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Evaluation of Feed Additives and Delivery Method for Receiving and Finishing Cattle

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EVALUATION OF FEED ADDITIVES AND DELIVERY METHOD FOR RECEIVING AND FINISHING CATTLE

BY

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THESIS ACCEPTANCE PAGE

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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LIST OF ABBREVIATIONS

AA	Amino Acid
ACP	Acyl Carrier Protein
ADF	Acid Detergent Fiber
ADG	Average Daily Gain
AFBW	28% Estimated Empty Body Fatness
AOAC	Association Of Official Agricultural Chemists
ATP	Adenosine Triphosphate
B1	Thiamin
B12	Cyanocobalamin
B3	Niacin
B5	Pantothenic Acid
B6	Pyridoxin
B7	Biotin
B9	Folate
β-AA	Beta-Adrenergic Agonist
BRD	Bovine Respiratory Disease
BW	Body Weight
CH_4	Methane
Cl	Chloride
cm	Centimeters
Co	Cobalt
CoA	Coenzyme A
CP	Crude Protein
Cr	Chromium
Cu	Copper
cwt	Hundredweight
d	Day
DDGS	Dried Distillers Grains Plus Solubles
DM	Dry Matter
DMI	Dry Matter Intake
DNA	Deoxyribonucleic Acid
DOF	Days On Feed
EBF	Empty Body Fat
FBW	Final Body Weight
FDA	Food and Drug Administration
Fe	Iron
g	Grams
G:F	Gain to Feed
h	Hour

HCW	Hot Carcass Weight
hd	Head
I	Iodine
K	Potassium
Kg	Kilogram
Km	Kilometer
m	Meter
MDGS	Modified Distillers Grains Plus Solubles
Mg	Magnesium
mg	Milligram
mL	Milliliter
mm	Millimeter
Mn	Manganese
Mo	Molybdenum
Na	Sodium
NASS	National Agricultural Statistics Service
NDF	Neutral Detergent Fiber
NE	Net Energy
NEg	Net Energy for Gain
Nem	Net Energy for Maintenance
Ni	Nickel
P	Phosphorus
PBS	Phosphate Buffered Saline
PET	Production-enhancing technologies
PPM	Parts Per Million
RCBD	Randomized Complete Block Design
REA	Ribeye Area
RH	Ractopamine Hydrochloride
RNC	Ruminant Nutrition Center
RPBV	Rumen Protected B Vitamin
RY	Calculated Retail Yield
S	Sulfur
SCFP	Saccharomyces Cerevisiae Fermentation Products
Se	Selenium
SERF	Southeast Research Farm
t	Ton
TM	Trace Mineral
TMR	Total Mix Ration
VFA	Volatile Fatty Acids
YCP	Yeast Culture Product
YG	Calculated Yield Grade

USDA	United States Department of Agriculture
USDA YG1	Yield Grade 1
USDA YG2	Yield Grade 2
USDA YG3	Yield Grade 3
USDA YG4	Yield Grade 4
USDA YG5	Yield Grade 5
ZH	Zilpaterol Hydrochloride
Zn	Zinc
ZnCl	Zinc Chloride
ZnO	Zinc Oxide

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ABSTRACT

EVALUATION OF FEED ADDITIVES AND DELIVERY METHOD FOR RECEIVING AND FINISHING CATTLE

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Trace minerals (TM) and vitamins are essential to improve production output and efficiency in beef cattle. The first experiment was conducted to investigate if the delivery method of TM and *Saccharomyces cerevisiae* yeast culture influence growth performance and hepatic TM content of receiving steers. The second experiment evaluated the effect of supplemental protected B - vitamins to finishing steers. Experiment 1 used Charolais x Angus steers calves (n = 192; 256 ± 14 kg) in a 49-d receiving experiment. Within 36h of weaning, steers were weighed, allotted to 24 pens (n = 8 steers/pen; 8 pens/treatment) and randomly assigned to treatments: traditional receiving diet (Con); traditional receiving diet plus the “stress-pack” directly in the diet (Force); traditional receiving diet plus a low-moisture, cooked molasses block fortified with the “stress-pack” (Tub). The “stress-pack” was offered the first 28 d of the 49-d experiment. Hepatic biopsy samples were collected from a subsample of steers (n = 14 steers) on the day of weaning and subsequent samples were collected from the same steer (n = 1 steer/pen) on d 14, 28 and 49 for hepatic TM concentration determination. A treatment × day interaction ($P \leq 0.01$) for hepatic Cu concentration was noted. Force had greater hepatic Cu ($P \leq 0.05$) compared to Tub and Con at each sampling point. Tub had greater hepatic Cu compared

