cattle if the use of antibiotic growth promoters is eliminated in the Unites States. As a result, antimicrobial growth promoters need to be replaced by non-antimicrobial drug alternatives (Gaggìa et al., 2010b).

Feed efficiency and productivity in ruminants can be improved through ruminal fermentation manipulation. Jouany & Morgavi (2007), identified four main targets to be

optimized with the use of feed additives: improve fiber digestion, reduce the risk of lactic acid accumulation and ruminal acidosis, reduce feed protein degradation, and increase protein duodenal flow, improve energy balance through reduction in methane production and greater propionate synthesis. As we mentioned before, ionophores are able to alter ruminal fermentation, decrease methane production and ruminal proteolysis, and increase propionate synthesis and protein flux to the small intestine (Marques & Cooke, 2021), without negative impact on fiber digestion (Azzaz et al., 2015).

In the need of a substitute for ionophore antibiotics to avoid a negative effect on system production efficiency, some natural feed additives have risen as possible replacements. Alternatives to ionophores are organic acids, essential oils, enzymes, probiotics, prebiotics. These natural feed additives are characterized by its beneficial effect on gut microflora, digestibility, and health (Gaggìa et al., 2010b; Placha et al., 2022)

Probiotics are live microbes that can be fed as supplements to improve animals' microbial balance. In adult animals, with developed and stable microbiota are recommended during transition periods (Jouany & Morgavi, 2007). Bacterial probiotics are used to maintain acid lactic production, improve propionate synthesis, or as antimicrobials against pathogen species as *Echerichia colii* (Krehbiel et al., 2003; Nocek et al., 2002; Peterson et al., 2007). However, the most common probiotics used are live yeast (*Aspergillus orizae* and *Saccharomyces cerevisiae*), prepared as a dry mix of live cells, growth media, and inert matrix (Jouany & Morgavi, 2007). Yeast are aerobes organisms and need to be continuously supplied to ruminants to achieve an effective concentration (10^5 CFU/g rumen content) (Jouany, 2006; Jouany & Morgavi, 2007).

Supplementing cattle with live yeast products, especially *Saccharomyces cerevisiae*, on diets with high concentration levels, improves feed efficiency, feed digestibility, weight gain and immune system, due to a decrease of rumen pathogens prevalence. It also prevents health disorders, reduces lactate production, minimizes ammonia losses, stabilizes ruminal pH, improve organic matter and fiber digestibility, increase volatile fatty acid concentration and ruminal energy utilization, and improve meat quality as a final product (Desnoyers et al., 2009; Gaggìa et al., 2010b; Shurson, 2018; Vohra et al., 2016). Productive results achieved with the use of probiotics tend to be inconsistent, with an important influence of type of diet, ingestion rate, passage rate, and individual ruminal ecosystem (Jouany & Morgavi, 2007).

A proposed model on how these probiotics work is based on an interaction/association between yeast and microbes present in the digestive ecosystem, creating a "micro-consortium" structure (Jouany, 2006). Yeasts need oxygen to survive and maintain their metabolic activity in the anaerobic ruminal environment. Fresh particles of ingested feed will be surrounded by oxygen, yeast will utilize this oxygen to metabolize sugars, and produce ethanol, glycerol, peptides, and amino acids, that will be used by other bacteria (Jouany, 2006). At the same time, oxygen consumption improves the anaerobiosis of the ruminal environment which is favorable for cellulolytic bacteria growth and activity (Jouany, 2006; Jouany & Morgavi, 2007). Another positive effect is related to the capacity of *Saccharomyces cerevisiae* to accumulate and release malate. Malate is a dicarboxylic acid that will enhance lactate uptake from *Selenomonas ruminantium* (Nisbet & Martin, 1991) and that will act as an electron sink for hydrogen in the succinate-propionate pathway (Martin, 1998), stimulating the synthesis of propionate

from lactate. This could explain the positive effect of yeast on propionate synthesis, and ruminal pH.

Greater ruminal pH enhances cellulose digestion, increasing feed digestibility and feed energy utilization (Russell & Strobel, 1989) and reduces ruminal acidosis risk (Callaway et al., 2003), promoting feed intake, nutrient supply, and animal performance (Jouany, 2006). Beneficial use of yeast supplementation is mainly associated with growth promotion of cellulolytic ruminal bacteria, and fiber intake and digestion (Shurson, 2018; Vohra et al., 2016).

When yeasts are autolyzed in the rumen, all the cytosol content, including vitamins, minerals and proteins, and part of the cell wall will be utilized and promote growth of the associated bacteria in the micro environment (Jouany, 2006). Yeast autolyzed products are classified as prebiotics. Prebiotics are a nondigestible ingredient that will stimulate growth or activity of specific microbiota that is beneficial for the host health status (Pandey et al., 2019). Yeast cell wall available compounds are β-glucan, mannan oligosaccharide and D-mannose, three carbohydrates structures that provide health and performance benefits to cattle, based on their ability to modulate immune system and bind pathogenic bacteria (Ganner & Schatzmayr, 2012; Nocek et al., 2011).

Mannan-oligosaccharides promote the growth of beneficial lactic acid bacteria, and enhance humoral immunity against gram negative bacteria, limiting the proliferation of *Salmonella sp.* and decreasing colonization and cytotoxicity of *Escherichia coli* (Baines et al., 2011; Patel & Goyal, 2012); and β-glucans has immune modulatory effect, associated with T cells to antigens or cytokines responsiveness and proliferation (Nocek

et al., 2011). This could improve cattle growth performance and feed conversion as a final result (Shurson, 2018).

Autolyzed yeast seems to increase dry matter intake and chewing activity (Kröger et al., 2017), counteracting microbial imbalance and removing toxic compound form the digestive tract caused by sub-acute ruminal acidosis (Humer et al., 2018). Yeast cell wall components act as a ligand for gram negative bacteria, removing them from digestive tract (Neubauer et al., 2018; Nocek et al., 2011), decreasing starch fermenting and ammonia utilizing bacteria (*Bacteroides spp.*), and act as a substrate for cellulolytic bacteria (*Ruminococcus* spp.) promoting its growth (Neubauer et al., 2018), even in a high-concentrate diet regimen. These effects are translated in a greater fiber apparent digestibility and the maintenance of a physiological ruminal pH, that again contributes with cellulose degradation (Kröger et al., 2017; Lei et al., 2013). Lei et al. (2013) also reported a positive effect on average daily gain and feed efficiency, and a reduction in acute phase proteins in beef steers. The reduction in acute phase proteins, as haptoglobin and serum-amyloid A, is associated with a suppression of inflammatory reaction through lipopolysaccharides (LPS) binding, avoiding translocation into circulation (Lei et al., 2013). Biogenic amines and LPS are microbe-derived toxic compounds, biomarkers of dysbiosis, and associated with the presence of sub-acute ruminal acidosis. Supplementation of yeast cell wall components showed a reduction of these toxic compounds (Humer et al., 2018; Lei et al., 2013), which demonstrates it positive effect on ruminal pH and inflammatory response in cattle.

Phytogenic additives: Condensed tannins and coccidiosis

Plants and its secondary metabolites has been used for its medicinal properties since ancient times (Jamshidi-Kia et al., 2018) with an increasing demand during last decades (Briskin, 2000) focused on human health. Recently, these compounds started to gain popularity on livestock production based on the consumers concerns around antibiotics and hormones use in food-producing animals, and its health and environmental hazards (Rochfort et al., 2008).

Phytogenic additives appear as an alternative to synthetic chemicals to improve animal health, especially in ruminants (Rochfort et al., 2008). These plant secondary metabolites (PSM) have also the ability to improve performance, feed quality, and quality of the final product, fulfilling the role of growth promoters, antimicrobials, antioxidants, gut function modulators, immunomodulators or anti-inflammatories (Pandey et al., 2019). It has been identified more than 80,000 beneficial compounds derived from plants, that can be classified in different ways depending on chemical composition, action, biological origin, or use (Hashemi & Davoodi, 2010). Based on its chemical composition can be classified in alkaloids, essential oils, saponins, acids, steroids, tannins, among others.

Tannins are polyphenols compounds, that have been classified as anti-nutritional components, due to its negative effect on nutrient digestibility, feed intake, diet palatability, and performance (Naumann et al., 2017). These polyphenols are one of the most common anti-nutritional factors widely present in various feed types, as forages, and forestry and agro-industrial byproducts (Bhat et al., 2013; Makkar, 1993).

Tannins are classified in two big groups: hydrolysable tannins and condensed tannins. Hydrolysable tannins, can be subdivided in galloglucoses, gallotannins, and

ellagitannins, depending on their chemical structure, and are characterized for being astringents and antioxidants (Koleckar et al., 2008; Smeriglio et al., 2017). However, this type of tannins are considered toxic for ruminants, causing digestion inhibition and hepatotoxic and nephrotoxic effects (Naumann et al., 2017). Condensed tannins (CT), are polymers of flavonoids, largely resistant to microbial degradation (Huang et al., 2018), and commonly found in legume forages, seed coats, and leaves of browse plants, and rarely in grass (Waghorn & McNabb, 2003).

Tannins are characterized by their ability to bind proteins (Bhat et al., 2013; Mueller-Harvey, 2006; Naumann et al., 2017). Interaction between CT and proteins in the saliva form CT-protein complexes that will negatively impact palatability due to astringency (Naumann et al., 2017). When we think in the effect on nutrient digestibility, CT-protein complexes decrease protein ruminal degradation; CT can also bind to structural carbohydrates and starch, decreasing their digestion and affecting total DM digestibility (Besharati et al., 2022; Mueller-Harvey, 2006).

Reduction of protein ruminal degradation could increase duodenal flow of dietary protein, and decrease nitrogen excretion as urea in the urine (Mueller-Harvey, 2006). This will result in greater amino acid absorption, leading to grater performance. However, this positive effect will depend on the tannin's protein-binding activity and dissociation ability post rumen. Formation and dissociation CT-protein complexes seems to be pH dependent, with formation happen at pH 3.5-7.5, and dissociation at pH <3.5, which coincides with abomasum pH (Bhat et al., 2013; Mueller-Harvey, 2006).

Antibiotic activity is one of the main functions for which PSM had been studied; especially in ruminants where bacterial gut fermentation is essential for nutrition, but also

produces non-desired secondary compounds as methane (Rochfort et al., 2008). Tannins can inhibit growth of cellulolytic and proteolytic bacteria, rumen methanogens, and cause protozoal defaunation in the rumen, affecting protein and fiber digestibility, but also decreasing ammonia N concentration and methane production (Bodas et al., 2012). Growth inhibition mechanisms could be related with tannins astringency, inhibiting extracellular enzymes, causing substrate deprivation, or directly affecting metabolism, iron deprivation, or inhibition of oxidative phosphorylation (Scalbert, 1991).

Condensed tannins effect could either be detrimental or beneficial depending on tannin's chemical characteristics, physical structure, protein-binding activity, and concentration in the diet (Rochfort et al., 2008; Waghorn & McNabb, 2003). This will variate in different species, and in the ability of microbes to adapt to CT and avoid anti-nutritional effects (Naumann et al., 2017).

Perception about tannins and its effect on ruminants has been changing during the last decade, becoming widely studied for its potential use as feed additive in ruminants (Yanza et al., 2021). Inclusion of CT in ruminants' diet at low doses (2-4% DM) has shown beneficial effects on performance and health. It has been demonstrated the ability of CT to achieve greater protein nutrition through a reduction of protein ruminal degradability, reduce methanogenesis and methane production, reduce incidence and severity of bloat, and suppress gastrointestinal nematodes parasites (Getachew et al., 2008; McMahon et al., 2000; Min & Hart, 2003; Naumann et al., 2017).

Phytogenic additives appear as an alternative to synthetic chemicals to improve animal health and treat gastrointestinal diseases, especially in ruminants (Rochfort et al., 2008; Tamminen et al., 2018; Weyl-Feinstein et al., 2014) Tannins are characterized by

its effect on intestinal bacteria, nematodes, and protozoa (Huang et al., 2018). Post ruminal effects of CT, show an antiparasitic activity, but also would stimulate host resistance through protein metabolism, with a greater protein supply to the small intestine (Jouany & Morgavi, 2007).

An anthelmintic effect of CT has been demonstrated on gastrointestinal nematodes in sheep, goats and cattle (Athanasiadou & Kyriazakis, 2004; Tedeschi et al., 2021). Condensed tannins supplementation has also shown an inhibitory effect of *Eimeria spp.* (Tedeschi et al., 2021) the protozoan responsible of causing coccidiosis (Chapman et al., 2013). Oral supplementation of CT causes a reduction of *Eimeria spp*. oocyst excretion on rabbits (Parisi et al., 2018), goat kids (Fraquelli et al., 2015; Markovics et al., 2012), and lambs (Acharya et al., 2020; Burke et al., 2013; Saratsis et al., 2012), reducing parasite load and alleviating clinical signs of coccidiosis. Condensed tannins are also effective in controlling calves diarrhea caused by protozoan (Bonelli et al., 2018). These results showed the potential anti protozoal effects of condensed tannins to prevent and control coccidiosis, in addition to the anthelmintic effect on ruminants.

Coccidiosis, is a disease that affect all livestock species, causing major performance and productivity losses (Bangoura & Bardsley, 2020; Chapman et al., 2013). Cumulative incidence in beef cattle herd tend to be 100% (Jäger et al., 2005). Economic losses related with this parasite infection were estimated to be US$723 million worldwide (Fitzgerald, 1980), impacting the cattle industry with an annual cost of US$100 million or more. Economic impact is associated with subclinical and clinical signs of this disease and treatment cost (Keeton & Navarre, 2018; Lassen & Østergaard, 2012).

In cattle, young animals are more susceptible to be infected with *Eimeria spp.* parasite (Ernst et al., 1984). Main sign of clinical coccidiosis is the presence of diarrhea and *Eimeria spp*. oocysts in the feces (Bangoura & Bardsley, 2020). Depending on *Eimeria* specie pathogenicity this disease could cause slow growth, morbidity, and even mortality (Epe et al., 2005). There are 13 different species that affect bovines, but only two have the highest pathogenicity: *Eimeria bovis and Eimeria zuernii* (Daugschies & Najdrowski, 2005).

Life cycle of *Eimeria* has 3 phases, two internal phases that include asexual and sexual reproduction, and one external or environmental phase of sporulation (Bangoura & Bardsley, 2020). Once symptoms are detected, animals had been infected since 2-4 weeks ago, and are shedding new oocysts in the environment (Keeton & Navarre, 2018). At excretion, oocysts need to become infective, and this process (sporulation) will take 1-3 days in warm and humid conditions (Bangoura & Daugschies, 2018). Once infective, oocysts will infect other animals through fecal-oral infection route. Adults animals develop certain immunity after its first infection, but become oocysts reservoir further contaminating the environment for the next naïve animals (Bangoura & Bardsley, 2020; Joachim et al., 2018; Keeton & Navarre, 2018).

Environmental load is one of the main infection sources for *Eimeria spp*. Oocyst resilience ensure the presence of the parasite in the site in diverse climate conditions (pH, temperature, oxygen levels) (Chapman et al., 2013; Marquardt et al., 1960). Once this parasite is present at the farm it quickly spread between animals, for which coccidiosis should always be considered as a herd disease (Bangoura et al., 2022; Bangoura & Bardsley, 2020). This disease is also commonly considered as an endemic problem

(Carrau et al., 2018). Infection pressure is greater in highly frequented places, as drinking through and feed bunks, and overcrowded paddocks and feedlot facilities, that increase accumulation and direct contact with feces (Daugschies & Najdrowski, 2005; Keeton & Navarre, 2018; Noack et al., 2019).

Control of coccidiosis is based on animal treatment (Bangoura et al., 2022), or in the reduction of infection pressure (Bangoura & Bardsley, 2020). Preventive treatment will be based on reduction of parasite load and oocysts excretion. However, the use of coccidiostats, despite being effective, need to be applied to all animals even those showing no symptoms (Keeton & Navarre, 2018). This practice makes selection pressure that could contribute to antimicrobial resistance, and increase the risk of chemical residues in final product (Bangoura et al., 2022; Lassen & Østergaard, 2012; Saratsis et al., 2012). An alternative will be the use of nonpharmacological treatments to decrease environmental contamination, through CT supplementation. Use of a natural product as CT has the potential to reduce parasite load, environmental contamination, without the risk of generating drug resistance.

Chapter 3 : Effect of injectable trace mineral supplementation on grazing multiparous beef cows overall mineral status, fertility, and subsequent offspring birth weight and weaning weight

Abstract

The objective of this study was to evaluate the effect of an injectable multi-mineral complex supplementation on grazing beef cows overall mineral status and fertility, and subsequent offspring performance. Two experiments were conducted to evaluate two protocols of injectable trace mineral supplementation during fall and spring breeding season. In both experiments, all cows had *ad libitum* access to diet, water, and mineral supplementation in the form of mineral blocks. Cows were enrolled in a 7-day CO-Synch+CIDR fixed-time artificial insemination (FTAI, d0) protocol, followed by natural service for approximately 65 d breeding season. Experiment 1, 1,128 Angus crossbred multiparous cows at 9 different locations during fall breeding season, were stratified by days post-partum (82 ± 18 d) and randomly assigned to one of two treatments: 1) one dose (6 mL) of an injectable trace mineral containing zinc (60mg/ml), copper (15mg/ml), selenium (5mg/mL) and manganese (10mg/ml) (Multimin® 90, Multimin USA, Ft. Collins, CO) on d -10 (MIN; n = 560); or 2) no trace mineral injection (CTRL; n = 568). No treatment effects were detected in days post-partum (P = 0.94), BCS ($P \geq 0.79$) and estrus detection (P = 0.64), neither in mineral status of copper, manganese, selenium, or zinc ($P \geq 0.28$). In addition, FTAI pregnancy rate (57%) and overall pregnancy rate (92.6%) were similar ($P > 0.1$) between treatments. Experiment 2, 986 Angus crossbred multiparous cows at 8 different locations during spring breeding season, were randomly assigned approximately 30 days before calving (d -120) to one of two treatments: 1) two doses (6 mL) of Multimin® 90 at d -120 and d -10 (MIN; n = 494);

or 2) no trace mineral injection (CTRL; n = 492). No differences between treatments were detected in days post-partum (P = 0.87), BCS ($P \geq 0.83$), estrus expression (P = 0.92), or mineral status of copper, manganese, selenium, or zinc, before (P = 0.20) and after treatment (P = 0.17). There was a treatment effect ($P \geq 0.01$) in FTAI pregnancy rate, increasing pregnancy rate 7.7% (CTRL=47%, MIN= 54.7%), with an interaction with BCS (P=0.001). Cows with BCS ≤5 achieved greater pregnancy rate at FTAI when receiving two doses of injectable trace minerals supplement. However, no difference was detected in the overall pregnancy rate (89.6%, P=0.89). In both experiments, calves were weaned at approximately 7 months of age, and 205-day adjusted weaning weight (AWW) was calculated based on birthday, age at weaning and age of dam. No differences between treatments were detected for birth BW (33.7±1.01 kg; P = 0.50) and AWW (212.2±5.0 kg; P = 0.25) when only one dose of injectable trace mineral was administered to the dam in early lactation. Similarly, when two doses of injectable trace mineral were administered birth BW (35.6±1.1 kg; P = 0.70) and AWW (259.4±6.1 kg; P = 0.83) did not differ between treatments. Therefore, injectable trace mineral supplementation did not improve overall mineral status in multiparous beef cows or influenced offspring growth performance. However, when administrated in two doses, 30 days before calving and 10 days before FTAI, increased pregnancy rate at FTAI, with greater impact in cows with low BCS.

Introduction

Beef cow-calf operations in north America rely mainly on pasture grazing and hay feeding, with none or minimum grain supplementation (Mcbride & Mathews, 2011). These production systems depend on forage availability and quality to cover their

essential nutrient requirements. Mineral deficiencies for grazing cattle have been reported over the world (McDowell, 1996). Especially for trace minerals, forage is the most significant contributor (Arthington & Ranches, 2021), and its availability varies with weather, soil type and fertilization, and forage species (Greene, 2016; Mcbride & Mathews, 2011).

For beef cattle, essential trace minerals are chromium, cobalt, copper, iodine, iron, manganese, molybdenum, nickel, selenium, and zinc (NRC, 2000). Among these, only selenium, copper, zinc, manganese, iodine, and cobalt have practical relevance when defining mineral supplementation. Trace minerals deficiencies are associated with impaired immune system, weakened metabolism, decrease in reproductive hormones synthesis, muscle degeneration, placenta retention, calves birth defects and lower birth weight, abortion, and low fertility (Arthington & Ranches, 2021; Greene, 2016). However, health issues, productive losses, and reproductive failure associated with trace minerals deficiencies or excesses, are commonly unnoticed in farm operations (Genther & Hansen, 2014; Greene, 2016; Olson, 2007).