to Con on d 14 and 28 ($P < 0.05$), but was similar to Con on d 49 ($P > 0.10$). Force tended ($P = 0.08$) to have greater DMI compared to Tub from d 1 to 14. From d 15 to 28, steers offered "stress-pack" had greater DMI ($P = 0.01$) and tended ($P = 0.07$) to have greater ADG compared to Con by 12.5%. From d 29 to 49, "stress-pack" steers had greater DMI ($P = 0.01$) and Force consumed 6.9% more DM compared to Tub ($P = 0.01$). Cumulative DMI ($P = 0.01$) and ADG ($P = 0.05$) was greater for Force compared to Tub by 5.4% and 9.4%, respectively. These data indicate that application of a "stress-pack" in diets offered to newly-weaned cattle enhanced production responses, but delivery method influences DMI and daily gain. In experiment 2, steers (n = 246; initial shrunk BW = 411 ± 25.8 kg) from two sources, were used in a 126-d experiment to evaluate use of a rumen-protected B – vitamin blend (RPBV). Within 48 h after arrival, steers were individually weighed and processed. Steers were then allotted to one of 24 pens (n = 10 to 12 steers; 8 pens/treatment) and randomly assigned to 1 of 3 treatments: 1) No RPBV; 2) RPBV1 at 1 g/steer·d^{1}; 3) RPBV2 at 2 g/steer·d^{1}. During the first 14 d cattle received two transition diets. From d 15 to 126 cattle were fed the final diet containing [dry matter (DM) basis]: 53% dry-rolled corn; 23% corn silage; 20% MDGS; and 4% suspended supplement. No differences ($P \geq 0.13$) were found for DMI, live final BW, ADG, or G:F. Carcass-adjusted final BW, ADG, and G:F were not influenced by treatment ($P \geq 0.59$). Hot carcass weight (HCW), dressing percentage, marbling score, kidney-pelvic-heart fat, or BW at 28% empty body fat did not differ among treatments ($P \geq 0.11$). Ribeye area (REA) was altered (quadratic effect, $P = 0.02$) by treatment; steers from RPBV1 had decreased REA compared to others. Additionally, calculated yield grade (YG) and calculated retail yield (RY) were altered (quadratic effect, $P \leq 0.01$) by

treatment; steers from RPBV1 had increased YG and decreased RY compared to others. Estimated empty body fatness tended (P = 0.06) to be greater from steers fed RPBV compared to control. Overall, USDA YG distribution was altered by dietary treatment (P = 0.01). The proportions of YG1 and YG5 carcasses were unaffected by treatment, but there was a shift in the proportion of carcasses that graded YG2, YG3, and YG4 among treatments. Distribution of USDA Quality Grade was not altered by treatment (P = 0.53). No differences were observed on liver abscess prevalence or severity among treatments. The use of RPBV altered carcass muscularity and rib fat accumulation influencing the overall YG distribution. However, RPBV did not appreciably influence any cumulative growth performance measures or liver abscess outcomes in these steers.

Key words: B vitamin, beef, growth, liver, steers, trace mineral.

Chapter 1 : LITERATURE REVIEW

1.1. US Beef Production

It is critical to enhance the efficiency of the beef production system in order to meet human needs, according to the Food and Agriculture Organization of the United Nations (FAO, 2006), as meat demand is predicted to grow 1.7% per year to 2030 and by 1.0% per year to 2050. In order to meet the nutritional demands associated with this growing population, agricultural production must increase by 70% (FAO, 2009), leading farmers to be dynamic in terms of methods of raising and feeding cattle as well as exploring new market opportunities by flexible practices, for example using byproducts to feed the animals and thereby creating additional value to the food chain.

The most intensive cycle of production is the feedlot phase. The primary goal of this system is to optimize animal weight gain by reducing production cycle time and boost productivity per area and per animal (Carrer et al., 2013). The feeding period is normally 100 to 300 days with an arrival weight of 272 to 363 kg and harvest occurring at an age of 15 to 28 months and 590 to 680 kg. Most animals fed for slaughter are crossbreds, with Angus influence in 60% or more (Samuelson et al., 2016; Drouillard, 2018). Almost 90% of beef is consumed domestically, and the target carcass is highly marbled, but not over-fattened (Drouillard, 2018).

According to the cattle inventory released in January 2022, by the National Agricultural Statistics Service (NASS) the production is predominantly in the five-state region, 19.9% Texas, 19.2% Nebraska, 17.8% Kansas, 8.0% Iowa, and 7.9% in Colorado. These states account for 72.8 % of animals on feed. The Northern Plains (Minnesota,

Nebraska, North Dakota, and South Dakota) account for 25% of the total cattle on feed (NASS, 2022). In the United States, cattle fed for slaughter reached 14.7 million head in January 2022 across all feedlots and when compared to January 2021, the inventories are slightly higher. Feedlots with a capacity of 1,000 or more head accounted for about 82% of total feedlot livestock, slightly higher from the previous year (NASS, 2022).

1.2. Growth Promoting Technologies

Specialization and concentration of the U.S. beef cattle and cattle feed production have substantially increased the efficiency of beef production in terms of cost per unit produced. Technological advances have been the primary reason for the increase in cattle efficiency; improvements in reproductive management, genetic development, exogenous growth promoting compounds, vaccines, antimicrobials, as well as feed processing strategies; have allowed U.S. producers to focus on growth efficiency and health of cattle which in turn can decrease production costs (Capper, 2011; Drouillard, 2018).

Growth promoting technologies are widely applied to beef cattle production as steroidal hormones with anabolic activity and orally active β-adrenergic agonists used as feed additives. Growth promoting implants are used at different phases of production and have been approved for use in the U.S. since 1956 (Smith and Jonson, 2020). These production-enhancing technologies (PETs) are a vital component to improving sustainability and economic competitiveness compared to other protein sources in the meat industry. Implants can be classified by potency (low, medium, and high), or in accordance of the compound estrogenic (estradiol or zeranol), androgenic (testosterone or trenbolone acetate), or a combination of both (Johnson and Beckett, 2014). The objective of growth promoting implants is to enhance muscle growth, increase dry matter intake

(DMI), average daily gain (ADG; 8% to 28%), chemically mature body weight, final body weight (FBW) and, hot carcass weight (HCW), improve feed conversion (G:F; 5% to 20%) and also reduce the total cost of production. However, implants can decrease USDA marbling scores which makes it necessary to feed cattle to a heavier weight in order to achieve the same marbling score compared to a non-implanted animals (Duckett and Andrae, 2001; Johnson and Beckett, 2014; Kamanga-Sollo et al., 2010; Parr et al., 2011). Growth promoting implants are used in both the growth and finishing stages of beef production (Drouillard, 2018). Parr et al. (2011) reported that implanted cattle had a greater DMI and those that received more than one implant had greater DMI over the single implanted animals during the feedlot phase.

β-adrenergic agonists (β-AA) are a class of non-steroidal feed additives that induces skeletal muscle hypertrophy (increase in the size of cells) by increasing protein synthesis; consequently, ADG and feed efficiency are improved (Baile and McLaughlin, 1987; Boler et al., 2012; Hosford et al., 2015). The most commonly used β-AA is the beta-I adrenergic agonist, ractopamine hydrochloride (RH), which was approved by the FDA in 2003 to be fed to finishing cattle. Ractopamine hydrochloride is used during the last 28 to 42 d on feed with a daily dosage varying from 100 a 480 mg/animal daily (Boler et al., 2012; Samuelson et al., 2016). Because β-AA cause hypertrophy but do not increase the DNA to protein ratio, it results in an un-sustained hypertrophy of skeletal muscle, thus it is important to offer β -AA only in the finishing period (Smith and Johnson, 2020).

Zilpaterol hydrochloride (ZH) is another β-AA that targets the beta-II receptor and is more potent than RH. It was first approved in 2006 by the FDA, but only started to

be commercialized in 2007 (FDA, New Animal Drug Application 141-258). The approved rate of inclusion of zilpaterol hydrochloride is 8.3 mg/kg (DM basis) during the finishing phase for 20 to 40 d, followed by a 3-d withdrawal period before slaughter (Delmore et al., 2010). The supplementation of ZH results in improved feed efficiency without an increase in DMI. It increases ADG, HCW, and lean tissue deposition. However, this compound is seldom used currently because of restrictions imposed by major abattoir companies (Delmore et al., 2010; Drouillard, 2018).

Another class of feed additives that are used to enhance production efficiencies are ionophores. They are a type of non-medically important antibiotic that affects the movement of ions across the membranes of gram-positive bacteria inside of the rumen resulting in a greater proportion of gram-negative bacteria and the modulation of ruminal fermentation (Schelling, 1984). Monensin is an ionophore that was approved in the mid-1970s and has been widely used in feedlots since 1975 (Duffield et al., 2012). It has been demonstrated that the addition of monensin in the diet enhances feed utilization and reduces occurrence of ruminal acidosis and bloat in feedlot cattle mainly due to the suppression of lactate producing bacteria and the reduction of daily variance in feed intake. Additionally, monensin changes the ratio of volatile fatty acids (VFA) in the rumen increasing propionate synthesis while decreasing acetate and butyrate production (Bergen and Bates, 1984; Stock et al., 1995). As a consequence of the effect of this ionophore, a higher net revenue and better economic efficiency can be expected (Zhang et al., 2021). Duffield et al. (2021) conducted a meta-analysis that evaluated the effects of monensin in beef cattle. It was demonstrated that the inclusion of monensin in the diet ranged between 3 to 98 mg/kg of feed and the average inclusion was 28.1 mg/kg of feed.

Overall, monensin supplementation reduced DMI, but improved both ADG and G:F in growing and finishing beef cattle. The reduction in DMI was more notable when applied to cattle fed concentrate-based diets when compared to forage-based diets, because of the greater propionate synthesis and delivery of propionate to the liver, that can modulate feed intake in a negative manner (Duffield et al., 2012, Allen et al., 2009). Additionally, beta-oxidation is inhibited during meals by propionate and high ATP levels, hence, this delays hunger (Allen et al., 2009).

Multiple studies have found that feeding monensin reduces CH_4 emissions by 6.5 to 12% (Odongo et al., 2007; Vyas et al., 2018). Considering all the recent environmental worries around enteric emissions, mainly methane (CH_4), which is a fermentation byproduct eructated by the ruminant and the contribution of CH_4 to global warming renewed interest related to methane has surfaced (Gerber et al., 2013; Knapp et al., 2014). Methane eructation represents a loss of up to 12% of feed energy losses (Hess et al., 2004). Monensin represents a viable alternative to improve feed conversion and hence ruminant performance by decreasing ruminal CH_4 production targeting the gram positive (methanogenic) bacteria.