Supplementation of these trace minerals, required in small amounts (mg/kg), with variation in requirements and supply over the year and location, is challenging. Supplementation of trace minerals through a subcutaneous injection allow us to control the dosage administrated to each animal individually (Arthington et al., 2014), increase the uptake of these minerals into blood stream, and avoid the formation of insoluble complexes in the rumen (Genther & Hansen, 2014; Stokes et al., 2019).

Dam mineral status also impacts offspring health and performance, through nutrient transfer via placenta to the fetus, and via colostrum and milk quality post-calving

(Van Emon et al., 2020). Deficiencies in Cu, Mn, Se, and Zn will impair performance and immune response (Stokes et al., 2019). Cows supplemented with Co, Cu, Mn, and Zn since the second trimester, increased their liver concentration of Co, Cu, and Zn, and cause a greater liver concentration of Cu and Zn in their offspring (Marques et al., 2016). However, the effect of trace mineral supplementation of the dam on calf health and performance had not been extensively studied.

Our hypothesis was that one or two doses of an injectable trace mineral complex supplementation, would be able to cover zinc, copper, selenium, and manganese requirements, improving fertility and overall mineral status of grazing multiparous beef cows receiving mineral supplementation in form of mineral blocks due to an additional effect between two different mineral sources with different bioavailability. Consequently, injectable trace mineral administrated at late gestation and/or during lactation, will improve calves' performance due to a greater mineral transfer, and greater concentration in colostrum and milk. Therefore, the objective of this research was to evaluate the effect of an injectable trace mineral supplementation for multiparous beef cows on fertility, mineral status, and subsequent calf pre-weaning performance. Two trace mineral supplementation protocols were investigated, a single dose prior to breeding and two doses one pre-calving and the second pre-breeding.

Materials and methods

All animals were managed in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Experiment 1: Animals, diets, treatments, and sampling

A total of 1,128 Angus crossbred multiparous beef cows at 9 different locations in the state of Virginia, were enrolled in this experiment (Table 3-1). Experimental period included, approximately, 116 days during a Fall breeding season from November/December 2018 through February/March 2019. Diet varied among locations but was similar between treatments within location. Diet was offered *ad libitum* and was composed of grass, hay, corn silage, corn grain and commodity pellets (Table 3-2). All cows received mineral supplementation in the form of mineral blocks, regardless of treatment assignment. Feeds and mineral blocks were sampled and conserved frozen until posterior mineral content analysis (Veterinary diagnostic laboratory, Michigan State University). In addition, cows had *ad libitum* access to water.

Table 3-1. Number of cows per location and treatment (CTRL: no trace mineral injection; MIN: one dose, 6mL, of an injectable trace mineral supplement on d-10) in experiment 1.

Location	CTRL	MIN
1	48	48
2	99	97
3	74	77
4	77	78
5	63	63
6	42	40
7	47	45
8	66	68
9	54	46
Total	570	562

Cows were enrolled in the 7-day CO-Synch + controlled internal drug release (CIDR) fixed-time artificial insemination (FTAI) protocol. In brief, 100- μg injection of GnRH (2 mL Factrel; Zoetis Animal Health, Florham Park, NJ) at CIDR (1.38 g P4; Zoetis Animal Health) insertion (d –10) with 25-mg injection of PGF2α (5 mL Lutalyse;

Zoetis Animal Health) at CIDR removal (d –3) followed by injection of 100- μg GnRH and TAI (d 0) by 66 h after CIDR removal. A total of 4 different sires and multiple AI technicians were used for FTAI but were evenly distributed between treatments. Ten days following FTAI, cows were exposed to natural service with fertile bulls that had previously passed a breeding soundness exam for an approximately 65 d breeding season. Within location, cows were stratified by days post-partum (DPP) and at the initiation of the FTAI protocol randomly assigned to one of two treatments: 1) one dose (6 mL) of an injectable trace mineral containing zinc (60mg/ml), copper (15mg/ml), selenium (5mg/ml) and manganese (10mg/ml) (Multimin® 90, Multimin USA, Ft. Collins, CO) on d -10 (**MIN**; n = 560); or 2) a negative control with no trace mineral injection (**CTRL**; n = 568).

Table 3-2. Average mineral content of the diet offered in Experiment 1.

	Mineral			
Feed type	Selenium, ug/g	Copper, ppm	Manganese, ppm	Zinc, ppm
Hay	0.05	5.78	70.60	29.88
Supplement	0.76	25.17	87.67	82.97
Ad-libitum Mineral	25.58	1961.14	3264.88	3279.63

Individual cows body condition scores (BCS) were recorded at days -10, 53 and 93 (end of breeding season). Estrus expression prior to FTAI was determined based on the activation of an estrus detection aid (Estrotect; Rockway Inc., Spring Valley, WI), which was placed in each cow on day -3 and recorded on day 0 as either activated (50% or more of patch removed, estrus) or non-activated (less than 50% of patch removed, not in estrus). Pregnancy diagnosis was performed through transrectal ultrasonography (EVO II, 5.0-MHz linear multi-frequency transducer, Ibex, E.I. Medical Imaging, Loveland,

CO) at 63 (FTAI pregnancy rate) and 106 days after FTAI (final pregnancy rate). Calf birth weight was collected using a scale within 24h of birth and calf sex was determined. Calves were weaned at approximately 7 months of age, individual calf weaning weight was determined and 205-day adjusted weaning weight (AWW) was calculated based on birthday, age of weaning and age of dam.

Liver biopsies were collected on days - 10 and 63 in a subgroup of 140 cows total, evenly distributed between treatments and collected at each location, to determine mineral status. Liver biopsy procedure was conducted using a Tru-Cut type biopsy device as described by Swecker (2014). After collection, liver samples, of at least 50 mg, were placed in a 6-mL trace element tube (BD Vacutainer Trace Element Serum plus Blood Collection Tube), snap-frozen and stored in liquid nitrogen until mineral content analysis was performed (Veterinary diagnostic laboratory, Michigan State University).

Experiment 2: Animals, diets, treatments, and sampling

A total of 986 Angus crossbred multiparous beef cows at 8 different locations in the state of Virginia, were enrolled in this experiment (Table 3-3). Experimental period included, approximately, 225 days during the Spring calving and breeding seasons from April to September of 2019. Diet varied among locations but was similar between treatments within location. Diet was offered *ad libitum* and was composed of grass, hay, sorghum grain and commodity pellets (Table 3-4). All cows received mineral supplementation in the form of mineral blocks, regardless of treatment assignment. Feeds and mineral blocks were sampled and conserved frozen until posterior mineral content analysis (Veterinary diagnostic laboratory, Michigan State University). In addition, cows had *ad libitum* access to water.

Table 3-3. Number of cows per location and treatment (CTRL: no trace mineral injection; MIN: two doses, 6mL each, of an injectable trace mineral at d-120 and d-10) in experiment 2.

Location	CTRL	MIN
1	135	131
2	92	94
3	18	23
4	23	21
5	77	76
6	47	56
7	46	43
8	54	50
Total	492	494

Within location (Table 3-3) cows were stratified based on predicted calving date and approximately 30 days prior to calving were assigned to one of two treatments: 1) two doses (6 mL) of an injectable trace mineral supplement (Multimin® 90), at d -120 and d -10 (MIN; n = 494); or 2) a negative control with no trace mineral injection (CTRL; n = 492).

Following calving, cows were enrolled in the 7-day CO-Synch +CIDR FTAI protocol (day -10) as previously described in Experiment 1. Estrus expression and pregnancy diagnosis (FTAI at day 65, and final at day 105) was determined as previously described. Cow BCS was determined at day -120, -10 and 105 Calf birth weight was collected using a scale within 24h of birth and calf sex was determined. Calves were weaned at approximately 7 months of age, individual calf weaning weight was determined and 205-day AWW was calculated based on birthday, age of weaning and age of dam.

Table 3-4. Average mineral content of the diet offered in Experiment 2.

	Mineral			
Feed type	Selenium, ug/g	Copper, ppm	Manganese, ppm	Zinc, ppm
Hay	<10	6.54	68.55	24.25
Supplement	<10	19.63	111.63	79.57
Ad-libitum Mineral	<100	1232.46	2400.10	2399.77

Liver biopsies were collected on d -120 and d 65 in a subgroup of 60 cows total, evenly distributed between treatments and collected at each location, to determine mineral status, following the same procedure of experiment 1.

Statistical analysis

In both experiments data were analyzed as a complete randomized design, with animal (cow and calf) as the experimental unit. The SAS (version 9.4; SAS/STAT, SAS Inst. Inc., Cary, NC) statistical package was used for all statistical analyses, with the MIXED and GLIMMIX procedures to analyze quantitative and binary data, respectively. The covariance structures were selected based on the lowest Akaike information criterion values. For pregnancy and estrus expression the model included the fixed effects of treatment, location, BCS and all interactions. Artificial insemination sire and AI technician were distributed evenly among treatments; therefore, these variables were not included in the models. For calf birth weight, weaning weight and adjusted weaning weights the model included the fixed effects of treatment, sex, location, and all interactions. Statistical differences were considered significant at $P \leq 0.05$ and tendencies were determined if $0.05 < P \leq 0.10$.

Results

Experiment 1

Cow initial BW was 594 ± 77 kg for CTRL and 599 ± 77 kg for MIN treatment. Mineral content of the diet offered per location is described in Table 3-2. As designed, no treatment differences were detected in DPP ($P = 0.94$), with 82 days in CTRL and MIN (Table 3-5). No treatments differences were either detected for BCS ($P \geq 0.79$) and estrus expression ($P = 0.64$) (Table 3-5). However, there was a location effect over DPP ($P < 0.0001$), BCS ($P < 0.0001$) and estrus expression ($P = 0.0002$), but no treatment or treatment × location interaction.

Table 3-5. Productive and reproductive parameters of cows receiving one dose of an injectable trace mineral supplement prior to breeding, in Experiment 1.

	Treatment			
Item	**CTRL**	**MIN**	**SEM**	***P*-value**
Days postpartum	82	82	3	0.94
BCS, day -10	5.7	5.8	0.2	0.87
day 53	5.1	5.2	0.1	0.79
day 93	5.2	5.3	0.2	0.81
Reproductive parameters	% (No.)			
Estrus expression	42.6 (242/568)	41.3 (231/560)	3.7	0.64
Pregnancy rate				
Fixed-time AI	58.1 (330/568)	55.9 (231/560)	3.6	0.45
Overall	92.1 (523/568)	93.0 (521/560)	2.8	0.54

Mineral status of copper, manganese, selenium, or zinc was not different, before ($P \geq 0.62$) and after treatment ($P \geq 0.22$) (Table 3-6). No effect of treatment was detected in FTAI pregnancy rate ($P = 0.45$) or overall pregnancy rate ($P = 0.54$) (Table 3-5). Nevertheless, there was an effect of estrus expression ($P < 0.0001$) and initial BCS ($P = 0.03$) on FTAI pregnancy rate. Cows with BCS equal or less than 5 had decreased FTAI

pregnancy rate (Figure 3-1), compared to cows that had BCS greater than 5; however, no treatment × BCS was detected ($P = 0.38$).

Table 3-6. Liver concentration of Cu, Mn, Se, and Zn in liver biopsies of cows receiving (MIN) or not (CTRL) an injectable trace mineral supplementation prior to breeding, Experiment 1.

	Treatment			
	CTRL	**MIN**	**SEM**	***P*-value**
Copper, μg/g dry				
Initial (d-10)	205.10	196.22	24.70	0.80
d63	231.90	244.10	21.22	0.69
Manganese, μg/g dry				
Initial (d-10)	11.80	11.90	0.89	0.86
d63	12.45	16.56	2.66	0.28
Selenium, μg/g dry				
Initial (d-10)	1.19	1.21	1.20	0.95
d63	1.16	1.29	0.07	0.22
Zinc, μg/g dry				
Initial (d-10)	128.70	134.70	8.65	0.62
d63	130.60	136.10	7.74	0.62

No differences between treatments were detected for calf birth weight ($P = 0.50$) and adjusted weaning weight ($P = 0.25$) when only one dose of injectable trace mineral was administered to the dam at early lactation (Table 3-7).

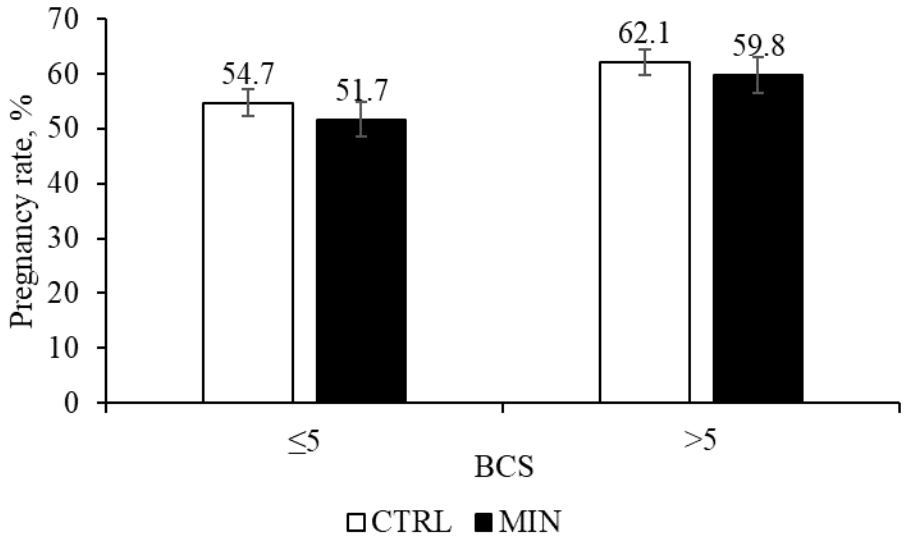

Figure 3-1. Fixed-time artificial insemination pregnancy rate by body condition score of cows receiving one dose of an injectable trace mineral supplement prior to breeding, in Experiment 1. Effect of treatment $P = 0.50$, BCS $P = 0.03$ treatment*BCS $P = 0.38$.

Table 3-7. Offspring birth weight and adjusted weaning weight (WW) in experiment 1 and 2.

	Treatment			
	CTRL	**MIN**	**SEM**	***P*-value**
Experiment 1				
Birth weight, kg	33.67	33.63	1.01	0.50
Adjusted WW, kg	213.2	211.1	4.98	0.25
Experiment 2				
Birth weight, kg	35.6	35.5	1.08	0.70
Adjusted WW, kg	258.9	259.9	6.13	0.23

Experiment 2

As in experiment 1, no differences between treatments were detected in DPP ($P = 0.87$) with 75 d in CTRL and 74 d in MIN. Estrus expression ($P = 0.92$) and initial BCS ($P \geq 0.83$) was similar between treatments (Table 3-8). Mineral status determined from liver biopsies, of copper, manganese, selenium, or zinc was similar between treatments before ($P \geq 0.20$) and after treatment ($P \geq 0.17$) (Table 3-9).

Interestingly, there was a treatment effect in FTAI pregnancy rate ($P = 0.01$), with an increase of 7.7% in FTAI pregnancy rate for the MIN group (Table 3-8). Also, there was a treatment by BCS interaction ($P = 0.001$, Figure 3-2), where MIN cows with BCS ≤ 5 achieved a greater pregnancy rate at FTAI when compared to CTRL group. Overall pregnancy rate, including FTAI and natural service, was similar between treatments ($P = 0.89$).

Table 3-8. Productive and reproductive parameters of cows receiving two doses of an injectable trace mineral supplement, first dose prior to calving and the second prior to breeding, in Experiment 2.

	Treatment			
Item	**CTRL**	**MIN**	**SEM**	***P*-value**
Days postpartum	75	74	4	0.87
BCS, day -120	5.1	5.1	0.3	0.91
day -10	5.4	5.5	0.2	0.83
day 105	5.6	5.6	0.1	0.86
Reproductive parameters	% (No.)			
Estrus expression	40.2 (198/492)	40.1 (198/494)	3.1	0.92
Pregnancy rate				
Fixed-time AI	47.0 (231/492)	54.7 (270/494)	2.8	0.01
Overall	89.4 (440/492)	89.7 (443/494)	2.4	0.89

Similarly to experiment 1, when two doses of injectable trace mineral were administrated calf birth weight (35.6±1.1 kg; $P = 0.70$) and adjusted weaning weight (259.4±6.1 kg; $P = 0.83$) did not differ between treatments (Table 3-7).

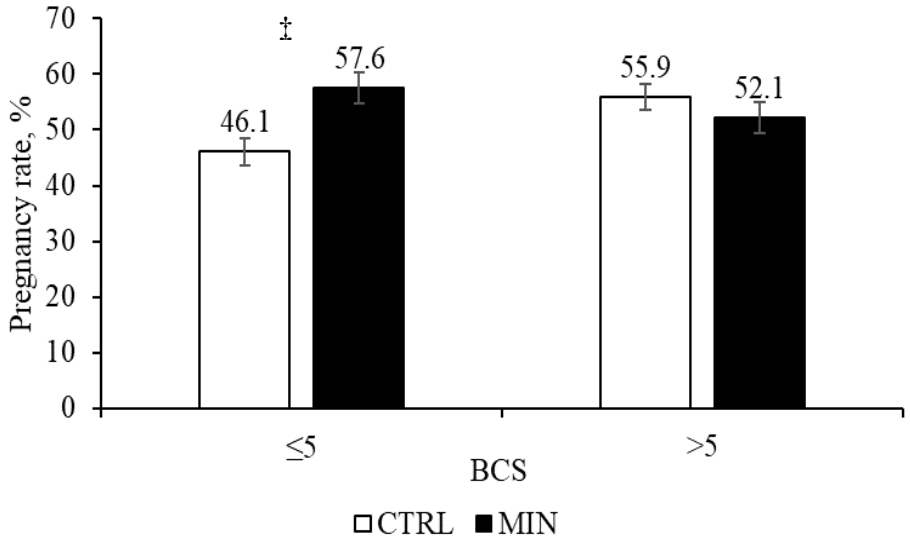

Figure 3-2. Fixed-time artificial insemination pregnancy rate by body condition score of cows two doses of an injectable trace mineral supplement, first dose prior to calving and the second prior to breeding, in Experiment 2. Effect of treatment $P = 0.50$, BCS $P = 0.58$, ‡treatment*BCS $P = 0.001$.

Table 3-9. Liver concentration of Cu, Mn, Se, and Zn in liver biopsies of cows receiving (MIN) or not (CTRL) an injectable trace mineral supplementation prior to breeding, Experiment 2.

	Treatment			
	CTRL	**MIN**	**SEM**	***P*-value**
Copper, µg/g dry				
Initial (d-10)	160.18	141.61	24.70	0.80
d65	183.60	260.55	21.22	0.69
Manganese, µg/g dry				
Initial (d-10)	11.38	10.50	0.89	0.86
d65	8.95	10.76	2.66	0.28
Selenium, µg/g dry				
Initial (d-10)	1.13	1.12	1.20	0.95
d65	1.01	1.09	0.07	0.22
Zinc, µg/g dry				
Initial (d-10)	114.75	101.52	8.65	0.62
d65	105.20	100.80	7.74	0.62

Discussion

Cows' mineral status and reproductive performance

Nutritional status is essential to determine productive and reproductive performance. Trace minerals are associated with health and reproductive performance, affecting not only the maternal side but also the fetal growth and development, as well as neonatal health (Van Emon et al., 2020). However, trace mineral status is not commonly assessed (Van Emon et al., 2020), even when trace minerals deficiencies have been frequently reported in livestock grazing systems (McDowell, 1996; Olson, 2007).

Requirements of trace minerals are in mg/kg or µg/kg and change over the production cycle (Greene, 2016), what makes even more difficult to define requirements and exact supply of these minerals through feedstuff. In the United stated, forage-based systems are commonly deficient in Cu, Se, and Zn (Olson, 2007). To define animal mineral status, body reservoir should be measured. Nevertheless, trace mineral concentration in the blood is not always representative. Homeostatic control mechanisms will maintained minerals concentration in plasma during periods of inadequate intake (Herdt & Hoff, 2011) until reserves are completed depleted (Kincaid, 2000). Liver is the tissue that best represents mineral status in the animal (Olson, 2007), as it is its major storage organ (Counotte et al., 2019).

In experiment 1, initial liver concentrations (µg/gDM) of Cu and Zn were adequate, with marginal levels of Mn and Se for CON and MIN cows. After treatment, this classification was maintained, except for Mn and Se values for MIN that became adequate levels (Kincaid, 2000). In experiment 2, initial liver concentrations of Cu and

Zn were adequate, and concentration of Mn and Se were marginal for CON and MIN cows (Kincaid, 2000). This categorization was maintained even after treatment.