Saccharomyces cerevisiae fermentation products (SCFP) have long been integrated in domestic animal diets, particularly when it comes to ruminant nutrition, such as calves, lactating cows, and feedlot animals. Use of SCFPs have demonstrated a positive influence in the ruminal environment by stimulating the growth of beneficial microorganisms (Geng et al, 2015). *Saccharomyces cerevisiae* fermentation product is a byproduct of the fermentation process containing compounds such as B vitamins, amino acids, and nucleic acids that impact the development and growth of various types of

ruminal microorganisms, not depending of the live culture for the physiological response by the rumen bacteria and therefore the animal (Callaway and Martin, 1997; Mohammed et al, 2018; Wagner et al., 2016). Several studies have suggested that the use of SCFP has a positive effect on growth performance such as ADG and BW. Further, SCFP has been shown to improve fiber digestion, feed efficiency, DMI, rumen and small intestine general health and has the ability to alter immune responses (Alugongo et al., 2017; Geng et al, 2015; Mohammed et al, 2018). The total ruminal anaerobic and cellulolytic bacteria have been shown to be increased during the period that animals were fed SCFP, making it reasonable that the improvement of fiber digestibility can be obtained by using SCFP (Mohammed et al, 2018).

2. RECEIVING, GROWING AND FINISHING PERIOD

2.1 Receiving period

The receiving period is arguably the most stressful period in a calves' life, because of weaning and transportation to the feedlot, introduction upon arrival to a new source of water and feed, and also exposed to a new social hierarchy, potentially leading to a depression in DMI and increased susceptibility to opportunist diseases (Galyean and Hubbert, 2014; Preston, 2007). To decrease morbidity and mortality, but also increase growth performance, a plethora of supplements and medications can be administered before or after arrival at the feedlot (Preston, 2007).

Feed intake is critical in newly weaned calves. The degree of stress, previous plane of nutrition, genetics and health status can depress DMI. Many authors consider that the animals need to eat 2 to 3% of their BW in DM in the first 14 days (Galyean and

Hubbert, 2014) and if animals do not reach that target during that time, they are more likely to be infected with a pathogen. Therefore, DMI can be used to track the course and risk of disease.

2.1.1 Bovine Respiratory Disease

Many diseases are important to consider during the receiving period; however, Bovine Respiratory Disease complex (BRD) is acknowledged to be the most economically significant one for beef cattle producers (Blakebrough-Hall et al., 2020, Dubrovsky et al., 2020). Wang et al, (2018) estimated that lower livestock productivity, increased veterinarian treatment expenses, and death losses cost the sector $165 million annually.

Cattle with BRD have poorer growth performance and decreased carcass quality, which leads to greater production costs and a poor return on investment (Dubrovsky et al., 2020). Bovine Respiratory Disease is a multifactorial disease that includes the triad, 1) environmental components, 2) host factors, and 3) various microorganisms that can lead to a severe respiratory syndrome. Bacterial pathogens of interest include: *Pasteurella* (*Mannheimia) haemolytica, Pasteurella multocida*, and *Haemophilus sommus*, *Mycoplasma bovis* among others (Duff and Gaylean, 2006). Viral pathogens are the leading cause of secondary bacterial infection, they are bovine respiratory syncytial virus, bovine parainfluenza-3, bovine coronavirus, bovine herpesvirus type 1, bovine viral diarrhea, influenza C and D, Bovine adenovirus serotype 7 (Studer et al., 2021).

2.1.2 Receiving Diet

Considering that beef calves are accustomed to forage-based diets, supplying long-stemmed roughage once they are weaned and moved to the feedlot is a standard technique to enhance consumption of milled-feed (Galyean and Hubbert, 2014). The high roughage diet used during the starting period at the feedlot is fed based on the perceived advantage of decreasing morbidity and mortality (Rivera et al., 2005), boosting the immune system, and also improving performance, and therefore the overall economic outcome (Holland et al., 2010).

Related to concentrate-based diets, Lofgreen (1988) suggested that the best diet strategy for newly received cattle is to offer a 50 to 75% milled feed or 25 to 50% of roughage for the first month, agreeing with Rivera at al. (2005) that used mixed model regression methods to identify the optimal roughage inclusion level for receiving cattle.

To compensate the low feed intake of newly received cattle a higher CP should be offered to prevent the negative effects on performance and to assist the immune system (Waggoner et al., 2009). Samuelson et al. (2016) reported that the crude protein (CP) level in the receiving diets varies from 13.4 to 14.5% (DM basis), that typically meet the protein requirement of receiving calves.

2.2 Transition Period

The transition period is necessary to adapt the ruminal epithelium and optimize the type of microorganisms to rapidly ferment and effectively utilize starch (Cheng et al., 1998; Owens et al., 1998). During the transition period, the addition of highly

fermentable carbohydrates and the reduction of roughage level results in a shift in the ruminal microbiome where the number of cellulolytic bacteria reduces (Tajima et al., 2000) and amylolytic and lactate-utilizing bacteria increases. Additionally, the ruminal pH declines and the molar proportions of VFA shifts as dietary concentrate increases (Mackie et al., 1978).

The VFA production, concentration, ratio, pH, and ruminal bacterial species varies upon the feedstuff (Russel, 1998). The VFAs are synthetized as a co-product of microbial fermentation of feedstuff in the rumen and are the main source of energy for ruminants (Nozière et al., 2010). Additionally, the amount and proportion of VFAs formed in the rumen has consequences for the efficiency of energy utilization and production of CH_4 (Nozière et al., 2010). The acetate to propionate ratio is generally less for cereal grains than for feed ingredients containing a greater concentration of fiber (Fulton and Klopfenstein, 1979). When the ratio of acetate to propionate decreases, CH_4 production lessens, and the carcass carbon retention increases (Nozière et al., 2010; Wolin, 1970).

The most common method of diet transition is the multiple step-up until the final diet, where on each step concentrate will be increased and roughage will be decreased every 3 to 7 d (Samuelson et al., 2016), is to allow the ruminal microorganisms to adjust gradually to a low ruminal pH to minimize subacute acidosis and intake variation (Choat et al.,2002). Other method is to restrict or limit feed intake of high-concentrate diets; this has also shown potential reductions in subacute acidosis (Soto-Navarro et al., 2000).

2.3 Growing and Finishing Period

The finishing period starts after the transition from a higher roughage diet to a higher concentrate diet to optimize gain and performance. According to the Samuelson et al. (2016) survey, corn and wheat are the most common grain used as an ingredient in finishing diets and usually included in a range of 50 to 90% of the diet (DM basis), being lower compared to past years (Vasconcelos and Galyean, 2007) in part because of the increased use of grain byproducts like distillers grains plus solubles (DDGS) where the inclusion level averages 17% or greater (Vasconcelos and Galyean, 2007; Samuelson et al., 2016).

A common practice is to include a small percentage of roughage in high-grain finishing diets in order to prevent digestive disorders such as acidosis and to maximize feed intake (Galyean and Hubbert, 2014). According to Samuelson et al. (2016), the most used source of roughage for finishing cattle is corn silage followed by corn stalks and alfalfa hay.

It is a common practice in U.S. feedlots to at least meet and often times to exceed CP requirements by maintaining a fixed CP inclusion throughout the finishing period. To overfeed protein is considered a low-risk diet strategy, in attempt to avoid protein deficiency and reduced growth performance caused by mistakes in mixing and delivery. (Cowley et al., 2019). However, CP levels higher than 13% on DM basis might not appreciably influence gains in finishing cattle (Gleghorn et al., 2004).

Compared to the late 1990s, sources of CP have changed, where a significant component of the shift is due to an increase in grain-based production of bioethanol.

Finishing diets that historically used cottonseed meal, soybean meal, alfalfa, or urea as protein sources, now use less expensive products such as ethanol production byproducts (Gleghorn et al., 2004; Vasconcelos and Galyean, 2007; Samuelson et al., 2016). Beef cattle have been successfully fed as much as 40% of ration DM as wet or dried DGS, it can increase DMI (Al-Suwaiegh et al., 2002), with no effect on beef tenderness or palatability (Roeber et al., 2005).

3. Trace Minerals and Supplementation Methods

Supplements can be delivered to cattle in a variety of forms, including mixing in the diet using either liquid or dry supplement forms, or self-fed supplements such as pressed block or low-moisture block delivery systems (Bailey and Jensen, 2008). Supplementation can result in improved animal performance because of increased nutrient intake and consumption (Delevatti et al., 2018)

According to NASEM (2016), there are at least 17 minerals required for beef cattle, which are classified as macrominerals and microminerals. The first category is provided to the diet in grams daily, while the microminerals, also called trace minerals (TM), are provided in the diet in milligrams daily. Macrominerals such as phosphorus (P), potassium (K), sulfur (S), magnesium (Mg), sodium (Na), and chloride (Cl) and TM such as cobalt (Co), copper (Cu), chromium (Cr), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn), are considered essential for beef cattle (NASEM, 2016; Arthington and Ranches, 2021). Updates to from 1984 to 2016, had Ni and Cr included as essential trace elements, but without a specific requirement. The requirement for Co, Cu increased in the updated version, while I, Fe, Mn, Mo, Se, and Zn stayed the same or were decreased, despite the fact that in 2007 beef

cattle grew at a rate 44% faster than cattle reared in 1977. This pattern of larger cattle size and increased growth has not changed since 2007, with advances in beef cattle growth rates and carcass weights continuing to rise (Capper, 2011).

In general, minerals play a variety of roles in the body, including normal tissue growth. Minerals regulate osmotic pressure and acid-base balance and are involved in several enzyme functions and cell regulation, being a structural component for tissue and enzymes, and also aid in improving immune function. (NASEM, 2016; McDowell, 2003; Underwood and Suttle, 1999). Mineral deficiency can suppress or completely block important metabolic pathways, resulting in a variety of clinical symptoms with different levels of severity. However, minor deficiencies have non-specific symptoms that are difficult to identify (Radwińska and Żarczyńska, 2007).