Marginal concentration of Mn and Se did not cause a reduction in reproductive or productive performance. Even though, liver Se concentration is used to define Se status, muscle would be a better tissue to measure this trace mineral (Kincaid, 2000). Also, during middle and late pregnancy this trace mineral will be partitioned through the fetus as the priority (Herdt & Hoff, 2011). In the case of Mn, concentration of Mn in the liver is not always a clear reflection of Mn intake (Underwood and Suttle, 1999), and liver levels under 1 ug/g are actually considered deficient (Herdt & Hoff, 2011), which is far below from our values.

In both experiments, liver Cu adequate concentration reflects long term availability of bioavailable Cu from the diet and the presence of Cu reserves (Herdt & Hoff, 2011). Ruminants tend to store cupper avidly due to a higher risk of deficiency (Underwood and Suttle, 1999). Zinc concentration is not a clear reflection of intake (Herdt & Hoff, 2011), but if a severe restriction is occurring liver reserve will deplete over time, which again is not the case here.

Accordingly to NRC (2000), requirements of trace minerals for gestating and lactating cows are: 10 mg Cu, 40 mg Mn, 0.1 mg Se, and 30 mg Zn, kgDM consumed. If we analyze mineral content of feed offered, as a mix of pasture and supplement, cows could be restricted in Se and Cu availability. However, these low levels of Se and Cu should be covered by free-choice trace mineral supplementation offered to both groups (Table 3-2, Table 3-4).

An excess of trace minerals supply, like Cu, Se, and I, can be toxic (Swecker, 2014) or cause detrimental effects on reproductive performance (Stokes et al., 2019). A correction of the deficiency or an excess of mineral supplementation would not always have a positive response. Sometimes, deficiency in trace minerals is more related with health issues than reproductive failure (Olson, 2007). And response shows a greater variability when initial trace mineral status is adequate (Daugherty et al., 2002).

When initial liver concentration of Cu, Mo, and Zn doesn't show a deficiency, trace mineral supplementation would cause a minimal or none benefit in terms of reproductive performance (Arthington & Swenson, 2004). Supplementation of inorganic or organic source, of Cu, Co, Mn, and Zn, increased Co, Cu, and Zn liver levels. However, it did not have an impact over reproductive performance (Marques et al., 2016).

In the other hand, Olson et al. (1999) reported a decrease in pregnancy rate (15%, $P < 0.01$) when Cu, Co, Mn, and Zn supplementation, offered during 60 days before breeding, was two times the required amount (NRC, 2000). This response could be attributed to a subclinical toxicity, or a mineral imbalance due to a Cu and Zn interaction with Mn that could happen at absorption or post-absorption level (P. A. Olson et al., 1999). Same response was achieved in heifers with several doses of injectable trace minerals, with lower pregnancy at AI (53 vs 67%), but no difference in overall pregnancy rate (Stokes et al., 2019), even with an increase in Se and Cu reservoir in the liver, and lower losses of Cu during gestation with greater fetus demand close to calving.

Similarly, Daugherty et al. (2002) using two doses of Multimin90 plus vitamin E, 30d pre calving and 21d pre breeding, reported an increase in liver Cu concentration,

which last up to 160 d after last injection. However, no response in Se and Zn concentration in liver was detected, due to initial adequate status. Neither an effect over pregnancy rate was detected. The lack of negative response suggests that injectable trace minerals appear to not cause toxicity.

Results obtained in both of our experiments showed no difference in estrus expression, and overall pregnancy rate, as response to trace mineral supplementation. Pregnancy values obtained were expected values for cows with good body condition score. This suggests the absence of a toxic effect caused by the extra mineral supplementation through injection. Injectable Cu, Mn, Se, and Zn supplementation, at the initiation of estrus synchronization protocol showed an increase in pregnancy rate for embryo transfer at d 23 (48% vs. 36%, P=0.015) and d48 (43% vs. 30%, P=0.005), demonstrating and increase in embryonic survival (Sales et al., 2011).

Supplementation of inorganic or organic + inorganic Cu, Mn, and Zn, *ad-libitum* in a free-choice system, 80d before calving until 120d after calving, showed no difference in estrus expression (~80%), or pregnancy rate at FTAI, when compared to a non-supplemented group. However, this response was affected by the year, with an increase in pregnancy rate when cows were deprived of trace mineral supplementation for over a year (Ahola et al., 2004). Similarly, cows supplemented with Co, Cu, Mn, and Zn, with a mix of organic + inorganic source, showed an increase in pregnancy rate and a reduction in calving interval, especially in cows under 4 years old. This response could be attributed to the presence of an organic source of minerals or the fact that these group received a greater amount of minerals compared to the group only getting inorganic minerals (Arthington & Swenson, 2004).

These responses suggest that initial trace mineral status will reduce the response to an extra supplementation, and that reproductive performance will depend on overall nutritional management (Marques et al., 2016); achieving good results when cows are in good nutritional status regardless of extra mineral supplementation. Accordingly, when analyzing trace mineral supplementation effect on pregnancy at FTAI, there was a positive response in cows receiving two doses of injectable trace mineral, particularly in those with BCS under the optimal values (≤5). These results showed an increase of 7.7% in FTAI pregnancy rate for MIN cows. This response represents almost a 25% increase when considering only cows with BCS ≤5.

A very similar protocol to experiment 2, with two doses of injectable trace minerals, offered at 105 d before calving and 30d before AI, plus *ad-libitum* access to free choice trace mineral supplement, also showed no differences in estrus expression (P >0.51), greater pregnancy at AI in favor to the treated cows (P = 0.05, 50 vs 61%), and no difference in overall pregnancy rate (92%) (Mundell et al., 2012).

Trace mineral injectable supplementation was beneficial to cows with low BCS, increasing AI pregnancy rate when compared to the control group. These results emphasize that trace mineral supplementation has an impact when animals are restricted energetically or under a mineral deficiency. Supplementation of inorganic or organic source, of Cu, Co, Mn, and Zn, did not improve body weight or BCS during gestation when supplemented during late gestation, when control diet have adequate levels of the minerals (Marques et al., 2016). Same response was reported by Daugherty et al. (2002), when two doses of Multimin plus vitamin E was supplemented twice, pre-calving and

pre-breeding. Mineral source and feeding method, restricted or *ad-libitum* does not seem to have an effect on BCS and BW (Arthington & Swenson, 2004).

Stokes et al. (2019), reported no difference in BCS after repeated injections of trace minerals in heifers during gestation; without changes in BCS over time showing a good nutritional status. The same response was found in our experiments, where supplementation of trace minerals pre-calving and/or pre-breeding did not improve BCS.

However, in experiment 1, without difference between treatments, BCS decrease from day of AI to 60d post-breeding. Ahola et al. (2004), also reported no difference in BW and BCS, when cows received *ad-libitum* supplementation of Cu, Mn, and Zn, before and after calving. However, supplemented, and non-supplemented cows suffer a reduction in BW and BCS after calving, recovering initial status at mid-summer.

Calf performance

Supplementation of a trace mineral injection on the dam during early lactation or late gestation can impact calves' fetal development, and post-calving growth. Supply of trace minerals, as other nutrients, to the embryo will depend on maternal ability to transfer those, in order to achieve normal development (Sales et al., 2011). Copper supply during pregnancy will define neonatal reserves at birth through liver accumulation of this mineral (Underwood and Suttle, 1999).

In experiment 1, dams received one dose of trace mineral supplement during early lactation, while in experiment 2, received one dose at late gestation and one dose at early lactation. However, in both cases, no differences were found in birth weight or 205d adjusted weaning weight ($P \geq 0.25$). In both experiments, calves did not receive an injectable trace mineral supplement.

Same response was reported in the literature when mineral source come only from milk and feedstuff (Marques et al., 2016; P. A. Olson et al., 1999; Stokes et al., 2019), and even when calves received a dose of injectable trace mineral supplement at birth and 70d of age (Mundell et al., 2012). Mineral source, organic or inorganic, and method of feeding, limited or *ad-libitum*, did not affect BW of calves at weaning (Arthington & Swenson, 2004).

Lack of response in offspring performance could be explained due to the lack of response in trace minerals concentration of the dams. Nonetheless, even when liver status of the dam showed greater levels of Cu and Se due to injectable supplementation, this did not alter milk mineral composition, maintaining same concentration of Mn, Se, and Zn, when compared to non-supplemented cows via injectable trace minerals source (Stokes et al., 2019). Stokes and colleagues (2019), also found an increase in milk yield in cows supplemented with an injectable source of trace minerals, but again this didn't cause any difference in calves weaning weight and average daily gain.

Even though we did not measure milk quality and mineral content, it is possible this would not show any changes, due to a lack in response in weaning weight. Marques et al. (2016) reported no difference in birth weight, even with a greater concentration of Cu and Zn in calves' liver, and greater Co and Cu concentration in placenta cotyledons achieved when dams were supplemented with organic trace minerals (Cu, Co, Mn, and Zn) in late gestation. These results show an increase of trace minerals transfer from maternal to fetus tissue, but no apparent impact on fetus growth. Which again, was not found in both of our experiments.

Similarly, Daugherty et al. (2002), reported no difference in Cu and Zn liver concentration, and blood Se levels in the offspring, when dam was supplement with two doses of Multimin90 plus vitamin E, 30d pre calving and 21d pre breeding. No impact was detected on calves' birth weight, average daily gain, and weaning weight.

Arthington et al. (1995) showed that treatment Cu deficiencies with slow-release boluses does not have an impact on reproductive performance but decrease BW on calves through dam supplementation. Birth weight can also be affected when Mn levels are not adequate (Stokes et al., 2019). In the dams from both groups Mn levels were slightly deficient, however this does not cause a negative impact on birth weight.

A decrease in AWW and kg weaned calf/cow exposed, was reported when calves had access to the same inorganic or organic free-range supplementation of Cu, Mo, and Zn that were receiving the dams before and after calving (Ahola et al., 2004). The effect of mineral supplementation on the dam and/or on the calf, in offspring performance is still unclear, with high variation between years, mineral status, and trace mineral source.

Birth weight in the fall breeding season was 33.7 kg, while in the spring was 35.6 kg, without difference between treatments. These values are normal compared with Angus calves birth weight (38kg; Casas et al., 2012), and birth weight recorded in the same locations during 12 years (Fall 34.1kg, Spring 34.4kg; Goncherenko et al., 2023).

Calves AWW, were on average 212kg and 259kg for Fall and Spring breeding season. Again these values showed no limitation in growth when compared to Angus calves AWW (235kg; Casas et al., 2012), and AWW recorded in the same locations (Fall 228kg, Spring 246kg; Goncherenko et al., 2023).

Implications

The lack of response to an extra mineral supplementation in BCS and overall pregnancy rate found in our experiments can be attributed to a short period of time between supplementation and breeding, an adequate initial mineral status in the herd in both breeding seasons, or the adequate BCS of the herd overall which was translated in achieving excellent results for FTAI (>50%), and overall pregnancy rate (>90%). However, when two doses of injectable trace minerals were administered a positive response of FTAI pregnancy rate was detected. Specifically, the injectable mineral supplementation was most beneficial for cows with BCS $\leq$ 5.

Conclusions

Offspring performance was not affected by trace mineral treatment over the dams at late gestation and/or early lactation A single dose of injectable trace minerals before artificial insemination did not improve pregnancy rate or overall mineral status for grazing beef cows. However, two doses of the injectable trace mineral, 30 days before calving and 10 days before FTAI, did improve FTAI pregnancy rate, especially in cows with low BCS.: Supplementing a yeast-derived product to feedlot cattle: Impacts on performance, physiological responses, and carcass characteristics.

Chapter 4 : Supplementing a yeast-derived product to feedlot cattle consuming monensin: Impacts on performance, physiological responses, and carcass characteristics

Abstract

Feed additives are included into finishing diets to improve cattle growth and feed efficiency, resulting in enhanced productivity and profitability in feedlot systems. Yeast-derived products and monensin have analogous and complementary benefits to rumen function and cattle production. Hence, this experiment evaluated the impacts of supplementing a yeast-derived product (Celmanax; Church & Dwight Co., Inc.; Princeton, NJ, USA) during the finishing period on performance, physiological responses, and carcass quality of feedlot cattle consuming monensin. Eighty-nine Angus steers (13 months of age) were housed in a feeding facility 15 days prior to the beginning of the experiment, which was equipped with Calan gates for individual feed delivery. Steer BW was collected on days -3, -2, and -1, averaged, and represented steer initial BW (446 ± 5 kg of BW). On day 0, steers were ranked by initial BW to receive a finishing diet containing (CEL) or not (CON) 16 g/steer daily (as fed basis) of the yeast-derived product. The finishing diet was a total-mixed ration (TMR) including (DM basis) 22% corn silage, 70.2% ground corn, 5% soybean meal, 1.8% mineral and vitamin mix, and 1% urea. Monensin was included in the TMR at 30 mg/kg of DM. The yeast-derived product was mixed with 100 g of soybean meal and top-dressed daily into the TMR of CEL steers, whereas 100 g of soybean meal was top-dressed daily into the TMR of CON cohorts. Steers received TMR and treatments until slaughter, after 132.5 ± 1.2 days on feed. Intake of the TMR was recorded daily from day -15 until slaughter. Steer BW was also recorded for 3 consecutive days prior to slaughter and averaged for final BW. Feed

efficiency was calculated from each steer according to TMR intake and BW gain during the experiment. Blood samples were collected on days 0, 64, and the day prior to slaughter, and analyzed for plasma glucose, haptoglobin, insulin, leptin, and insulin-like growth factor (IGF)-I. Carcass parameters were recorded upon slaughter. No treatment effects were detected ($P \geq 0.25$) for BW gain, TMR intake, feed efficiency, and carcass quality traits. No treatment effects were also detected ($P \geq 0.39$) for plasma concentrations of glucose, insulin, leptin, and IGF-I, whereas mean plasma haptoglobin concentrations tended ($P = 0.09$) to be less in CON vs. CEL steers. Collectively, including a yeast-derived product into a finishing diet containing monensin did not improve performance, physiological responses, and carcass quality of feedlot cattle.

Introduction

Feed additives are included into finishing diets to improve cattle growth and feed efficiency, resulting in enhanced productivity and profitability in feedlot systems (Callaway et al., 2003; Russell & Strobel, 1989; Vohra et al., 2016). Yeast-derived products, such as yeast cultures and extracts, are natural feed additives that can be classified as probiotics (live yeast) or prebiotics (non-living yeast). Supplementing cattle with live yeast, especially *Saccharomyces cerevisiae*, has been shown to improve feed intake, dietary digestibility, BW gain, and feed efficiency by promoting rumen function. More specifically, live yeast derivatives reduce ruminal lactate production, alleviate dietary protein loss as ammonia, and stabilize ruminal pH (Desnoyers et al., 2009; Gaggìa et al., 2010b; Shurson, 2018). Supplementing cell wall compounds from non-living yeast, including β-glucan, mannan-oligosaccharide and D-mannose also promote cattle

performance response by similarly improving rumen health and function (Patel & Goyal, 2012; Shurson, 2018).

Nonetheless, monensin remains the most common feed additive used across the globe to improve performance and feed efficiency in finishing cattle (Samuelson et al., 2016; Strydom, 2016). In the rumen, this ionophore increases propionate synthesis and reduces ruminal accumulation of methane, ammonia, and lactate, promoting dietary digestibility and nutrient utilization (Callaway et al., 2003; Russell & Strobel, 1989). Ionophores also stabilize ruminal pH by lessening feed intake variation, but also limit feed intake (Stock et al., 1995). Given the analogous and complementary benefits of yeast derivatives and ionophores, we hypothesized that inclusion of yeast-derived products into finishing diets containing monensin would result in additional benefits to cattle performance and efficiency. To test this hypothesis, the objective of this experiment was to evaluate the impacts of supplementing a commercial source of yeast culture + enzymatically hydrolyzed yeast products (Celmanax; Church & Dwight Co., Inc.; Princeton, NJ, USA) during the finishing period on growth, feed intake, physiological responses, and carcass quality of feedlot cattle consuming monensin.

Materials and Methods

This experiment was conducted at the Virginia Polytechnic Institute and State University - Shenandoah Valley Agricultural Research and Extension Center, McCormick Farm, located in Raphine, VA, USA. All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Virginia Polytechnic Institute and State University - Institutional Animal Care and Use Committee (#17-212). The research was replicated over 2 periods, from November

13th, 2017, to April 22nd, 2018 (replicate 1), and from June 8th to November 5th, 2018 (replicate 2). During replicate 1, minimum, maximum, and average environmental temperatures were -3, 9, and 3°C, respectively, average humidity was 65%, and a total of 35.5 mm of precipitation was recorded. During replicate 2, minimum, maximum, and average environmental temperatures were 14, 25, and 19°C, respectively, average humidity was 77%, and a total of 104.7 mm of precipitation was recorded.

Animals and dietary treatments

Across both replicates, 89 yearling Angus steers (13 months of age) were assigned to this experiment (replicate 1, n = 42; replicate 2, n = 47). During each replicate, steers were housed in a feeding facility equipped with Calan gates (American Calan Inc., Northwood, NH, USA) 15 days prior to the beginning of the experimental period (day 0) for acclimation to pens and feed bunks. More specifically, steers were ranked by full BW collected on day -16 (426 ± 4 kg), and allocated to 1 of 4 half-covered, concrete-floor pens (8.4 × 12 m) in a manner that average BW was equivalent across pens. Each pen contained 12 Calan gates for individual feed delivery. Three transitioning total-mixed rations (TMR) were offered to steers prior to the beginning of the experiment (day 0), and all included monensin (Table 4-1). Steers did not receive growth-promoting implants during the experiment.

Steer full BW were again recorded on day -3, -2 and -1, and averaged to serve as initial BW (446 ± 5 kg). On day 0, steers within each pen were ranked by initial BW and randomly assigned to receive the finishing TMR containing (CEL; n = 21 and 24 for replicates 1 and 2, respectively) or not (CON; n = 21 and 23 for replicates 1 and 2, respectively) the yeast-derived product (Celmanax; Church & Dwight Co., Inc.), which

was offered at 16 g/steer daily (as-fed basis) according to manufacturer's recommendation (Church & Dwight Co., Inc.) and previous research in feedlot cattle (Salinas-Chavira et al., 2018). Celmanax consists of the liquid medium used to grow strains of *Saccharomyces cerevisiae*, and includes dead cell walls, the medium, and an undetermined number of live yeast cells. Enzymatically hydrolyzed *S. cerevisiae* cell wall and its metabolites, including mannan-oligosaccharide and β-glucan components are added to the liquid medium, which is dried on a grain-based carrier (by proprietary processes; Church & Dwight Co., Inc).

The TMR was offered once daily at 0800 h in amounts to ensure ad libitum consumption and yield 15% (as-fed basis) of orts from the previous feeding. The yeast-derived product was mixed with 100 g of soybean meal (as-fed basis) and top-dressed daily into the finishing TMR of CEL steers, whereas 100 g of soybean meal (as-fed basis) was top-dressed daily into the TMR of CON cohorts. Monensin (Rumensin 90; Elanco, Greenfield, IN, USA) was included in the finishing TMR at 30 mg/kg of DM (Table 4-1). Steers received treatments and the fattening TMR until slaughter, which was determined according to the availability of a commercial packing facility and steer BW (heaviest steers slaughtered first). As a result, steers were assigned to slaughter on 2 separate dates within each replicate, which were balanced by treatment. During replicate 1, 9 CON and 10 CEL steers were slaughtered after 123 days receiving treatment (DRT), whereas 12 CON and 11 CEL steers were slaughtered after 151 DRT. During replicate 2, 15 CON and 15 CEL steers were slaughtered after 125 DRT, whereas 8 CON and 9 CEL steers were slaughtered after 130 DRT. No incidence of morbidity or mortality were noted during both replicates.

Sampling

Samples of the TMR were collected weekly, dried in a forced-air oven at 55°C for 72 h, and stored for posterior nutrient analysis via wet chemistry procedures in a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA) as described by Silva *et al.* (2017). Samples from each transition TMR were analyzed individually, whereas samples of the finishing TMR were pooled across all weeks prior to nutritional analysis Calculations for net energy for maintenance and gain were calculated according to equations proposed by the National Research Council (2000). Nutritional profiles of TMR offered during transition and finishing periods are described in Table 4-1.

Steers full BW were also recorded over 3 consecutive days and averaged to represent intermediate BW (days 63, 64 and 65 of the experiment) and final BW (3 days preceding slaughter). Initial and final BW were used to calculate ADG (kg/day) during the experiment. Individual feed intake was recorded daily for each steer from day -15 until slaughter by weighing offered and non-consumed TMR. Daily samples of the offered and non-consumed TMR were dried for 72 h at 55°C in forced-air ovens for DM calculation. Total BW gain and TMR intake during the experimental period of each steer were used to calculate feed efficiency (g of BW gain/kg of DM intake). At the commercial packing facility, hot carcass weight (HCW) was collected upon slaughter. After a 24-h chill, trained personnel assessed LM area, marbling score, and yield grade.