The effectiveness of TM absorption is affected by dietary variables such as fiber content and ruminal pH (Whitehead et al., 1985; Waghorn et al., 1990). Also, interactions between specific TMs form metal complexes that become insoluble or unavailable to the animal (Spears, 2003), for example the three-way interaction between Cu, Mo and S. Thiomolybdates are formed through the interaction of molybdate with sulfide, and when Cu is added, they form insoluble complexes that do not release copper even under acidic conditions (Allen & Gawthorne, 1987).

3.1 Cobalt

Cobalt (Co) is a metallic element required for rumen microbial metabolism in order to produce CH_4, acetate, and methionine. It is also a part of the structure of cobalamin that contains up to 4.5% of Co, popularly known as vitamin B12, which is a

crucial co-factor for gluconeogenesis, metabolism of carbohydrates, lipids, amino acids, and nucleic acids in the ruminant (González-Montaña et al., 2020; Waterman et al., 2017). Research suggests that Co improves fiber digestibility by 1) acting as a divalent cation, a link between negatively charged microbes and similarly charged fiber particles or 2) physical destruction of fibrous material (Bishehsari et al., 2010; Casper et al., 2021; Lopez-Guisa & Satter 1992).

Legumes have a higher Co level than grasses, and the effectiveness of supplemental Co is dependent on the biological availability of the mineral. For example, Co oxide has a poorer biological value than Co carbonate or Co sulphate (Waterman et al., 2017). For growing and finishing cattle the Co requirement is 0.15 mg/kg, and the maximum tolerable concentration is 25 mg/kg (NASEM, 2016).

Vitamin B12 concentration can be used evaluate Co status concentrations range from 3.0 to 220.0 $\mu g \cdot g^{-1}$ of fresh tissue weight (Sivertsen & Plassen, 2004). Vitamin B12 concentrations equivalent to or less than 0.10 $\mu g \cdot g^{-1}$ wet weight, or accumulation of homocysteine in plasma suggest Co deficiency (Stangl et al., 1999). However, analogues may limit access to serum B12 levels (Halpin et al., 1984).

Insufficient Co levels cause alterations in the composition of ruminal microbiome, which inhibits cellulose degradation and CH_4 synthesis, therefore contributing to metabolic disorders (Radwińska & Żarczyńska1, 2014). Growth and immune function are also negatively affected by Co deficiency (Schwarz et al., 2000; Sharman et al. 2008). Poor coat condition and skin problems are other signs and in severe cases, the nervous system can be damaged, leading to depression and neurological disorders amongst other things (Fisher and MacPherson, 1991).

3.2 Copper

Copper (Cu) is a component of many enzymes, some of the most important ones are cytochrome C oxidase and superoxide dismutase that play critical roles in aerobic metabolism, also Cu is a component of ceruloplasmin, and lysyl oxidase. There are many studies showings that Cu impacts lipid metabolism in ruminants (Felltman, 1991; Ward and Spears, 1997; McDowell, 2003; Engle, 2011; Radwińska & Żarczyńska1, 2014) and affects forage digestibility (Lopez-Guise and Satter, 1991).

According to NASEM (2016), the Cu requirement is 10 ppm (DM basis) for growing and finishing cattle, and 40 ppm (DM basis) is the maximum tolerable concentration. However, Cu requirements are highly dependent on concentration of Mo and S in the diet. If the concentration of those elements exceeds 2 ppm and 0.25% respectively, 10 ppm of Cu might not be sufficient (NASEM, 2016). Other elements, such as lead (Pb), cadmium (Cd), Zn, Ca and Fe can also inhibit the availability of Cu, because these can form an insoluble complex, there is unavailable for absorption by animals (Radwińska & Żarczyńska1, 2014; NASEM,2016). Various different sources of Cu can be used to supplement cattle diet, varying in origin and bioavailability, such as copper-sulfate, copper oxide, tribasic chloride, or attached to an amino acid. Copper oxide powder is essentially unavailable in cattle due to its inability to dissolve in acidic circumstances, nevertheless, when administered orally to animals with a gradual release, Cu oxide can be used to increase absorption (Suttle, 2012).

Since Cu is present in many enzymes and serves many different functions in the body, deficiency that is also called hypocupremia can cause anemia, lethargy, lack of growth and development, bone deformities, lameness, neurological problems, weakness,

diarrhea, and arguably the most well-known sign, changes in coat color. (Suttle, 2012; Radwińska & Żarczyńska1, 2014; NASEM, 2016).

To understand how Cu can become toxic, it is important to consider the way that it is excreted. In non-ruminant species the biliary elimination of this mineral is adequate and most of it is bound to metallothioneins in the liver, these species can tolerate high amounts of dietary Cu (Suttle, 2012). However, the ability for Cu biliary excretion in ruminants is quite limited, and only a small amount of copper is bonded to metallothionein. Once hepatic storage capacity is overloaded, a sudden and generally fatal hemolytic crisis occurs (Saylor & Leach, 1980; Bremner, 1998, Suttle, 2012). Copper toxicity has long been recognized as a health hazard for intensively reared sheep, and the only treatment relied on Cu chelation with parenteral TM, given as tetra thiomolybdate (Suttle, 2022). In bovines, Cu toxicity is a result of excessive supplementation with diets containing more than 40 mg/kg of Cu on a DM basis (NASEM, 2005; NASEM, 2016); however, the liver can store large amounts of Cu before the animal start to show signs of toxicity, concentrations around 125 mg/kg showed negative effects on animal performance (Engle and Spears, 2000). Gummow (1996) tested the effects of chronic Cu toxicity, which resulted in rumen stasis, bloat, and depression.

3.3 Manganese

Manganese (Mn) plays many metabolic roles, including bone formation (Graham, 1991) and as a cofactor for enzyme pyruvate carboxylase, an essential enzyme in gluconeogenesis; arginase, that is responsible for urea synthesis in the liver; and superoxide dismutase, which is critical in preventing cellular oxidative stress. Manganese

also is an activator of many different enzymes such as pyruvate carboxylase, lipase, and superoxide dismutase. (Crossgrove and Zheng, 2004; NASEM, 2016).

NASEM (2016) recommends 20 mg/kg of Mn for growing and finishing cattle, with a maximum dosage of 1000 mg/kg. However, Mn absorption, like other minerals, is affected by dietary antagonists, these antagonists are Fe (Hansen et al., 2010), Cu and Zn (Graham, 1991). Additionally, Ca and P also interfere with the absorption of Mn, but the extent of this interaction is minor (Spears, 2003).

Mn can be supplemented in the diet as manganese sulfate (MnSO4), manganese oxide (MnO, MnO_2), and numerous organic forms, such as Mn proteinate or attached to an amino acid, altering the bioavailability for the animal (Wong-Valle et al., 1989; NASEM, 2016), the $MnSO_4$ is the more biologically accessible than MnO (Wong-Valle et al., 1989; Graham, 1991). Mn deficiency cause bone deformities, poor reproductive performance (Hurley and Keen, 1987), and negatively impact lipid and carbohydrate metabolism (Graham, 1991).

High Mn levels can be harmful to ruminants, and the consequences are mainly associated with decreased rates of gain or negative interactions with iron and calcium. Reduced hemoglobin concentrations and change of the usual micro flora, with resultant reduction in volatile fatty acid synthesis, have also been seen (Graham, 1991).

3.4 Zinc

Zinc (Zn) is an essential nutrient required for many enzyme systems including those involved with the cellular signaling process, regulation of DNA synthesis and mitosis, as well as its role as serving as a structural element and/or a regulatory factor

(Miller, 1970; Beyersmann and Haase, 2001). In addition, it is related with other enzymes associated with protein and carbohydrate metabolism, both of which are important for development of immune function, including the development and activity of neutrophils and natural killer cells (Miller, 1970).

According to the NASEM (2016), the Zn requirement for growing and finishing cattle is 30 mg/kg and maximum tolerable is 500 mg/kg; however, feedlot nutritionists commonly recommend 100 mg/kg (Samuelson et al., 2016) to obtain maximum animal performance.

There are many options to supplement Zn in the diet, inorganic form as zinc sulfate (ZnSO4) (Samuelson et al., 2016), zinc oxide (ZnO), zinc glycinate, attached to an amino acid (organic source), and zinc chloride (ZnCl), all of which have varying bioavailability (Spears and Kegley, 2002; Sridhar et al., 2015; Shaeffer et al., 2017).

Zinc deficiencies in ruminants are primarily related dietary ingredients with reduced Zn concentrations, or secondarily, by Zn antagonists (Cu, Mg, Ca, P, and divalent iron compounds) and amino acid deficiency (Radwińska & Żarczyńska1, 2014). The symptoms of mild deficiency are depressed feed intake and reduced rate of growth due to poor nutrient use (Miller, 1970). However, severe Zn deficiency may impair protein synthesis and non-activation of Zn-dependent enzymes leading to parakeratosis. Common symptoms of this disease include skin issues, peel, scab, itch and hair loss, also oral and nasal inflammation with excessive salivation, swelling of the gums and teeth grinding (Miller, 1970; Alves et al., 2012).

4. B Vitamins

Vitamin B complex molecules are water-soluble and function as cofactors to many metabolic processes, including energy, carbohydrate, lipid, and amino acid (AA) metabolism, cellular signaling, DNA synthesis, and also function as a co-factor of enzymes (Zinn et al., 1987; Morrison et al., 2018; Kaur at al., 2019).

Vitamin B molecules are largely synthesized by ruminal bacteria during the process of fermentation of feedstuffs and it has long been believed that this was enough to provide adequate B vitamin amounts for the host (NASEM, 2001). However, the B vitamin production depends upon the type of the diet, level of fermentable carbohydrates, nitrogen concentration and roughage quality. For example, high concentrate diets decrease thiamin (B1) and increase niacin (B3) synthesis (Conrad and Hibbs, 1954). Considering the modern ruminant genetic potential and all factors that can affect B vitamin synthesis, dietary supplementation may be warranted to meet the nutritional demands (Carper, 2011; Deters et al., 2021).