Table 4-1. Ingredient composition and nutrient profile (DM basis) of total mixed ration offered during the experiment[a].

Item	A	B	C	Finishing
Ingredient, %				
Corn silage	52.0	42.0	32.0	22.0
Concentrate[b]	48.0	58.0	68.0	78.0
Nutrient profile				
Net energy for maintenance, Mcal/kg	1.87	1.91	1.95	1.99
Net energy for growth, Mcal/kg	1.22	1.26	1.30	1.33
NDF, %	23.7	21.3	18.9	16.5
ADF, %	12.2	10.6	9.00	7.41
CP, %	12.1	12.7	13.3	13.9

[a]A = day −15 to −10; B = day −9 to −5; C = day −4 to −1; and Finishing = day 0 to slaughter. Cattle had free-choice access to the total mixed ration and water throughout the experimental period.

[b]Concentrate contained (DM basis) 90% ground corn, 6.4% soybean meal, 2.3% mineral mix (Tennessee Farmers' Cooperative; Lavergne, TN, USA), and 1.3% urea. The mineral mix contained 30% Ca, 1% P, 21% NaCl, 0.25% Mg, 800 mg/kg of Cu, 12 mg/kg of Se, 2400 mg/kg of Zn, 160,000 IU/kg of vitamin A, 22,000 IU/kg of vitamin D3, and 615 IU/kg of vitamin E. The concentrate also contained sodium monensin (Rumensin; Elanco Animal Health, Greenfield, IN, USA) at 30 mg/kg of DM.

Blood samples were collected from all steers, before the TMR feeding of the day, via jugular venipuncture on days 0, 64 and the day prior to slaughter. Blood was collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA) containing freeze-dried sodium heparin. After collection, blood samples were placed immediately on ice and centrifuged at 2,400 × g for 15 min for plasma harvest and stored at -20°C on the same day of collection. Samples were analyzed for

concentrations of haptoglobin (Cooke & Arthington, 2013), glucose (colorimetric kit #G7521; Pointe Scientific, Inc., Canton, MI, USA), insulin (porcine radioimmunoassay kit #PI-12K; EMD Millipore Corporation, Billerica, MA 01821, USA; Kaufman *et al.*, 2018), insulin-like growth factor (IGF)-I (enzyme-linked immunosorbent immunoassay kit #SG100; R&D Systems, Inc., Minneapolis, MN, USA; Cooke *et al*., 2012), and leptin (multi-species radioimmunoassay kit #PI-12K; EMD Millipore Corporation; Oliveira *et al.*, 2016). The intra- and interassay CV were, respectively, 6.7 and 2.8% for glucose, 2.9 and 3.7% for haptoglobin, and 1.9 and 7.8% for IGF-I. Insulin and leptin were analyzed within a single assay and intra-assay CV were 8.4% and 8.5%, respectively.

Statistical analysis

Steer was considered the experimental unit for all analyses. Quantitative data were analyzed using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC, USA), whereas binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.) with a binomial distribution and logit link function. All data were analyzed using Satterthwaite approximation to determine the denominator df for tests of fixed effects, with steer (treatment × pen × replicate), pen(replicate), and replicate as random variables. Model statements for BW, ADG, feed efficiency, and carcass parameters contained the effects of treatment. Model statements for DMI and plasma variables contained the effects of treatment, day, and the resultant interaction. Moreover, average DMI from day -15 to 0 and plasma results from day 0 were included as independent covariate in each respective analysis. The specified term for all repeated statements was day, with steer (treatment × pen × replicate) as subject. The covariance structure used was compound symmetry, which provided the smallest Akaike information

criterion. All results are reported as least square means. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Repeated measures are reported according to main treatment effect if the treatment × day interaction was $P > 0.10$.

Results

As designed, no treatment differences were detected ($P \geq 0.75$) for initial BW and days receiving dietary treatments (Table 4-2). No treatment differences were also detected ($P \geq 0.61$) for intermediate BW, final BW, as well as ADG during the experimental period (Table 4-2). Feed intake did not differ ($P = 0.25$) between CON and CEL cattle; hence, feed efficiency was also similar ($P = 0.74$) between treatments (Table 4-2).

No treatment differences were detected ($P \geq 0.39$) for mean plasma concentrations of glucose, insulin, IGF-I, and leptin. However, mean plasma concentration of haptoglobin tended ($P = 0.09$) to be greater in CEL steers during the experimental period (Table 4-3).

Across treatments, day effects were detected ($P \leq 0.04$) for plasma concentrations haptoglobin, IGF-I, insulin, and leptin, but not ($P = 0.46$) for plasma concentrations of glucose (Table 4-4). Additionally, no treatment differences were also detected ($P \geq 0.37$) for carcass traits, including hot carcass weight, LM area, marbling score, yield grade, and proportion of carcasses grading Low Choice and above (Table 4-2).

Table 4-2. Performance parameters and carcass traits of feedlot cattle consuming a diet containing monensin and supplemented (**CEL**; *n* = 45) or not (**CON**; *n* = 44) 16 g/steer daily of Celmanax (Church & Dwight Co., Inc.; Princeton, NJ, USA) during the finishing period[a].

Item	**CON**	**CEL**	**SEM**	***P*-value**
Days receiving treatments	132.8	132.3	5.8	0.79
BW parameters[b]				
Initial BW, kg	443.7	446.3	15.1	0.75
Intermediate BW, kg	552.3	554.7	11.8	0.82
Final BW, kg	629.8	629.4	10.9	0.97
ADG (initial to final), kg/day	1.40	1.38	0.04	0.61
Feed intake, kg/day (DM basis)	10.59	10.37	0.13	0.25
Feed efficiency, g/kg	134	133	4	0.74
Carcass characteristics[c]				
Hot carcass weight, kg	374	377	8	0.64
LM area, cm^2	85.2	86.8	3.2	0.37
Marbling	513	503	19	0.69
Yield grade	3.05	3.00	0.12	0.66
Carcasses grading Low Choice and above, %	88.6	82.2	5.3	0.39

[a]Cattle received the final diet within inclusion of treatments from day 0 until slaughter.

[b]Cattle full BW was recorded over 3 consecutive days at the beginning (days −3, −2, and −1), middle (days 63, 64, and 65), prior to slaughter, and averaged for BW assessment.

[c]Marbling score: 400 = $Small^{00}$, 500 = $Modest^{00}$; 600 = $Medium^{00}$. Carcass grading according to USDA (1997).

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Table 4-3. Physiological responses of feedlot cattle consuming a diet containing monensin and supplemented (CEL; $n = 45$) or not (CON; $n = 44$) 16 g/steer daily of Celmanax (Church & Dwight Co., Inc.; Princeton, NJ, USA) during the finishing period[a].

Item	CON	CEL	SEM	*P-value*
Plasma glucose, mg/dL	83.1	82.7	4.8	0.87
Plasma haptoglobin, mg/mL	0.238	0.337	0.040	0.09
Plasma IGF-I, ng/mL	241	234	9	0.39
Plasma insulin, µIU/mL	43.0	45.4	2.6	0.51
Plasma leptin, ng/mL	16.1	16.7	1.3	0.69

[a]Cattle received the final diet within inclusion of treatments from day 0 until slaughter (132.5 ± 1.2 days on feed). Blood samples were collected on days 0, 64, and prior to slaughter. Results from day 0 were used as independent covariates within each respective analysis; hence, values reported are covariately adjusted least square means. Moreover, all values reported are least square means according to main treatment effects, given that no treatment × day interactions were detected ($P \geq 0.19$).

Table 4-4. Physiological responses of feedlot cattle during the finishing period[a].

Item	day 0	day 64	prior to slaughter	SEM	*P-value*
Plasma glucose, mg/dL	81.6	82.2	83.6	5.2	0.46
Plasma haptoglobin, mg/mL	0.193 ^{y}	0.323 ^{x}	0.249 ^{xy}	0.036	0.04
Plasma IGF-I, ng/mL	285 ^{x}	250 ^{y}	222 ^{z}	6.0	< 0.01
Plasma insulin, µIU/mL	26.0 ^{z}	49.4 ^{x}	38.6 ^{y}	2.4	< 0.01
Plasma leptin, ng/mL	13.7 ^{y}	16.3 ^{x}	16.5 ^{x}	0.85	0.01

[a]Cattle received finishing diets for 132.5 ± 1.2 days. Blood samples were collected on days 0, 64 and the day prior to slaughter. Values with different superscripts (x, y, z) differ ($P \leq 0.05$).

Discussion

Monensin has been extensively investigated and known to improve beef cattle productivity, mainly by enhancing rumen fermentation efficiency, reducing energy loss as methane, and lessening variation in feed intake (Callaway et al., 2003; Russell & Strobel, 1989; Stock et al., 1995). The yeast-derived product supplemented to CEL steers

also increased performance of feedlot cattle (Ponce et al., 2012; Salinas-Chavira et al., 2018; Silva et al., 2018) by improving rumen function (Nde et al., 2014). More specifically, *S. cerevisiae*-derived products benefit fiber degradation and microbial protein synthesis (Salinas-Chavira et al., 2015), while alleviating lactate and ammonia accumulation in the rumen (Desnoyers et al., 2009; Gaggìa et al., 2010b; Shurson, 2018). Together, these outcomes are known to improved feed intake and dietary nutrient utilization, leading to increase cattle performance responses (Allen, 1996; Nocek et al., 2011). Nonetheless, combining both additives herein did not yield in synergistic effects to improve TMR intake, feed efficiency, and overall performance, indicating that CEL failed to improve rumen function of feedlot cattle receiving monensin. Mir and Mir (1994) also reported that combining *S. cerevisiae*-derived products and ionophore had no benefits to performance responses of feedlot cattle compared with cohorts receiving these additives singly, although lasalocid was the ionophore investigated. Perhaps monensin improved the ruminal environment to an extent that negated the potential benefits of CEL, although cattle not receiving monensin nor CEL were not evaluated herein to fully support this rationale.

Alternatively, others have reported positive effects in feeding CEL to feedlot cattle receiving finishing diets containing monensin, mainly by increasing feed consumption. Salinas-Chavira et al. (2015) noted improved feed intake and ADG, but only when cattle were exposed to heat stress conditions. Ponce et al. (2012b) also reported increased ADG and feed intake when adding CEL to monensin-containing diets fed to newly weaned cattle upon feedlot arrival. Similar outcomes were noted by Salinas-Chavira et al. (2018) in calf-fed Holstein steers during growing and fattening periods.

Stressful conditions such as heat stress or feedlot arrival are known to depress feed consumption in cattle (Cooke, 2017), whereas Holstein cattle are more susceptible to health and metabolic disorders in the feedlot compared with traditional beef breeds (Duff & McMurphy, 2007). Feeding monensin to high-stress or metabolically challenged cattle can further depress their feed intake and overall performance (Duff & Galyean, 2007). In turn, cattle assigned to this experiment were yearling Angus steers previously adapted to feedlot practices, managed in a temperate climate with negligible incidence of heat, metabolic, and general stressors. Therefore, one can speculate that CEL mostly benefits feed intake and subsequent ADG, alleviating the intake limiting effects of monensin, in cattle experiencing stressful and challenging conditions. Accordingly, the yeast-derived product evaluated herein has been shown to modulate other body functions besides the rumen such as the immune system, contributing toward improved overall health in beef and dairy cattle (Nocek et al., 2011; Ponce et al., 2012; Silva et al., 2018).

Similar carcass parameters and plasma concentrations of glucose, insulin, IGF-I, and leptin between CEL and CON cattle corroborate the feed intake, efficiency, and ADG results. Circulating concentrations of these substances are positively associated with plane of nutrition and BW gain in beef cattle (Ellenberger et al., 1989; Hersom et al., 2004). Hot carcass weight, LM area, and carcass marbling are largely regulated by nutrient intake and growth rates during the finishing period in cattle with similar background and genetic merit (Camfield et al., 1999; Hassen et al., 1999). Moreover, circulating IGF-I and insulin are directly involved in muscle development and lipogenesis, respectively (Ellenberger et al., 1989; Etherton & Evock, 1986), whereas circulating leptin directly correlates with carcass marbling and quality grade (Geary et al.,

2003). Day effects noted across treatments for plasma concentrations of leptin, insulin, and IGF-I denote such changes in lipogenic activity, nutrient availability, and diet utilization by cattle during the finishing period (Hersom et al., 2004), whereas the lack of variation in plasma glucose can be associated with the rigorous homeostatic regulation of circulating glucose in ruminants (Bickerstaffe et al., 1974).

A trend for increased mean plasma haptoglobin concentrations was noted with CEL inclusion. This acute-phase protein is a common biomarker for inflammatory processes, including disruption of the ruminal ecosystem and subsequent release of microbial endotoxins to the bloodstream in cattle consuming high-concentrate diets (Gozho et al., 2005; Marques et al., 2012). The day effect detected for plasma haptoglobin concentrations across treatments may be associated with increased systemic inflammation caused by the finishing diet as the experiment progressed, or by other inflammatory stimuli not controlled by the research design (Cooke, 2017) although morbidity and mortality were not observed herein. Moreover, the acute-phase response typically impairs cattle performance by demanding a significant amount of body resources, increasing maintenance requirements, and decreasing nutrient intake (Cooke, 2017), although treatment differences noted for plasma haptoglobin were not accompanied by altered feed intake, feed efficiency, and ADG. Alternatively, the acute-phase response is a major component of the innate immune system, and increased haptoglobin concentrations may be associated with heightened immunocompetence (Cooke, 2017). Others have also reported increased acute-phase reaction, denoting improved immunity, in cattle supplemented with products derived from *S. cerevisiae* (Brandão et al., 2016). While the exact reason to why CEL increased plasma

concentration of haptoglobin herein is unknown and did not impact performance responses, these outcomes further suggest the immunomodulatory properties of yeast-derived products (Nocek et al., 2011; Ponce et al., 2012; Silva et al., 2018).

Conclusions

Inclusion of CEL into finishing diets containing monensin did not improve performance, physiological responses, and carcass characteristics of feedlot cattle. These outcomes were contrary to our hypothesis, which was based on potential additive effects of both feed ingredients to ruminants consuming high-concentrate diets. Perhaps CEL would be of greater benefit to feedlot cattle experiencing stressful conditions, given its aptitude in promoting feed intake in stressed cattle. Hence, additional research is warranted to elucidate the benefits of CEL and other yeast-derived products in finishing diets, and their potential to synergize or perhaps substitute traditional feed additives such as monensin.

Chapter 5 : Impact of supplementation of a yeast derived product on growth and performance of beef calves

Abstract

The objective of this work was to evaluate the impact of a yeast derived product (Celmanax; Church & Dwight Co., Inc.; Princeton, NJ) supplementation on beef calf acute phase response after stress accumulation, and feedlot performance, substituting monensin as growth promoter. A total of 44 castrated male beef calves (249 ± 31 kg BW, 296 ± 18 days of age) were stratified by BW and randomly assigned to one of two treatments: supplementation of 1 kg/hd/d of corn gluten feed (Control; n=22); or supplementation of 1 kg/hd/d of corn gluten feed and 14 g/hd/d (as fed-basis) of a yeast derived product (Treatment, YDP; n=22). Steer calves received their treatment for 30 days, using an automated feeding system (SmartFeed PRO, C-Lock Inc., Rapid City, SD), that allowed to manage all animal together in a fescue pasture paddock while receiving their individual treatment. Steer calves also had *ad-libitum* access to the fescue pasture, grass hay, and water. Supplement feed intake was measured daily, and BW was measured every 14 days. At d30, calves were weighed and loaded into a commercial livestock trailer to be transported for a total of 6 hours, until feedlot arrival. Once in the feedlot, animals were again weight, classified by BW and assigned to two treatment in a 2*2 factorial arrangement, generating 4 treatments: 1) background phase without YDP supplementation + finishing phase with monensin (CC, n = 11); 2) background phase without YDP supplementation + finishing phase with YDP (CT, n = 11); 3) background phase with YDP supplementation + finishing phase with monensin (TC, n = 11); and 4) background phase with YDP supplementation + finishing phase with YDP (TT, n = 11).

Once in the feedlot, animals were assigned to a pen equipped with Calan gates and automated scales. Feed intake and BW were measured daily. Blood samples were taken before and after transportation, at d3 and d7 after arrival, and at d50, 85 and 120, once finishing diet started, to measure acute phase protein concentration. The backgrounding phase did not cause an impact on steers performance, reaching the same final body weight (256 ± 6.73 kg for CEL and Control; P = 0.98). Acute phase proteins were not affected by treatment, or treatment combination before and after transportation ($P \geq 0.16$). Supplementation of YDP, substituting monensin, caused a decrease in DMI (kg/d, P=0.015), and an increase in feed efficiency (gain:feed ratio, P=0.008) in the whole period at the feedlot phase. In conclusion, supplementation of a YDP during 30 days prior to transportation did not cause any changes in performance or acute phase proteins of backgrounded beef steer calves; but YDP showed the potential to decrease substitute the use of monensin in highly concentrated diets.

Introduction

Weaning, commingling, transportation, and the feedlot receiving phase are all major stressful events that calves will be exposed to during their growing phase (Eicher et al., 2010; Meléndez et al., 2020; Swanson & Morrow-Tesch, 2001; Wiegand et al., 2020). Cattle experiencing stress during early life can have impaired immune response and performance in the future (Duff & Galyean, 2007; Swanson & Morrow-Tesch, 2001), with a detrimental effect on animal well- being (Enríquez et al., 2011). As evidence of the impact of stress on health, when calves are weaned and transported within the same day mortality rate caused by respiratory diseases doubles compared to calves that are not weaned immediately before transportation (Hodgson et al., 2005).

Feed and water deprivation, as commonly happens during transportation, will impact on acute phase response and cattle performance (Marques et al., 2012). Transportation and entering the feedlot receiving phase increases plasma concentration of proinflammatory cytokines and acute phase proteins as evidence of a stress response to these events, which will later affect feed intake and performance (Cooke, 2017). The feedlot receiving phase, which occurs during the first 4 to 6 weeks after arrival, is also critical to determine the subsequent health and performance during the finishing phase (Duff & Galyean, 2007). Immunocompetence of the animal becomes essential during this phase to avoid health and growth performance impairment.

Nutritional management is an alternative to improve calf performance by controlling stress and modulating the innate immune response (Carvalho et al., 2019; Duff & Galyean, 2007), using dietary supplements as immunomodulators like yeast β-glucans (Eicher et al., 2010), essential fatty acids, or anti-inflammatory drugs (Cooke, 2017). Natural feed additives like prebiotics and probiotics have been shown to improve beef cattle performance and health on stressed cattle (Duff & Galyean, 2007). Supplementing cattle with probiotics, such as live yeast products, especially *Saccharomyces cerevisiae*, on diets with high concentrate levels improves feed efficiency, feed digestibility, weight gain and enhances the immune system, due to a decrease of rumen pathogens prevalence (Desnoyers et al., 2009; Gaggìa et al., 2010b; Shurson, 2018; Vohra et al., 2016). Prebiotics, such as yeast cell wall compounds, β-glucan, mannan oligosaccharide and D-mannose, are three carbohydrates structures that provide health benefits to cattle with a positive effect in alleviating external and

metabolic stressors leading to improved cattle growth performance and feed conversion as a result (Humer et al., 2018; Lei et al., 2013; Shurson, 2018).

Nonetheless, yeast products are not only evaluated by their effect on the immune response, but also as an alternative to the use of antibiotic growth promoters, such as ionophores. Supplementation of a yeast derived product, composed of live yeast cells, yeast fermented metabolites, and enzymatically hydrolyzed yeast products will provide health benefits to the cattle, improving cattle growth performance and feed conversion. Our previous work with finishing steers receiving yeast derived products indicates an increased acute-phase reaction which was associated with heightened immunocompetence, suggesting the immunomodulatory properties of this yeast derived product (Pancini et al., 2020). Additionally, others have shown a reduction in morbidity of respiratory diseases, as well as an improvement in feed efficiency and average daily gain when a yeast derived product was supplemented to weaned calves during preconditioning period and/or the receiving phase (Danielo et al., 2020; Ponce et al., 2012).

Therefore, our hypothesis is that yeast derived product (YDP) supplementation during the backgrounding phase will improve calf performance and alleviate the stress response during transportation and receiving phase at the feedlot. Also, this positive effect will be prolonged during the finishing phase and will be greater when supplementation is continued, allowing for the substitution of the use of ionophores as growth promotants. The objective of this work was to evaluate the impact of a YDP supplementation on beef calf performance during the backgrounding and finishing phases, and the acute phase response following transportation and the receiving phase.

Materials and Methods

All experimental protocols involving cattle were reviewed and approved by the Institutional Animal Care and Use Committee of Virginia Polytechnic Institute and State University 21-095. The experiment was conducted at the Virginia Tech Kentland farm, Blacksburg, VA, and at the Shenandoah Valley Agricultural Research and Extension Center, McCormick Farm, located in Raphine, VA, from July through December 2022.

Animals and treatments

A total of 44 male castrated commercial beef calves (249 ± 31 kg BW, 296 ± 18 days of age), from the Virginia Tech herd were assigned to this experiment. The experimental period was divided in two phases, backgrounding, and finishing. The YDP utilized in this research was derived from the *Saccharomyces cerevisiae* (Celmanax, Church & Dwight Co., Inc.; Princeton, NJ, USA) and composed by yeast fermented metabolites, enzymatically hydrolyzed yeast products containing non-living yeast cell walls and an undetermined number of live yeast cells by proprietary processes.

Backgrounding phase: steers were stratified by BW and randomly assigned to one of two treatments: 1) supplementation of 1 kg/hd/d of corn gluten feed (CTRL; n=22); or supplementation of 1 kg/hd/d of corn gluten feed and 14 g/hd/d (as fed basis) of a yeast derived product (YDP; n=22). Steers were managed as a single group with *ad-libitum* access to fescue pasture and grass hay, and clean and fresh water. Treatments were administered using an automated feeding system (SmartFeed PRO, C-Lock Inc., Rapid City, SD) located in the pasture. Four automated feeders allowed each group to have access to their specific supplements while being managed as a single group during the total duration of the experiment. Daily intake of supplement was automatically measured

and limited by the feeders. Prior to the beginning of the experimental period, steers were trained to use the automated feeders for 30 days. Total background phase and treatment period lasted 30 days. Body weight was measured at the beginning of the experiment (day 0) and every 14 days using a livestock scale (XR ID5000, Tru-testTM Datamars, Mineral Wells, TX). At d30, steers were weighed, loaded into a commercial livestock trailer, and transported for a total of 6 hours, until feedlot arrival. At feedlot, steers were unloaded, weighed, and assigned to their correspondent pens and treatments.

Finishing phase: after arrival to the feedlot, steers were stratified by previous treatment and BW, and assigned to one of two treatments in a 2 × 2 factorial arrangement: 1) control group receiving a high concentrate diet with monensin as growth promotant feed additive (CTRL, n= 22), and 2) same high concentrate diet with top dressed YDP (14 g/hd/d, as fed-basis) as growth promotant feed additive (YDP, n=22). This arrangement generates 4 treatments: 1) background phase without YDP supplementation + finishing phase with monensin (CC, n = 11); 2) background phase without YDP supplementation + finishing phase with YDP (CT, n = 11); 3) background phase with YDP supplementation + finishing phase with monensin (TC, n = 11); and 4) background phase with YDP supplementation + finishing phase with YDP (TT, n = 11).

Steers were immediately housed in a semi-roofed feeding facility equipped with Calan gates (American Calan Inc., Northwood, NH, USA), and started receiving their treatments (14g/h/d of Celmanax) during the adaptation period for the Calan gates and diets. Finishing diet was a total mixed ration composed of 80% concentrate and 20% corn silage as fiber source in dry matter basis (Table 5-1). Concentrate portion of the diet was offered in a pelleted ration composed of 81.9% corn grain, 15% soybean meal, 2.5%

minerals and vitamins, and 0.6% urea. Control diet contains monensin in 30g/ton of feed incorporated in the pelleted ration, and treated animals receive the YDP (14g/h/d) top dressed daily into the total mixed ration in the feed bunk. Transition diets started with 50% silage, in a 50:50 diet, decreasing by 10% until getting to the final proportion for a total of four diets.

The feeding facility was equipped with 4 pens, each one housing 11 steers, with an automated scale (SmartScale, C-Lock Inc, Rapid City, SD) located in front of the water through. Individual feed intake and BW were recorded every day. Feed management was *ad-libitum* using clean bunk management and diet was offered once a day in the morning.

Sampling and measurements

In both phases, feed samples were taken weekly, and frozen at -20°C for posterior nutrient analysis. Samples were thawed and dried in a forced-air oven at 55°C for 3 days, to get DM content. Once dried were ground in a hammer-miller (Willey Mill) to pass a 1mm sieve to make a composite sample per feed. Composite samples were analyzed for nutrient content by wet chemistry procedures in a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA).

Table 5-1. Ingredient composition and nutrient profile (DM basis) of total mixed ration offered during the experiment to control (CTRL) and treated (YDP) group.

Item	**A**	**B**	**C**	**Finishing[1]**
Ingredient (%)				
Corn silage	50	40	30	20
Concentrate[2]	50	60	70	80
Item			CTRL	YDP
Nutrient profile				
Net energy for maintenance (Mcal/kg)			1.76	1.78
Net energy for growth (Mcal/kg)			1.16	1.18
Neutral detergent fiber (%)			17.7	16.4
Acid detergent fiber (%)			8.5	8.2
Crude protein (%)			12.0	12.1

[1] A, B, and C were transition diets, changing between them every day during a 20-day transition period. Finishing diet was offered for 70 days, until the end of the experimental period. Cattle had free-choice access to the total mixed ration and water throughout the experimental period.

[2] Concentrate contained (DM basis) 81.9% of ground corn, 15% soybean meal, 0.6% urea, and 2.5% mineral and vitamins (0.82% Ca, 0.39% P, 0.61% NaCl, 0.13% Mg, 28.3 ppm Cu, 0.35 mg/kg Se, 98.7 ppm Zn, vitamin D3 0.64 KIU/kg, vitamin A 4.6 KIU/kg, vitamin E 40.1IU/kg). The control diet contained monensin (Rumensin; Elanco Animal Health, Greenfield, IN, USA) at 38.7 g/ton of DM.

Blood samples were taken from each animal before and immediately after the 6-hour transportation, 72 hours later, and 7 days after arriving to the feedlot, and again at d50, 85, and 120 (end of experimental period), blood samples were taken from the coccygeal vein, and collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA) containing sodium heparin (148 USP units). Blood samples were placed on ice, and immediately centrifuged (2,400 × g for 15 min) for plasma harvest. Plasma samples were frozen in duplicate for posterior analysis of ceruloplasmin and haptoglobin concentrations.

Plasma haptoglobin concentrations were determined in duplicate samples by a biochemical assay measuring haptoglobin-hemoglobin complexing by the estimation of differences in peroxidase activity (Makimura & Suzuki, 1982). Results were obtained as arbitrary units resulting from the absorption reading at 450 nm. Same quality control standards used in the biochemical assay were analyzed by quantitative determination of bovine haptoglobin in plasma (bovine haptoglobin ELISA test kit; Life Diagnostics, Inc., West Chester, PA, USA). The concentrations of haptoglobin, based on the ELISA assay, ranged from 0.03 (low control) to 0.95 mg/ml (high control) with an intra-assay CV of 1.26 %. The ELISA standard curve was used to convert the arbitrary units obtained from the biochemical procedures into mg/mL (Cooke & Arthington, 2013) with the least detectable value of 0.03 mg/mL. Samples CV was 4.68%. Plasma ceruloplasmin oxidase activity was measured in duplicate samples using colorimetric procedures described by Demetriou et al. (1974). Ceruloplasmin concentrations are expressed as mg/dL as described by King (1965). Samples CV was 1.92%.

Dry matter intake was estimated based on feed offered minus refusals in the next morning, adjusted by DM content. Average daily gain (ADG) was determined according to the slope of the BW regression line, and feed efficiency, expressed ad gain:feed ratio, was calculated individually with feed intake and ADG registered values.

Statistical analysis

Data was analyzed in a completely randomized design, with the animal as the experimental unit. Quantitative data were analyzed using the PROC MIXED of SAS (SAS Inst. Inc., Cary, NC, USA), including the fixed effect of the treatment. In the finishing phase, a factorial arrangement was included in the model, including the effect of

treatment at backgrounding, at finishing phase, and resulting interaction between them. Dry matter intake and acute phase proteins were analyzed as repeated measurements, using the PROC MIXED of SAS (SAS Inst. Inc., Cary, NC, USA), including the fixed effect of each treatment, effect of moment of measurement (day or week), and resultant interactions. All results are reported as least square means. Significance was set at $P < 0.05$, and tendencies were determined if $0.10 < P > 0.05$.

Results

Background phase:

As designed, initial BW was not different between treatments (Table 5-2; P=0.99). Performance of steers during backgrounding was not affected by yeast supplementation (Table 5-2), without difference in ADG and final BW ($P \geq 0.52$). Average daily gain achieved seems normal for weaned grazing steers receiving low supplementation level. Supplement intake was lower than expected, with a target intake of 1kg/d, but was not different between treatments (P=0.37).

Table 5-2. Effect of treatment (CTRL= control, YDP=yeast derived product supplementation) on calves' performance at backgrounding phase.

	Treatment			
Item	**CTRL**	**YDP**	**SEM**	***P* value**
Body weight, kg				
Initial	238.8	238.9	6.36	0.99
Final	255.9	256.2	6.73	0.98
Average daily gain, kg/d	0.61	0.57	0.05	0.52
Supplement intake, DM kg/d	0.83	0.75	0.06	0.37
Transportation				
Weight loss, kg	12.42	12.57	0.85	0.90
Weight loss, %	4.88	4.91	0.31	0.94

Finishing phase:

There was a tendency of a greater ADG during the finishing phase when treatment 2 was the control diet with monensin, with a difference of 170g/d. When analyzing the whole period, that includes 20 days of diet transition and the finishing phase, there is a significant interaction between treatments at background and finishing phase for DMI and feed efficiency, without differences in ADG. This interaction means that when control was present either at backgrounding or finishing phase decrease feed efficiency in 0.05, which is translated in 1kg more feed needed per kg of growth. Same response for DMI, that when control was present at either moment it implied an increase in DMI of 1.9kg/d.

Table 5-3. Performance parameters of feedlot steers in each treatment combination, including the effect of backgrounding treatment (Pre) and finishing phase treatment (Post), during diet transition (20days), finishing diet, or whole period.

	Treatment					*P*-value		
Item	**CC**	**CT**	**TC**	**TT**	**SEM**	**Pre**	**Post**	**Pre*Post**
Body weight, kg								
Initial	253.7	256.9	259.6	259.2	9.55	0.67	0.88	0.85
Final	501.4	492.9	487.1	493.2	17.3	0.69	0.95	0.67
Average daily gain, kg/d								
Transition	1.50	1.60	1.60	1.49	0.16	1.00	0.99	0.52
Finishing	2.28	1.97	2.12	2.07	0.10	0.74	0.09	0.20
Whole period	2.18	2.04	2.08	2.07	0.09	0.72	0.38	0.44
Dry matter intake, kg/d								
Transition	7.90	7.83	7.67	7.74	0.28	0.59	0.99	0.81
Finishing	10.7	9.76	9.93	9.71	0.43	0.34	0.18	0.41
Whole period	9.98	10.3	9.88	8.13	0.30	0.0006	0.018	0.0015
Gain: feed, kg:kg								
Transition	0.19	0.20	0.20	0.19	0.02	0.75	1.00	0.49
Finishing	0.21	0.20	0.22	0.22	0.01	0.43	0.49	0.55
Whole period	0.22	0.20	0.21	0.26	0.01	0.09	0.43	0.008

Evolution of DMI:

Analyzing DMI as a repeated measure, there was a tendency (*P*=0.07) of a backgrounding treatment*day interaction during diet transition period (Figure 5-1), with significantly greater intakes for CTRL group at d18 (0.75 kg/d, *P*=0.045) and d19 (1.05kg/d, *P*=0.005).

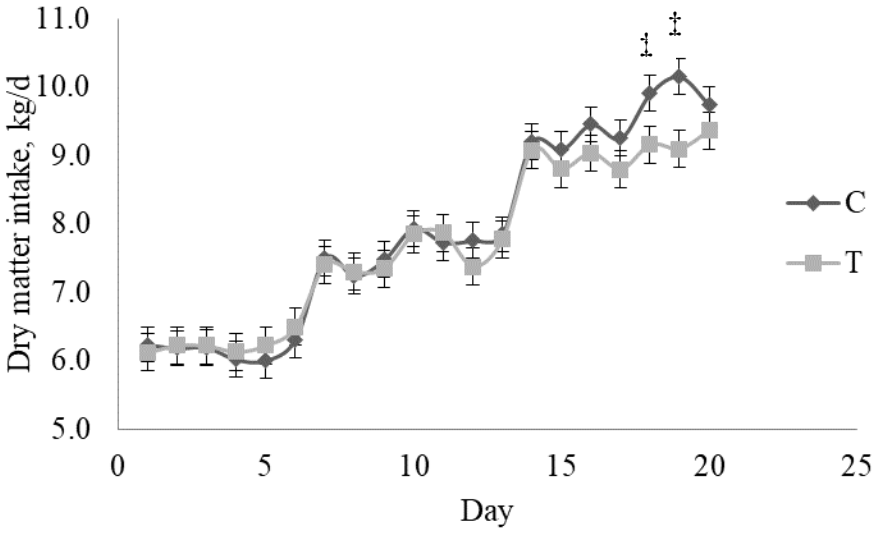

Figure 5-1. Evolution of dry matter intake (kg/d) during diet transition period (20 days) affected by backgrounding treatment (C= control, T= Yeast derived product supplemented group). Effect of treatment $P \geq 0.52$, ‡Day $P < 0.0001$, Treatment background*day *P*=0.07

Evolution of dry matter intake when receiving the finishing diet showed an interaction of finishing phase treatment*week ($P = 0.015$, Figure 5-2), with a significant difference in DMI during the last week of the experiment, with control group eating 0.98kg/d more than YDP group (*P*=0.032).

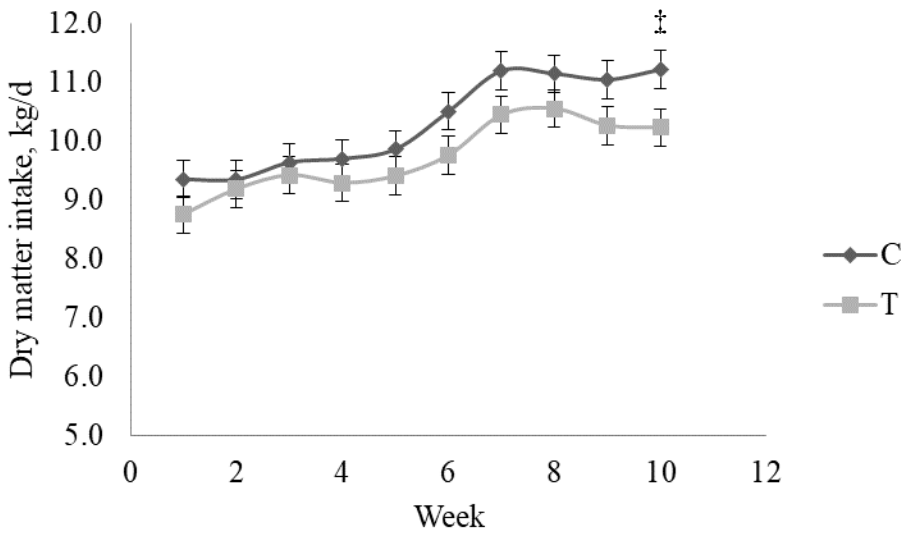

Figure 5-2. Evolution of dry matter intake (kg/d) calculated every week during the 70d receiving finishing diet affected by treatment at finishing phase (C= control, T= Yeast derived product supplemented group). Treatment $P \geq 0.19$, Week $P < 0.0001$, ‡Treatment feedlot*day $P = 0.0148$

Acute phase proteins:

Ceruloplasmin (Cp, mg/dL) concentration in plasma were not affected by YDP supplementation before or after transportation (Figure 5-3), neither an interaction ($P \geq 0.54$) was detected. There was a day effect ($P < 0.0001$), showing that transportation itself increased levels of Cp (18.89 vs 23.95 mg/dL before and after transportation respectively, $P = 0.0004$). After arrival Cp increase again at d3 but without being significatly different from values obtained after transportation (25.2 mg/dL, $P = 0.33$). Plasma concentration of Cp went up again when steers started eating the finishing diet (d50, Table 5-4), achieving greater increase on concentration than the one caused by transportation (+6.9 mg/dL, $P < 0.0001$). There was a tendency of a triple interaction between treatments and day 50 ($P = 0.09$, Figure 5-3). However, this interaction was only significative when finishing phase started ($P = 0.04$), with greater Cp levels for CT vs CC group (+6.3 mg*dL).

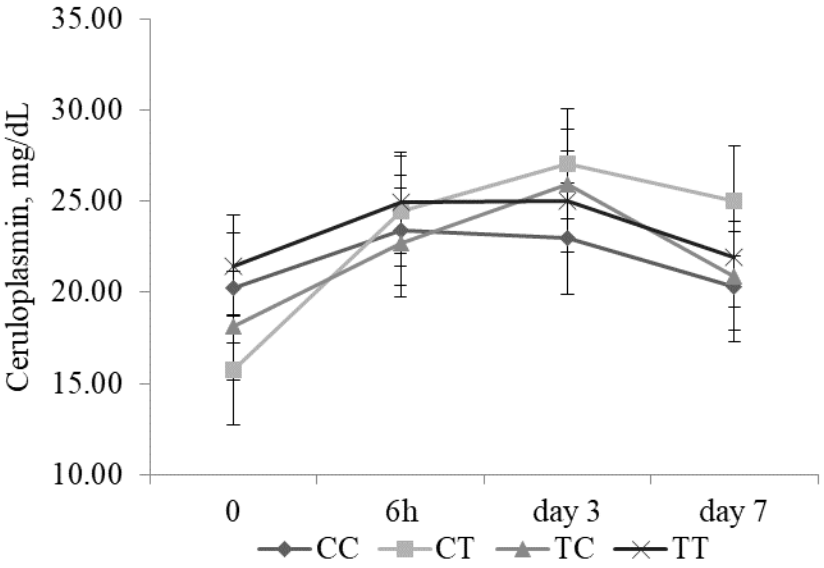

Figure 5-3. Concentration of plasma ceruloplasmin before (0) and after (6h) transportation, and at d3 and d7 since arrival at the feedlot, per treatment (CTRL= control group, YDP= yeast derived product supplementation during 30 days at backgrounding). Treatment P=0.539, Day P<0.0001, Treatment background*feedlot*day P=0.09.

Haptoglobin levels in plasma (Hp, mg/mL), were not affected by dietary treatment or treatment interaction, but again there was a day effect (P<0.0001) and a tendency of a backgrounding treatment*day interaction (P=0.052, Figure 5-4). Once finishing diet started, there was an increase in Hp concentration when compared TC with CC group (Table 5-4).

Backgrounding treatment caused a decrease in Hp plasma concentration at day 7 for animals receiving YDP supplementation (-0.08, P=0.031, Figure 5-4); however, this response was the opposite once the finishing diet started (Control -0.078, P=0.044, Table 5-4). In this case, transportation itself did not cause a significative change in Hp levels (P=0.57).

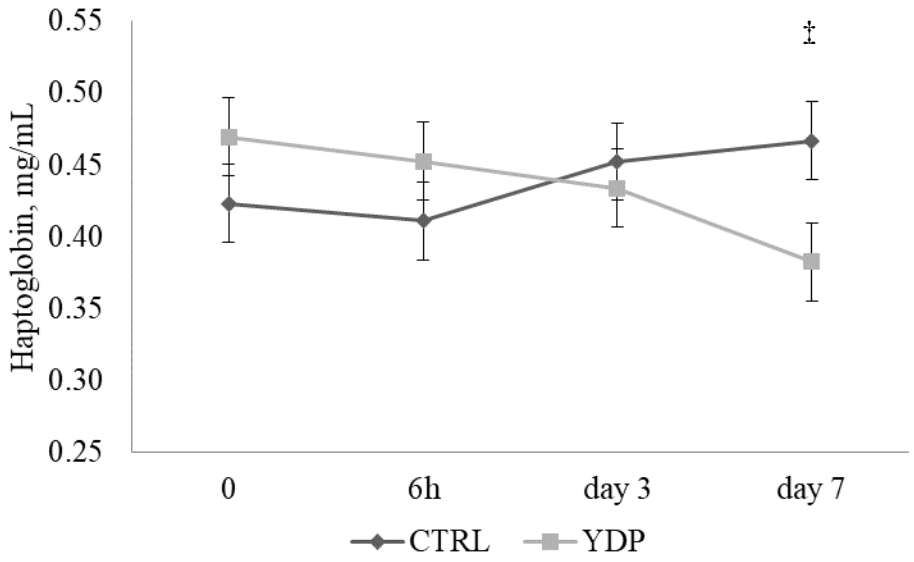

Figure 5-4. Concentration of plasma haptoglobin before (0) and after (6h) transportation, and at d3 and d7 since arrival at the feedlot, per treatment (CTRL= control group, YDP= yeast derived product supplementation during 30 days at backgrounding). Treatment *P*=0.1634, Day *P*<0.0001, Treatment*day *P*=0.052.

Table 5-4. Concentration of plasma acute phase protein in each treatment during finishing phase since finishing diet (80% concentrate-20% silage) was offered (d50) until the end of the experimental period (d120).

	Treatment[1]					
Item	**CC**	**CT**	**TC**	**TT**	**SEM**	***P*-value**
Ceruloplasmin mg/dL						
d50	28.3[a]	34.7[b]	29.4[ab]	30.9[ab]	2.18	0.016
d85	25.9	21.8	23.1	24.9	2.18	≥0.17
d120	28.8	26.9	30.9	26.9	2.18	≥0.19
Haptoglobin, mg/mL						
d50	0.27[a]	0.32[ab]	0.41[bc]	0.35[ab]	0.04	0.041
d85	0.34	0.34	0.36	0.35	0.04	≥0.73
d120	0.47	0.52	0.53	0.48	0.04	≥0.25

[1]CC = background phase without YDP supplementation + finishing phase with monensin
CT= background phase without YDP supplementation + finishing phase with YDP
TC= background phase with YDP supplementation + finishing phase with monensin
TT= background phase with YDP supplementation + finishing phase with YDP

Discussion

Yeast derived product supplementation during 30 days of the backgrounding phase did not cause any impact on steers performance. However, ADG (kg/d) achieve in both groups (0.59kg/d in average) was in a normal range of weight gain for backgrounded steers in grazing systems (0.6-0.8 kg/d) and dry lots (0.8kg/d) (Kumar et al., 2012).

Weight loss due to transportation is in average 1% per hour, during the first 3-4 hours of transport, decreasing at 0.1% per extra hour (Coffey et al., 2001). These values are going to be impacted by hydration level, feed restriction, animal weight, and climate condition, and mainly by length of transportation and management (Coffey et al., 2001; González et al., 2012). Our calves were transported for 6 hours, and the average weight loss was of almost 5% (Table 5-2), which could be considered as a normal weight shrink. Conditions during transportation were not extreme in temperature, length of transportation, or fasting, which could have helped to avoid an exacerbation of weight loss. Effect of pre-transport nutritional treatment is inconclusive (Coffey et al., 2001), and in this case did not have an impact. Once animals arrive to the feedlot, they had access to feed and water immediately which is helpful to cope with the stress of transportation (González et al., 2012). Treatment application at this moment was not thought to enhance performance, but to evaluate the impact of YDP supplementation on stress response after transportation and feedlot arrival.

Stress is the disruption of the homeostasis caused by one or various stimulus (Cannon, 1929). Consequently, after homeostasis is disrupted, different physiological mechanisms will be activated to return to that homeostasis, what is known as stress

response (Earley et al., 2017). During the stress response, cellular and humoral immune responses will be disturbed, increasing animal susceptibility to diseases, and affecting regulation of inflammatory responses (Mackenzie et al., 1997). Stress response will activate two axes, the hypothalamic-pituitary-adrenal (HPA) axis that culminates with the release of glucocorticoids from the adrenal cortex, and the sympathetic adrenal medullary (SAM) axis that end with the release of catecholamines from the adrenal medulla (Carroll & Forsberg, 2007; Chen et al., 2015). Increase of glucocorticoids synthesis and release will impact on nutrients metabolism, growth, regulation of stress, and regulation of the immune function (Wilson et al., 2017). Glucocorticoids can influence in innate immune system and induce pro-inflammatory responses (Chen et al., 2015). Activated immune cells as a response to an inflammatory process will secrete proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor-α, and interferon-γ) that will initiate an acute phase response (Arthington et al., 2003; Carroll & Forsberg, 2007). This will increase the synthesis and release of acute phase proteins synthesized at liver hepatocytes (Chen et al., 2015). Positive acute phase proteins used in cattle as indicators of infection response are: haptoglobin, ceruloplasmin, serum amyloid A, and fibrinogen (Carroll & Forsberg, 2007; Cooke et al., 2013). These acute phase proteins are mediators and released as a product of a pro-inflammatory response that can be measured directly in the plasma (Earley et al., 2017) and can be used as biomarker to detect an inflammatory response. Reduced levels of this protein are associated with decreased morbidity, mortality, and health cost during receiving phase (Step et al., 2008). Our previous work, in finishing feedlot steers, receiving this YDP as a supplement, indicate an increased acute-phase reaction which was associated with heightened immunocompetence,

suggesting the immunomodulatory properties of this YDP (Pancini et al., 2020). Even though the increase in haptoglobin concertation was not the expected response.

In the present study, supplementation of YDP at background or during feedlot phase did not cause significant changes in ceruloplasmin or haptoglobin levels. However, there was an impact of transportation itself and change of diet in ceruloplasmin plasma concentration. Transportation is one of the most common physical stressors in cattle, and can activate an acute phase response (Araujo et al., 2010; Arthington et al., 2003). This immunological response increases concentrations of acute phase proteins, as ceruloplasmin that will generally peak at d3 after transportation (Arthington et al., 2008; Mercadante et al., 2015). Registered increase in ceruloplasmin plasma concentration demonstrates that a 6-hour transportation caused a stress response in the steers. This response was not seen for haptoglobin.

Haptoglobin is an acute-phase protein produced by the liver with the function of modulates immune response and trapping microorganism and their products; plasma levels are used as a diagnostic tool to monitored health and identifies diseases, infections, inflammation or trauma reaction (Jain et al., 2011). Haptoglobin plasma concentration seems to be affected by backgrounding treatment, decreasing its concentration at d7 after transportation in steers that received the YDP supplementation. These results are aligned to what was expected for the treatment, alleviating stress response and decreasing this acute phase protein level, because of positive effect on immune status of yeast cell wall.

However, once steers start receiving the finishing diet with 80% of concentrate, haptoglobin levels increase in YDP treated animal at backgrounding. So, this effect was not carried over all adaptation period at the feedlot. Mannan oligosaccharide suppresses

pro inflammatory responses and β-glucan has an immune modulatory effect but need to be viable in the rumen to have effect (Nocek et al., 2011). Improvements on animal health and immune function are limited (Baines et al., 2011), because effect of prebiotics depends on not being degraded during fermentation and digestion in the gastro intestinal tract (Baines et al., 2011; Gaggìa et al., 2010a).

Haptoglobin elevated concentration levels in plasma are associated with stress response or nutritional challenges and influence negatively nutrient intake and growth (Cooke, 2017) as well as growth hormone production and catabolism muscle cells (Jain et al., 2011). In this case, it could be related to diet change, but it did not cause a negative effect on ADG or final performance (Table 5-3).

Yeast derived product inclusion in the diet at backgrounding, and continued inclusion during feedlot phase, allow to achieve the same ADG than the other treatments groups but with less feed intake, achieving a greater feed efficiency; being the TT group the most efficient one. Silva et al. (2018), in order to achieve a greater response in cattle health and performance during receiving phase, started the YDP supplementation 14 days before transportation. These authors reported a tendency to improve ADG and feed efficiency, without changes in physiological and acute phase responses. The lack of positive results was attributed to a restricted feed intake, and a low presence of concentrate in the diet (40%), that was not the case in the present experiment.

Yeast supplementation has been evaluated due to its effect on ruminal microbiota, digestibility and feed efficiency, that apparently will increase cellulolytic and lactate utilizing bacteria and protozoa population, maintaining a healthy ruminal pH even in concentrate diets (Baker et al., 2022). Yeast derived products are also characterized to

prevents health disorders, reduces lactate production, minimizes ammonia losses, stabilizes ruminal pH, improves organic matter and fiber digestibility, and increases volatile fatty acid (VFA) concentration and ruminal energy utilization (Desnoyers et al., 2009; Shurson, 2018). Additionally, Ponce et al. (2012) and Danielo et al., (2020) reported an improvement in feed efficiency and average daily gain (ADG), when a yeast derived product was supplemented to weaned calves during preconditioning period and/or receiving phase. In the other hand non-living yeast cell wall or mannan oligosaccharides supplementation in feedlot cattle tends to improve animal performance, showing an increase in daily weight gain and feed efficiency in feedlot receiving period (Young, 2012) as during finishing phase (Aragon et al., 2016). Also, it has demonstrated a positive effect on ruminal pH, rumen function and animal health. Greater ruminal pH enhances cellulose digestion, increasing feed digestibility and feed energy utilization (Russell & Strobel, 1989) and reduces ruminal acidosis risk (Callaway et al., 2003). Digestibility was not accessed in this study but would help to understand if YDP is causing an increase in digestibility, which allows to achieve a great performance with lower feed intake.

Feed intake tended to be greater for control group since transition period, which ended up with a difference in DMI at the end of the experimental period, for those animals receiving the control diet with monensin during finishing phase (Figure 5-2). This response was the opposite as data reported in the literature, where YDP inclusion in the diet increases dry matter intake (Ponce et al., 2012), particularly roughage intake (Nde et al., 2014). A low percentage of long fiber in the diet could had an effect on DMI and nutrient utilization.

Conclusion

Supplementation of a yeast derived product during the background phase for a 30-day period prior to transportation did not cause any changes in performance of beef steer calves. Following transportation, YDP supplementation failed to alleviate the acute phase response, but there was an effect 7 days after. At the finishing phase, yeast derived product supplementation showed the potential to decrease DMI and substitute the use of monensin in highly concentrated diets, achieving greater feed efficiency.

Chapter 6 : Supplementation of condensed tannins to grazing beef heifers during peripartum period affects natural coccidia parasite load in dam and offspring

Abstract

Coccidiosis is an enteric disease caused by a protozoan from the genus *Eimeria*, that affects all domestic livestock species, and it is characterized as being host-specific, a herd-disease, and an endemic problem. In cattle, young animals are more susceptible and predisposed to be infected. Infection occurs through sporulated oocysts ingestion from a contaminated environment (Mitchell et al., 2012). Adult animals act as a reservoir of oocysts that will continue to be excreted in the environment. It had been shown that nursing calves can acquire the infection from their dams. Control of coccidiosis is focused on decreasing the infection pressure. One alternative of control is the use of phytotherapy; specifically, the use of condensed tannins due to their anti-parasitic properties to decrease *Eimeria spp*. load and excretion. Data on the effects of preventive treatment of cows pre-calving and its effects on incidence of calf infection, health and performance is lacking. Our objective was to explore the effects of wood condensed tannins supplementation of beef heifers on their offspring incidence of coccidiosis, and growth performance in a pasture-based cow-calf operation system. A total of 30 pregnant beef heifers were classified by expected calving date, body weight (BW), and coccidia oocyst counts per gram of feces (OPG) at approximately 60d pre-partum, and randomly assigned to one of two treatment: 1) supplementation of 450g/h/d corn gluten feed (Control; n=14); or supplementation of 450g/h/d corn gluten feed and 20g/h/d of Quebracho colorado (*Schinopsis balansae*) condensed tannins (n=16; Treatment). All animals were managed as a single group, and heifers received treatment using an

automated feeding system (SmartFeed PRO, C-Lock Inc., Rapid City, SD) until 60d postpartum. Dam BW and fecal samples were collected every 30d. Offspring BW was determined at birth, and at 30d and 60d of treatment. Fecal samples were collected weekly after birth until 30d of age, and again at 60d of treatment. Supplement individual daily intake was similar between treatments (395±15.8 and 448±18.2 g/d for CT and Control, respectively; $P>0.10$). There was no difference in BW or weight gain for the dam or the offspring ($P>0.10$). Dam fecal OPG for *Eimeria spp*. was similar ($P>0.10$) at day -60 (161.5±8.5 OPG), and day 30 (107.5±9.4 OPG), but were greater ($P<0.05$) on day -30 for Control than CT (140.9±8.5 and 54.2±16.2 OPG, respectively). Calf OPG was similar on sample1 (0.0±0.0 OPG), sample 2 (283.1±25 OPG), and sample 4 (226.2± OPG); however, it was greater ($P<0.05$) for Control than CT calves on sample 3 (2,744.4±168.4 and 1,150.0±64.7 OPG, respectively). Oral supplementation of condensed tannins to periparturient grazing heifers has the potential to reduce coccidia load pre-calving for dam and their offspring postnatal.

Introduction

Coccidiosis is an intestinal disease that affects domestic livestock species caused by protozoan parasites of the genus *Eimeria* (Chapman et al., 2013). Each *Eimeria* specie is host specific (Bangoura et al., 2022), being *Eimeria bovis* and *Eimeria zuernii* the species considered pathogenic for young cattle (Daugschies & Najdrowski, 2005; Ernst et al., 1984). The incidence and severity of this disease is going to depend on the pressure of the infection, virulence of the *Eimeria* specie, and the immune status of the animal (Faber et al., 2002; Mitchell et al., 2012).

This parasite is characterized by its rapid multiplication and spread between animals, for which coccidiosis is always considered as a herd disease (Bangoura et al., 2022). Life cycle of *Eimeria spp*. involves two internal phases, and one external or environmental phase (Daugschies & Najdrowski, 2005). Transmission occurs via the fecal-oral route, where oocysts will be excreted in feces and will become infective once mature (Bangoura et al., 2022). This maturation process is called sporulation. Once the sporulated oocysts are ingested it will penetrate epithelial cells of the intestine, followed by asexual and sexual multiplication, producing a new generation of oocysts that will be spread in the feces (Chapman et al., 2013). Reproduction of this parasite is a long process (up to 3 weeks) that exclusively happens in intestinal endothelial cells (Hermosilla et al., 2012). Endothelial cells will be invaded and used for nutrition and development of exponential amount of parasites leading to tissue damage and clinical signs of this disease (Daugschies & Najdrowski, 2005).

In general, effective coccidiosis control is not based on complete elimination of the parasite, but in the reduction of the infection pressure and treatment of infected animals (Bangoura et al., 2022; Bangoura & Bardsley, 2020). Prophylactic and metaphylactic treatments of calves at risk has been shown to be efficacious in alleviating clinical symptoms and reducing environmental contamination (Daugschies et al., 2007; Romero et al., 2013). However, the use of anticoccidial drugs have the risk of generate antimicrobial resistance, as it has been extensively reported in poultry (Noack et al., 2019; Sangster, 2001). In general it is assumed that adult animals will play a minor role in the epidemiology of the disease, focusing on transmission between young animals on commercial farms (Bangoura & Bardsley, 2020). However, nursing calves, lambs, and

goat kids can acquire a primary infection from their mothers (Carrau et al., 2018; Faber et al., 2002). Data on the effects of prophylactic treatment of cows pre-calving and its effects on incidence of calf infection, health and performance is lacking.

Lately, the use of phytotherapy appear as an alternative to treat gastrointestinal diseases, based on antimicrobial properties of plant compounds (Tamminen et al., 2018; Weyl-Feinstein et al., 2014). Condensed tannins are plant polyphenols that have the ability to prevent bloat, impact methanogenesis and methane production, suppress gastrointestinal nematodes parasites, and improve protein nutrition through a reduction of protein ruminal degradability (Getachew et al., 2008; Min & Hart, 2003; Naumann et al., 2017) in ruminants. More specifically, chestnut tannins had been able to control calves diarrhea caused by protozoan when administrated orally (Bonelli et al., 2018). Similar results were found when feeding forages rich in condensed tannins reducing parasite load and alleviating clinical signs of coccidiosis in lambs (Burke et al., 2013; Saratsis et al., 2012), and goats (Markovics et al., 2012).

The potential exists that cows receiving condensed tannins supplementation during the last 60 days of gestation and the first 60 days post-partum might have reduced parasitic load, which will decrease environmental contamination and lessen the risk of neonatal calves to develop coccidiosis. Therefore, our objective was to explore the effects of wood condensed tannins peripartum supplementation of beef heifers on their offspring incidence of coccidiosis, and growth performance in a pasture-based cow-calf operation system.

Materials and Methods

All experimental protocols involving cattle were reviewed and approved by the Institutional Animal Care and Use Committee of Virginia Polytechnic Institute and State University. The experiment was conducted in the Beef Cattle center of Virginia Tech, Blacksburg, Virginia.

Animals and diets

A total of 30 Angus crossbred heifers, artificially inseminated during the spring breeding season, and diagnosed as pregnant, were enrolled in the study. Pregnancy diagnosis was performed through transrectal ultrasonography (Ibex portable ultrasound, 5.0-MHz linear multi-frequency transducer, Ibex, E.I. Medical Imaging, Loveland, CO) at 110 days after artificial insemination. At approximately 60 d pre-partum, determined by breeding and expected calving dates, heifers were stratified by body weight (BW) and coccidia oocyst counts per gram of feces (OPG), and randomly assigned to one of two treatments: 1) supplementation of 450 gDM/h/d corn gluten feed (Control; n=14); or 2) supplementation of 450 gDM/h/d corn gluten feed and 20 gDM/h/d of Quebracho colorado (*Schinopsis balansae*) condensed tannins (Treatment; n=16).

Treatments were administrated using an automated feeding system (SmartFeed PRO, C-Lock Inc., Rapid City, SD) that allowed individual access to the feed bunk and automated control over intake. Heifers, and calves, had *ad-libitum* access to native Virginia pasture, hay and corn silage, and clean water. However, only heifers had access to the automated feeder, to ensure calves were not having access to the treatment. Condensed tannins supplement was mixed with the corn gluten feed using molasses to avoid selection and improve palatability. Experimental period included approximately

120d during winter, from November to March 2022. Feeding started at 60d pre-partum until 60d postpartum. Both groups were managed together in a single pasture to increase the environmental pressure during the whole experimental period. Coccidiosis incidence was not artificially challenged. At the moment of calving, heifers were moved to the delivery area, a roofed pen inside the beef cattle barn, and spent one day before and one day after calving.

Measurements

Dam BW was determined at the beginning of the experimental period, approximately d-60 (pre-partum), at d-30 (pre-partum), and d30 and d60 post-partum. During those same moments fecal samples were collected from rectum grab and sent to the Virginia Tech Animal Laboratory Services (ViTALS, Virginia-Maryland College of Veterinary Medicine) for analysis. The McMaster egg counting technique was used to count the number of *Eimeria spp.* OPG in each fecal sample.

Offspring BW was determined at birth, and at 30d and 60d of treatment. Fecal samples were collected from the rectum, processed, and analyzed in the same way as the dam' samples. Calves were sampled weekly after birth until they approximately reach 30 days of age, and once again at the end of the experimental period. Incidence of diarrhea, as well as any other diseases, was monitored and recorded daily.

Concentrate intake was automatically measured every day. Allowed intake was set to be 450g DM/h/d. Intake was monitored to ensure all the cows had proper access to the feeder and avoid excessive intake. Concentrate, hay, and corn silage were sampled every week and conserved frozen. Feed samples were then dried in a forced-air oven at 55 °C for 72 h, and ground in a hammer-miller (Willey Mill) to pass through a 1-mm

screen. Composite samples of each component were made and stored for posterior nutrient analysis via wet chemistry procedures in a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA).

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA), with the fixed effects of treatment, initial BW as a covariate, and calf sex as random. Body weight and coccidial oocyst counts were analyzed as repeated measures with the effects of treatment, day, and treatment × day interaction, with day as the repeated measure. Significance set at $P < 0.05$, and tendencies $0.10 < P > 0.05$.

Results

Nutritional composition of feed offered is described in Table 6-1. Corn gluten feed offered to the treatment group was mixed with molasses; however, molasses addition did not cause an increase in net energy. This lack of difference was expected and desired to avoid an impact on cows' performance due to supplementation.

Table 6-1: Chemical characterization of diet offered.

Feed type	Hay	Silage	Corn gluten feed	Corn gluten feed+ molasses
Dry matter (%)	88.9	54.0	88.7	86.7
Crude protein (%)	7.2	15.6	16.6	15.9
NDF[1] (%)	79.4	39.7	36.7	35.8
TDN[2] (%)	52	71	70	70
NEm[3] (Mcal/kg)	0.9	1.63	1.65	1.65
NEg[4] (Mcal/kg)	0.35	1.01	1.03	1.06

[1]NDF: neutral detergent fiber
[2]TDN: total digestible nutrients
[3]NEm: net energy for maintenance
[4]NEg: net energy for gain

Supplement intake was not different between groups (395±15.8 and 448±18.2 g/d for Treatment and Control, respectively; $P = 0.67$, Table 6-2). Target intake was 450g per animal per day, for which we could say desired intake was reached, and tannins did not impact palatability.

Initial BW (day -60 relative to calving; 446±7.5 kg), as BW at the end of the experimental period, did not showed any difference between control and treatment groups ($P \geq 0.14$, Table 6-2). Evolution of BW showed a tendency to be greater for treatment group ($P = 0.08$) at 30 days post-partum (Figure 6-1). Average daily gain was not statistically different between groups ($P = 0.32$), despite a numerical difference in favor to treated group. Low weight gain could be associated with the winter season, where forage availability is limited.

Table 6-2. Grazing cows' performance and supplement intake during whole experimental period.

Item	Control	Treatment	SEM	*P*-value
n	14	16	-	
Body weight, kg				
Day -60	444.8	449.7	7.39	0.84
Day -30	485.7	511.3	7.89	0.19
Day 30	438.5	469.3	6.94	0.08
Day 60	455.1	480.6	6.38	0.14
Average daily gain, kg/d	0.06	0.20	0.03	0.32
Supplement Intake, kg/d	0.45	0.40	0.03	0.67

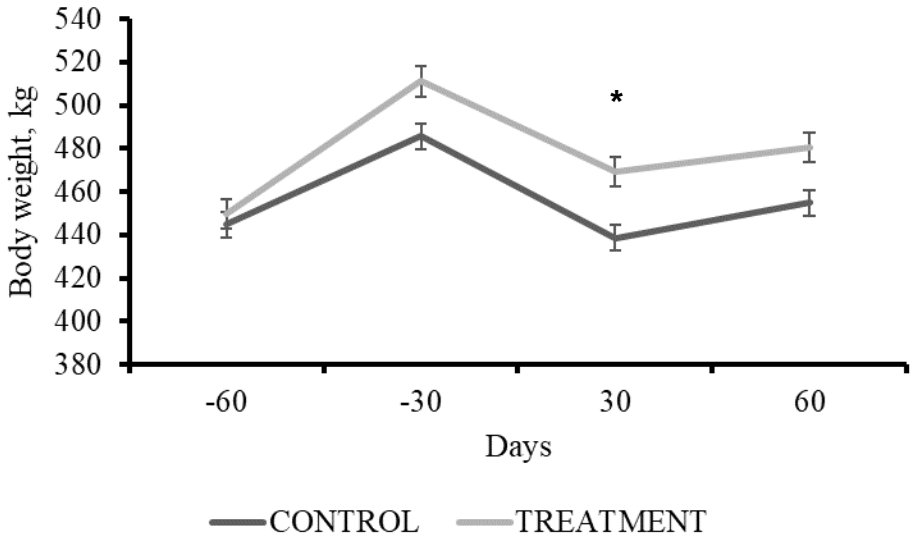

Figure 6-1: Evolution of dam body weight (kg) through the experimental period, showing a tendency ($P < 0.08$) to a greater BW at 30d post postpartum in favor of treatment group.

The parasite load was measured through *Eimeria spp.* OPG at 4 different moments. There was a treatment*day interaction (P <0.01), where treatment group showed a faster decrease in *Eimeria* OPG load from the initial level (Figure 6-2).

Dam fecal OPG for *Eimeria spp*. was similar (P>0.10) at day -60 (sample 1, 161.5±8.5 OPG), but were lower (P<0.05) on day -30 (sample 2) for treatment group when compared to control (54.2±16.2 and 140.9±8.5 OPG, respectively). This response allowed a decrease in parasite load close to parturition which could have an impact on offspring contamination. Oocysts were found in 63% of the heifers at the beginning of the experimental period. Prevalence of *Eimeria spp*. decreased for treatment group with values of 54%, 54%, and 8%; while control group was 54%, 85%, and 15%, at 30d pre-partum, and 30 and 60 d post-partum respectively.

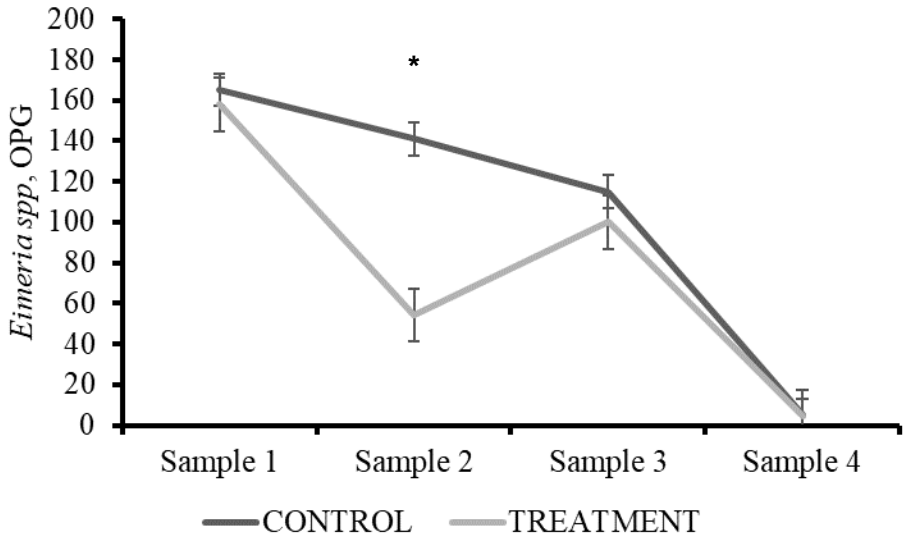

Figure 6-2. *Eimeria spp*. OPG in cows. There was no treatment effect ($P > 1.00$), treatment*day interaction $P<0.01$, and day effect $P<0.01$. With a significant difference between treatments on sample 2.

Calf birth weight and weight at the end of the experimental period were similar (Table 6-3, $P \geq 0.14$) between treatments (30.4±2.2 kg and 89.8±3.4 kg, respectively).

Table 6-3. Offspring performance from treated or control cows.

Item	Control	Treatment	SEM	*P*-value
n	13	13	-	
Body weight, kg				
Birth	31.6	29.9	0.82	0.56
Day 30	61.2	60.3	1.73	0.72
Day 60	86.8	92.8	3.42	0.14
Average daily gain, kg/d	0.91	0.92	0.01	0.87
Weight/day of age	1.47	1.37	0.08	0.21

This lack of response was translated to no impact on weight gain ($P = 0.87$). Calf *Eimeria spp.* OPG also showed a treatment*day interaction (Figure 6-3, $P <0.01$), where treatment group showed a lower parasitic load around 28-30 days of age (sample 3). No incidence of diarrhea or other disease was detected during the experimental period. Oocysts appear in calves at 23.6±8.8 days of age, with a prevalence of 23% and 8% for

control and treatment group. Incidence of oocysts increase at 28.4±9.8d, being 69% for both groups, and continue being high for control group with 62% of samples with oocysts, and 38% of treatment group samples with oocysts at the end of the experimental period with 62.7±13 days of age.

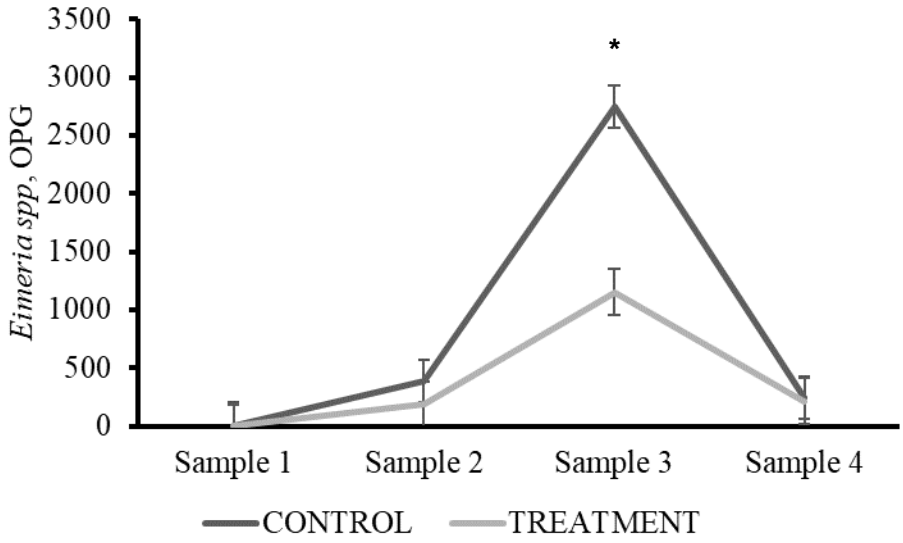

Figure 6-3. *Eimeria spp*. OPG in calves. There was no treatment effect ($P>0.1$), but there was treatment*day interaction and day effect ($P<0.01$), with a difference in OPG load at sample 3.

Discussion

Coccidiosis is an intestinal infection caused by a protozoan from the genus *Eimeria*, that affects all the livestock species (Chapman et al., 2013). This disease can cause major performance and productivity losses (Bangoura & Bardsley, 2020; Chapman et al., 2013) associated with long term impaired growth, delayed reach of puberty, and treatment cost (Keeton & Navarre, 2018; Lassen & Østergaard, 2012). Economic impact of coccidiosis in ruminant production is tremendous, with annual losses estimated to reach upwards to US$723 million worldwide (Fitzgerald, 1980).

In cattle, coccidiosis may produce clinical symptoms in animals between 3 weeks to 1 year of age (Ernst et al., 1984); however, it can infect animals of all ages. Coccidiosis symptoms are diarrhea, slow growth, morbidity, and even mortality (Epe et al., 2005). Once symptoms are detectable, animal had been already infected weeks ago and it is shedding new oocysts in the environment, because *Eimeria* cycle takes about 2-4 weeks to be completed (Keeton & Navarre, 2018).

Adult animals developed partial immunity after being infected, which decreases the level of oocyst excreted after reinfection (Joachim et al., 2018). However, they still act as a reservoir of oocysts that will continue being excreted in the environment (Keeton & Navarre, 2018). Younger animals are naïve up to their first infection, leading to a considerable multiplication of the parasite and excretion of high numbers of oocysts, further contaminating the environment for the next naïve animals (Bangoura & Bardsley, 2020; Joachim et al., 2018). Also, susceptibility will lead to greater infection and greater oocysts output (Romero et al., 2013).

Risk factors for infection of newborn calves includes inadequate colostrum intake, and environmental factors such as crowding of calving females and newborn calves, constant use of small buildings or pasture as maternity without adequate hygiene, and re-grouping of cow-calf pairs. Maternal antibodies seems to have a direct impact on OPG on calves, where greater antibodies level will decrease load and excretion of oocysts (Jäger et al., 2005). In the other hand, environmental load is one of the main infection sources for *Eimeria spp*. Once excreted, *Eimeria* oocysts need about 1 to 4 days to mature and become infectious. Infection pressure will be greater in animals that are grouped in the same place, like watering and feeding areas, feedlots, and stocked pastures (Daugschies

& Najdrowski, 2005; Keeton & Navarre, 2018; Noack et al., 2019). All husbandry practices with the objective to avoid accumulation or direct contact with feces will decrease incidence of this parasite (Jäger et al., 2005).

Once the environment is contaminated, the resilience of the oocyst ensure the presence of the parasite on the site (Chapman et al., 2013). Oocysts are able to sporulate in a great range of pH, temperatures, and oxygen levels, with low response to bactericidal control (Marquardt et al., 1960). Exposure of oocysts to high temperatures (>39C) and ultraviolet light for a prolonged period would inhibit sporulation (Marquardt et al., 1960). However, in the United States more than 50% of calves are born between February and April (USDA, 2020), where weather conditions would not be optimal to inhibit sporulation. In the present study all calves were born between January and February.

The focus of ruminant coccidiosis control lies on treatment of infected animals (Bangoura et al., 2022), or in the reduction of infection pressure to uncritical levels and endemic stability (Bangoura & Bardsley, 2020), rather than pathogen eradication. Some exposure to the parasite is needed for the animal to develop certain immunity (Keeton & Navarre, 2018). Also, *Eimeria spp*. cumulative incidence in beef cattle herd tend to be 100% (Jäger et al., 2005), for which this disease is commonly considered as an endemic problem (Carrau et al., 2018), and a herd disease (Bangoura et al., 2022).

Preventive treatments, as control treatments using coccidiostats, used on calves, can alleviate clinical symptoms and reduce oocysts excretion (Daugschies et al., 2007; Romero et al., 2013). However, preventive treatments (coccidiostats) used on young animals need to be applied to all animals, even to those showing no symptoms (Keeton & Navarre, 2018). Even when subclinical coccidiosis can impair growth performance, the

cost/benefit ratio of using a treatment needs to be estimated to ensure an economic benefit when applying it (Daugschies et al., 2007). Another concern for the general public is the risk of developing drug resistance and the presence of residues in animal products (Bangoura et al., 2022; Lassen & Østergaard, 2012; Saratsis et al., 2012).

Decrease infection pressure appears to be the most effective and safe measurement to control *Eimeria spp.* incidence and spread between animals. It has been demonstrated that infection in calves is greater when reared with the dam, especially in crowded systems (Faber et al., 2002; Tomczuk et al., 2015), due to oocysts being excreted in the delivery area (Joachim et al., 2018). Same was reported for lambs, being the ewe the main source of infection (Carrau et al., 2018).

One way to reduce environmental contamination is using plant compounds as nonpharmacological treatments. Tannins are characterized by its effect on intestinal bacteria, nematodes, and protozoa, which make it an antibiotic alternative (Huang et al., 2018). Tannins are classified as hydrolysable or condensed tannins based on their chemical structure. Condensed tannins are polymers of flavonoids, largely resistant to microbial degradation, commonly found in forages, legumes, and trees leaves (Huang et al., 2018). One of the most common commercial products are quebracho (*Schinopsis spp.*) wood tannins (Parisi et al., 2018).

Quebracho tannins supplementation caused a reduction in *Eimeria spp.* oocyst excretion on rabbits (Parisi et al., 2018), and goat kids, where it also improved their weight gain (Fraquelli et al., 2015). Acharya et al. (2020), also found a reduction in fecal oocyte count in treated (100g quebracho tannins/day) lambs and goat kids before and after weaning. However, this effect was not translated in a reduction of coccidiosis

clinical symptoms. Oral supplementation of chestnut tannins is also effective in controlling calves diarrhea caused by protozoan (Bonelli et al., 2018). Similar results were found when feeding forages rich in condensed tannins reducing number of excreted *Eimeria spp.* oocysts in lambs (Burke et al., 2013; Saratsis et al., 2012) and goats (Markovics et al., 2012). These results showed the potential anti-protozoal effects of condensed tannins to prevent and control coccidiosis when supplemented directly to the animal.

Our objective was to evaluate the impact of feeding quebracho tannins on dam parasitic load and its impact over offspring coccidiosis incidence. Maternal oocyst excretion will increase the risk of contamination, exposing young animal to sporulated oocysts since their first day of life (Acharya et al., 2020; Faber et al., 2002). Faber et al. (2002) reported an increase in infection prevalence, OPG, and oocysts excretion of *Eimeria spp.* during the prepartum period, same response reported in sheep.

Supplementation of quebracho condensed tannins in periparturient heifers had an impact on shedding oocysts on the heifers and on the newborn calves. Having in mind that *Eimeria spp.* cycle takes about 2-4 weeks to reproduce and be excreted (Keeton & Navarre, 2018); the reduction of OPG before parturition could help in reducing udder and environment contamination before the calves were born, which was translated in a faster decrease on OPG in calves born from the treated group. This response showed that the treatment was effective during the pre-partum period, avoiding an increase in *Eimeria spp*. prevalence.

Lucas et al. (2014) characterized the mid-Atlantic US region with a high incidence of *Eimeria spp*. parasites, present in more than 90% of the samples analyzed,

especially in replacement heifers and calves. Which was expected due to the greater susceptibility of younger animal versus mature cows (75%). So, the presence of the pathogen is real in forage-based beef cattle systems. However, no signs of clinical coccidiosis were detected in these animals. Count greater than 5000 OPG plus some signs could be directly related with the incidence of coccidiosis (Keeton & Navarre, 2018). However, OPG by itself cannot be directly related to the presence of coccidiosis. Pathogenicity of each *Eimeria* specie is different (Joachim et al., 2018). Lucas et al. (2014) found in calves an OPG range from 25 to 52000, and in heifers from 25 to 10925, without showing symptoms. However, for *Eimeria bovis* and *Eimeria zuernii*, an OPG value of 500 can be used to define the presence of clinical coccidiosis in the herd (Joachim et al., 2018).

The location where the experiment took place is characterized by a low incidence of clinical coccidiosis, with 63% of heifers presenting oocysts at the beginning of the experimental period. Our experiment was based on natural infection of this parasite, which can explain low initial levels of OPG in heifers, and the absence of coccidiosis symptoms in dams and calves as diarrhea and weight loss (Bangoura et al., 2022). Unfortunately, *Eimeria* species were not identified, but no signs of diarrhea or feces samples containing blood or tissues were observed. Also, calves did not show any signs of loss weight, reaching an excellent average weight gain for pre-weaned calves.

The fact that the oral supplementation of the condensed tannins influenced OPG levels even when the initial levels were low, showed a potential of the use of these plant compound to decrease contamination pressure and control *Eimeria spp*. infection and reproduction. In general, calves start showing coccidiosis symptoms at 3 weeks of age

(Ernst et al., 1984). Faber et al. (2002) reported first *Eimeria spp*. incidence in calves at 21d of age, with a continuous increase in this parasite prevalence until 63d old. A decrease in parasitic load at around 30 days of age (Figure 3), shows a positive effect of condensed tannins dam supplementation to alleviate parasitic load in the more susceptible moment for the offspring. Parasites seen at 3 weeks of age are a consequence of an earlier infection, probably immediately after birth (Joachim et al., 2018).

Conclusion

Oral supplementation of condensed tannins to grazing pregnant heifers generated a transient reduction in coccidia load pre-calving for dams and their offspring postnatal, when offered 60d pre- and pos-partum. This reduction in coccidia load demonstrates the potential of wood condensed tannins in parasite as an alternative natural control and coccidiostat.

Chapter 7 : Validation of an automated scale system for grazing and feedlot beef cattle

Abstract

Body weight (BW) is an essential measure in beef cattle production systems, used for various decision-making processes. Detecting changes in BW allows for estimation of weight gain curves, determine system profitability, and detect nutritional or health disorders reflected in weight loss. However, measuring BW requires animal movement and handling. Even non-harmful handling processes, such as restraining in a squeezy chute can trigger a stress response, negatively affecting animal performance. An automated scale, located in the pasture or in the feedlot pen, will reduce labor and animal labor handling, while ensuring an accurate BW estimation. Our objective was to evaluate the functionality and accuracy of an automated scale system (SmartScale, C-Lock Inc, Rapid City, SD), when compared to a conventional scale located at the cattle working facility. The automated scale is installed in front of the water through, and the system registers BW every time the animal approaches the water trough and automatically transmits data to a server via cellular network. The automated scale was evaluated in three experiments:1) Eight multiparous Angus crossbreed grazing cows (GC) were weighed in a 14-day interval for a period of 57 days with a conventional scale, while during the same period BW was measured daily with an automated scale. 2) Twenty-six multiparous Angus cows (FC) assigned to four concrete floor feedlot pens were weighed every 14 or 28 days, for a period of 88 days with a conventional scale, and daily with an automated scale. 3) Forty-four castrated yearling Angus crossbreed steers (FS) assigned to four concrete floor feedlot pens were weighed once a month during a 119-day period

with a conventional scale and daily with an automated scale. Correlation between weighing systems was evaluated through a linear regression (R Core Team, 2019), using average BW values for the whole period for each animal. The adjusted R^2 values were 0.99, 0.93, and 0.91, for GC, FC, and FS, respectively. In addition, the automated scale registered the time of day, time spent in the scale, and number of daily visits to the water through. In conclusion, an automated scale system accurately measures BW in grazing and feedlot systems for growing and mature beef cattle while reducing cattle handling, without disrupting feeding behavior, decreasing the probability of animal lesions, accidents and optimizing labor.

Introduction

Body weight (BW) is an essential measure in beef cattle production systems, used for various decision-making processes. Changes in BW can be used to detect health disorders, nutritional disorders associated with feed intake or feed quality, calculate growth rate, generate weight gain curves, measure feed efficiency, and estimate profitability of the production system (Berry & Crowley, 2012; Segerkvist et al., 2020). Variations in BW and growth rate will also affect reproductive success (Clanton et al., 1983; Roche et al., 2007).

A precision livestock production system requires routinely monitored BW (Laca, 2009). However, measuring BW frequently interferes with animal behavior and environment (Charmley et al., 2006), and relies on a scale and handling facilities around it (Otte et al., 1992). Even non-harmful handling events, like exposure to novel facilities or restraining for weighing, are considered psychological stressors and can generate a negative physical stress response, like hunger, thirst, or fatigue which in consequence

will negatively impact animal performance. (Grandin, 1997). The intensity of such stress response will depend on animal temperament and habituation to handling procedures (Cooke et al., 2012; Grandin, 1997).

Neuroendocrine stress response will activate the hypothalamic-pituitary-adrenal (HPA) axis that culminates with the release of glucocorticoids from the adrenal cortex (Carroll & Forsberg, 2007; Chen et al., 2015). Increase of glucocorticoid synthesis and release, will impact on nutrients metabolism, growth, susceptibility to diseases, reproductive success, regulation of stress and immune function (Cooke et al., 2012; Earley et al., 2017; Mackenzie et al., 1997; Wilson et al., 2017).

In grazing systems, especially rangelands, cattle are less used to people and new facilities, and restraining itself can cause similar stress levels than branding (Grandin, 1998), doubling basal cortisol levels in beef calves (Lay et al., 1992). Bristow and Holmes (2007) found that beef cows with greater cortisol levels spent more time vocalizing and standing, and less time ruminating. This behavior is a reflection of anxiety behavior, and has been related with less weight gain in beef heifers (Fisher et al., 1997) and steers (Purchas et al., 1980). In feedlot systems, cattle are more used to people, noises, and facilities. However, movement from the pen to weighing facilities can incur long trips, be labor intense, and cause heavy breathing, activating a stress response, reducing appetite and feed intake, and negatively impacting performance and feed efficiency.

Body weight can be accurately predicted using body measurements ($R^2 \geq 0.95$), like body length, wither height, hip width, and heart girth (Heinrichs et al., 1992), and the combination of these (Bozkurt, 2006). However, all these measurements require

restraining the animal, partially or completely, and result in great variability, especially in lightweight animals (Otte et al., 1992). Measuring BW automatically with animal identification technology using a walk-through system was successfully demonstrated by Peiper et al. (1993). However, it was necessary to control walking speed and animal flow to register a valid and accurate weight value (Alawneh et al., 2011).

Measuring BW every day without the need of moving the animals will ensure more accurate values for growth rate estimation when compared to BW measured between longer intervals. The possibility of having an automated scale located in the pasture, or feedlot pen, to measure BW regularly, remotely, and accurately, will reduce labor, decrease animal disturbance, and limit the resulting psychological and physical stress responses from animal-human interactions (Charmley et al., 2006; Peiper et al., 1993; Segerkvist et al., 2020). Therefore, the objective of this research was to evaluate and validate the functionality and accuracy of an automated scale system in comparison to a conventional scale to determine BW.

Materials and Methods

All experimental protocols involving cattle were reviewed and approved by the Institutional Animal Care and Use Committee of Virginia Polytechnic Institute and State University #20-161, 20-178, and 21-095. Three experiments were conducted in the Shenandoah Valley Agricultural Research and Extension Center of Virginia Tech, in Raphine, Virginia.

Weighing systems

Automated scale: The SmartScale (C-Lock Inc, Rapid City, SD) is an automated wireless scale system, which provides real-time animal weight. Animals are identified by the scale using a low-frequency RFID tag system. The automated scale was located in front of the water trough, and measured BW every time the animal drinks water and steps into the scale platform with both front feet (Figure 7-1). Data was transmitted directly to a server via cellular network in real time and included not only BW, but also number of visits, duration of the visit, and time of the day for each visit.

Conventional scale: A static scale (XR ID5000, Tru-test^{TM} Datamars, Mineral Wells, TX) attached to the restraining squeeze chute in the cattle handling facilities located in proximity to the pasture paddock (Experiment 1) and feedlot facilities (Experiments 2 and 3).

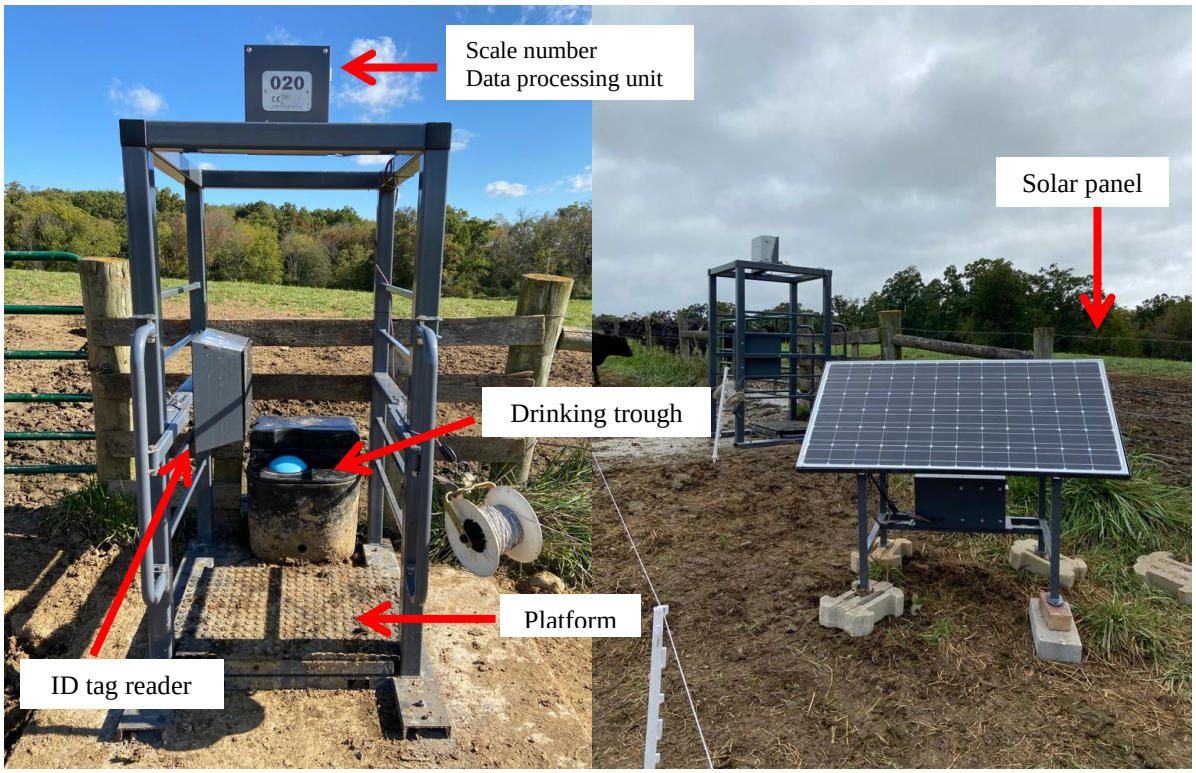

Figure 7-1. Automated scale located in the pasture with its solar panel by the side.

Upon exposure to the automated scale, animals were observed to determine latency time to approach the water and successfully drink water while being weighed. Visual observations were performed to verify the access of all animals to the scale without problems in the three first days at morning, afternoon, and evening.

Animals, BW measurements, and experimental design

Experiment 1: Eight multiparous Angus crossbreed grazing cows (GC) with *ad-libitum* access to a fescue pasture, grass hay, and free-choice mineral supplementation, were managed in the same paddock, with one drinking trough where the automated scale was located. Cows were weighed in a 14-day interval for a period of 57 days with a conventional scale, to ensure a good number of measurements with the traditional scale. Animals were walked onto the static scale located inside of the chute without any specific order. Body weight was recorded once system equilibrium was reached. At the same time, BW was registered every day in the automated scale located in the pasture in front of the water trough. This wireless system registered BW every time the animal approaches the water trough and automatically transmits it to a server via cellular network, generating one average value per day per animal. Body weight data were downloaded from the interface every week. Cows were identified using a radio frequency electronic identification (RFID) system (Allflex, DFW Airport, TX). Measurements of BW on the conventional scale, and automated scale were made on the same group of animals, at the same time for each of the experiments, to correlate the recorded values for same group of animals over time.

Experiment 2: Twenty-six multiparous Angus cows (FC) were randomly assigned to one of four semi-roofed concrete floor feedlot pens, each one equipped with one

drinking trough where the automated scale was located, and Calan gates (American Calan Inc., Northwood, NH, USA) for individual feed delivery. Cows were fed a total mixed ration (TMR) with high roughage proportion (88% corn silage), once a day in the morning. Feedlot cows were weighed every 14 or 28 days on a conventional scale, during an 88-day period, as previously described. Measurements of BW at the automated scale were recorded everyday as described for experiment 1.

Experiment 3: Forty-four castrated yearling Angus crossbreed steers (FS) were randomly assigned to one of four semi-roofed concrete floor feedlot pens, each one equipped with an automated scale in front of the drinking trough, and Calan-gate feeding system. Steers were fed a highly concentrated TMR (80% concentrate/20% corn silage), once a day in the morning. Feedlot yearling steers were weighed once a month during a 119-day period with a conventional scale, as previously described. Measurements of BW at the automated scale were recorded everyday as described for experiment 1.

Behavior

Automated scale automatically registered the number of visits per day, duration of the visit, and time of the day that each animal visits the scale. This information was used to characterize the behavior of the animal in the three experiments.

Statistical analyses

Correlation between weighing systems was evaluated through a linear regression (R Core Team, 2019), comparing average BW recorded during the whole experimental period for each animal. Every visit to the automated scale that generates a valid record

was included. In the model, weigh values from the automated scale were the dependent variable, while the predictor were the weights from the conventional scale.

Results

Experiment 1

Correlation between weighing systems using a linear regression showed an adjusted R^2 value of 0.99 (Figure 7-2), which means that values recorded using a conventional scale are going to explain in 99% values recorded automatically in this automated scale located at the pasture. To build a generalized model of prediction based on coefficients obtained, this will be y=0.99(x)-10.4. This means that the difference in prediction between both weighing systems will be only 0.91±0.72% of the cows BW. Finally, *P* value < 0.0001, indicates the significance of the model.

Behavior characterization for grazing cows, showed 2.93±1.22 visits per day, with an average time spent on the scale of 2.94+1.84 minutes, with a smaller probability to find an animal in the scale during night hours (1900-0700, Table 7-1).

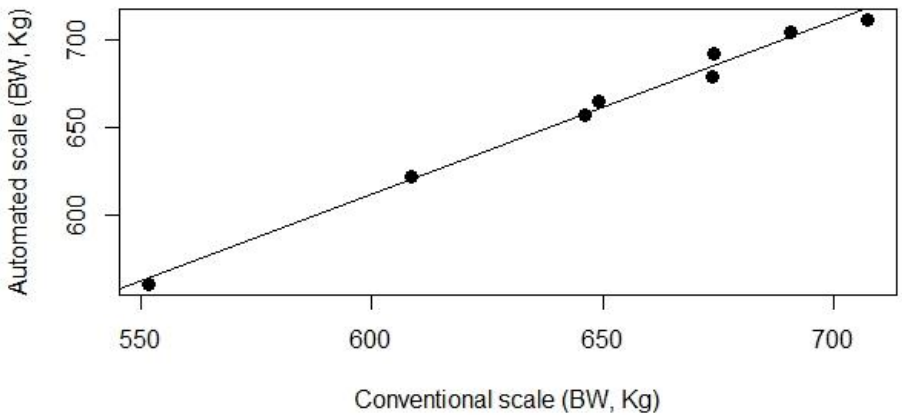

Figure 7-2. Correlation between conventional and automated scale for grazing mature cows. R^2 = 0.99; *P* < 0.0001.

Table 7-1. Probability (%) to find an animal in the scale for 24 hours period, for grazing cows (GC), feedlot cows (FC), and feedlot yearling steers (FS).

	Hours						
	07-09 am	09-11 am	11-13pm	13-15pm	15-17pm	17-19pm	19-07am
GC	14.9	14.6	20.0	16.7	20.3	9.7	3.9
FC	5.0	15.2	23.1	17	12.7	13.1	13.9
FS	9.8	12.8	12.4	11.0	12.6	15.1	26.2

Experiment 2

As experiment 1, linear regression showed an excellent adjustment, with and adjusted R^2 of 0.93 (Figure 7-3), meaning that 93% of weigh recorded with the automated scale are explained by the ones registered in the conventional scale, with a *P* value <0.0001. The generalized prediction model will be y= 0.95(x)+24.04, predicting values with an average variation of 2.5±1.6%BW.

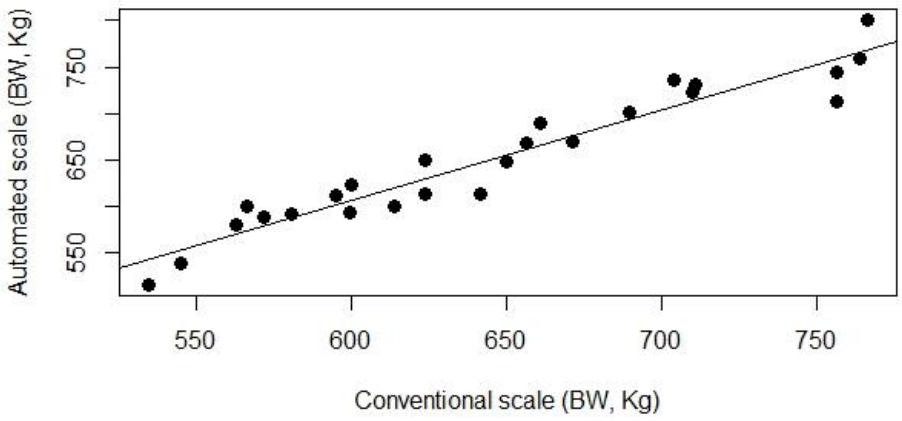

Figure 7-3. Correlation between conventional and automated scale for feedlot mature cows. $R^2 = 0.93$; $P < 0.0001$.

Behavior data obtained from the automated scale showed that mature cows in a feedlot system visit 2.72±2.66 times/day, with a duration of 2.22±2.19 minutes per visit. The probability of finding a cow at the scale was evenly distributed between day at night hours, increasing after 9 am, time of feeding (Table 7-1).

Experiment 3

Correlation between weighing systems for yearling steers showed an excellent and significative (P = 0.0001) linear relationship between values obtained by the conventional scale and values obtained by the automated scale, with an adjusted R^2 value of 0.91 (Figure 7-4). The generalized prediction model will be y =0.95(x)+24.51, with an average variation of 2.7±1.9%BW. In terms of behavior, yearling steers visited the scale 6.6±1.9 times per day, spending an average of 2.92±2.92 minutes/day. Distribution of probability of visit showed a similar distribution than experiment 2 (Table 7-1) with increasing values after feeding time (9am), but with greater probability to find an animal in the scale during night hours.

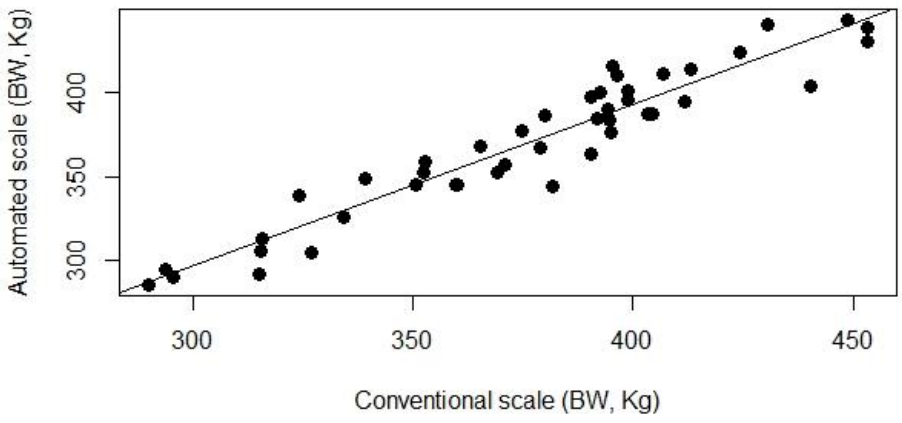

Figure 7-4. Correlation between conventional and automated scale for feedlot growing steers. R^2 = 0.91; P < 0.0001.

Discussion

Body weight is an essential measure to monitor weight changes and gain rate, that will directly impact productive and reproductive performance (Clanton et al., 1983). However, measuring BW regularly requires walking animals to and from the chute, which is labor intense, time consuming, and can be stressful (Charmley et al., 2006).

Nonetheless, adoption of technologies for measuring BW remotely will allow detection of short and long-term changes in performance associated with management decisions an environmental factors (González et al., 2014).

Smart farming is a concept that implies the use of innovative technology in the farm, to make processes more efficient and enhance profitability (Alawneh et al., 2011; Hansen et al., 2018). These technologies allow to closely monitor and manage individual animals using real time data to improve productivity and animal welfare (Hansen et al., 2018; Werkheiser, 2018). Adoption of such technologies has been greater for the dairy industry in comparison to the beef industry, including automated feeding and milking systems, sensors and activity monitors that allow for estrus detection and body temperature and rumination measures (Alawneh et al., 2011).

Use of walk-through scales, which do not require animal restraint, has been established as an accurate method to measure BW with a high correlation to conventional scales. However, these walk-through scales have limitations, including outlier detection and elimination, cow flow and walking speed, and the need of 7 days registries to make management decisions based on BW variations (Alawneh et al., 2011; Peiper et al., 1993). Walk-through scale systems were also tested in grazing systems, using water and minerals as an incentive for steer calves to get inside the weighing stations (Segerkvist et al., 2020). However, calves had to be trained on how to pass through a one-way gate to access the weighing station.

Implementation of a static scale but located in the pasture or feedlot water trough has shown a great potential as a new tool to estimate changes of body weight in real time.

These three different groups allow us to evaluate the effectiveness of an automated scale, in a grazing system vs a feedlot, mature vs growing animals, and different types of diets. The automated scale used herein has several advantages. First, this technology showed a great accuracy in estimating BW without disrupting natural behavior, there was no report of any problems or error from the server were detected during the experimental period, it does not require training before first exposition, and there is no need to build facilities or infrastructure around it, only a concrete pad in front of the water through.

These results make this automated scale a great technology to include in the farm. Especially in grazing systems, where moving animals, and weighing them is even more time consuming. The possibility of this scale being powered by a solar panel allows it to be placed in remote locations of the farm, which will be essential especially for rangeland production systems.

A disadvantage of this technology could be the need to have a specific water trough model, and the price when compared to a conventional scale. However, this price difference could be easily amortized by reduction in labor time, and stress, achieving a greater animal performance, but also greater labor conditions.

Behavior data is an extra tool offered by this system that could be used as an indicator of health or nutritional disorders, weather conditions, or even water intake if water flow could be measured at the farm. The minimum time needed for the scale to record a valid BW value was only 11 seconds, both in pasture and feedlot system, which make the system easy, fast, and reliable to use.

Conclusion

In conclusion, we successfully validated the use of an automated scale in grazing and feedlot system, getting excellent prediction results in both systems with different animal categories.

Chapter 8 : Conclusion and implications

Beef cattle production systems need to become more efficient, to be environmentally sustainable and profitable at the same time. Society concerns around beef production are based on environmental footprint, antibiotics resistance, and residues in final product. Our objective was to use nutrition and available technologies to enhance productivity and ensure animal welfare and health. Based on results obtained in chapter 3, we can conclude that the use of injectable sources of microminerals can be beneficial in grazing cows to enhance their reproductive performance, especially when their energy status measure through their body condition score is not optimal. Also, it is important to highlight that there is no need for an extra trace mineral supplementation when the cows are in good energetic and mineral status, which refutes the idea that minerals could be added in excess to ensure a benefit from it. This not only increases production costs but also increases the risk of toxicity.

Benefits of growth promoters especially in feedlot systems is well documented, hence its wide adoption worldwide. The ban on use of ionophores as antibiotic growth promoters in the European Union created the need for changes in production practices. First, it limited the use of ionophores in Europe, then it created an extra concern around these antibiotics from the public, consumers, and producers. Natural alternatives to ionophores exist and have been studied with inconsistent results. The use of yeast derived products, as a combination of prebiotic and probiotics, in high concentrate feedlot diets, as described in chapter 4 and 5, failed to improve performance and carcass characteristics when used together with monensin, but showed the potential to be the substitute of this ionophore without causing health disorders. The possibility to substitute monensin with a natural additive is a great opportunity to produce efficiently, achieving great feed

efficiency. The effect of this yeast derived product on acute phase response is still inconclusive.

In chapter 6, we evaluated the possibility of using plant secondary metabolites as an alternative to synthetic chemicals, due to its phototherapeutic properties, to substitute the use of antibiotic with high risk of develop resistance, as are the coccidiostats. We can conclude that the oral supplementation of condensed tannins in pregnant heifers was able to generate a transient reduction in coccidia load of the dam and in the offspring during the period with greater susceptibility to develop this disease. These results were transient, but also seem to be effective even when coccidia load in the environment was low, showing a potential to be used as a treatment alternative to control this parasite.

Lastly, in chapter 7 we validate the use of an automated scale to ensure accurate body weight measurements without interfering with animal environment. This technology allows us to reduce labor intensity and animal stress. All these alternative strategies explored in this dissertation showed to be successful and could be easily implemented to improve health, feed efficiency and reproductive performance of beef cattle, having an impact on productivity and profitability, while addressing public concerns with beef production practices.

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