4.1 Pantothenic Acid

Pantothenic acid or vitamin B5, is component of coenzyme A (CoA), and acyl carrier protein (ACP). Coenzyme A is a cofactor required for cell development and is involved in several metabolic activities, including phospholipid production, fatty acid synthesis and degradation, and the functioning of the Krebs Cycle. (Wojtczak and Slyshenkov, 2003; Leonardi and Jackowski, 2007).

Vitamin B5 can be found in many feedstuffs; however, the concentration on native B5 can be influenced by weather, species, vegetation stage and fertilizer used on the crop (Albers et al., 2002).

Pantothenic acid can be synthetized and degraded by ruminal microorganisms; therefore, it is difficult to measure the net amount of B5 in rumen due to it being in such a small quantity (Ragaller et al., 2011). However, Zinn et al. (1987) measured the difference between duodenal flow and B5 intake in an attempt to determine the ruminal synthesis on growing steers. The result was negative (-13.5 mg/day), meaning that no net B5 production in the ruminal environment occurred.

The requirement for this vitamin has not been set for beef cattle (NASEM, 2016). Additionally, no dietary factors are known that influence B5 availability (Roth-Maier et al., 2000).

4.2 Pyridoxine

Pyridoxine or vitamin B6, is important for the metabolism of amino acids as a component of the enzyme pyridoxal-5´-phosphate and kynureninase, also needed for the phosphorylation in carbohydrate metabolism (McDowell, 2000; Albers et al., 2002). Kynureninase plays a role in niacin synthesis by the breakdown of tryptophan. Additionally, according to NASEM (2007), vitamin B6 is required by ruminal protozoa for lysine synthesis, therefore enhancing lysine supply.

Vitamin B6 is widely distributed in animal and plant tissues such as cereals, milling byproducts, extracted oilseed meals and brewer´s yeast (Albers et al., 2002). The deficiency symptoms produced in all cases included lack of appetite, anorexia, poor gain

in weight, listlessness, and epileptic fits due to its impact on nerve pathology (Connor Johnson et al., 1949). Additionally, when fed in excess, it can be hazardous as excess vitamin B6 can lead to neurotoxicity in animals and humans (Connor Johnson et al., 1949; Albers et al., 2002).

4.3 Biotin

Biotin, often known as vitamin B7, is a co-enzyme that assists carboxylase function. These carboxylases are pyruvate carboxylase, propionyl CoA carboxylase, multiple CoA carboxylase, and acetyl CoA carboxylase I and II (Carling and Turner, 2019). The first three are mitochondrial enzymes, and acetyl-CoA carboxylase, which can be present in both mitochondria and the cytosol, is a rate-limiting step in the formation of long chain fatty acids (Fitzgerald et al., 1999). Gluconeogenesis, fatty acid production, propionate metabolism, and leucine catabolism are all mediated by the four carboxylase enzymes. (Chauhan and Dakshinamurti, 1988; McMahon, 2002; Carling and Turner, 2019).

The requirement for B7 has not been established for beef (NASEM, 2000) or dairy (NASEM, 2001) cattle. However, several studies support B7 supplementation, where they found improvements in milk yield and hoof health with biotin supplementation because the acidotic environment in the rumen may impair ruminal biotin synthesis, thereby increasing the need for supplementary biotin in high-producing cattle (Campbell et al., 2000; Fitzgerald et al., 2000; Lean and Rabiee, 2011).

Biotin is widely distributed in feedstuffs, but the bioavailability of biotin varies depending upon the dietary source; in wheat 5% is bioavailable, in corn it is 100% of biotin is bioavailable (Baker,1995).

More data is necessary to clarify B7 deficiency symptoms in cattle since B7 is widely produced by the ruminal and intestinal microflora. However, symptoms like brittle horns, grooves and cracks in the hooves of cattle, sheep and horses can be expected during B7 deficiency (Albers et al., 2002).

4.4 Folate

Folate, also known as folic acid or vitamin B9, has a variety of roles, including being donors and acceptors of one-carbon units and therefore cell division and amino acid metabolism. Folate is involved in the re-methylation of homocysteine to methionine (also requires vitamin B12) (Ragaller et al., 2008), is an essential part of the methylation cycle, neurotransmission modulation, gene expression, as well as an impact on immunological response (Zahoor Khan et al., 2020). Although is know that folate is important to metabolism. However, little is known about folate concentrations and bioavailability in feed. There can be a significant loss of folate activity during harvesting, storage and processing of feed (Gregory, 1989; Albers et al., 2002).

The forage:concentrate ratio in the diet may influence ruminal synthesized proteins to some extent by changing the microbial flora and data on microbial folate production is limited (Ragaller et al., 2008). The NASEM (2016) tried to estimate requirement values of folate for cows, however they had to extrapolate cow requirements using swine data and average vitamin contents found in milk (NASEM, 2001). Folate deficiency leads to anemia, granulocytopenia, and lymphocytopenia in rats, in cattle it can cause fertility disorders (Albers et al., 2002; Abe et al., 2012).

4.5 Cobalamin

Cobalamin also called B12, but technically B12 refers only to cyanocobalamin. Vitamin B12 is the generic name used to refer to a group of compounds that have B12 activity, such as cyano-, hydroxyl-, methyl- or deoxy adenosyl-cobalamin (Smith et al., 2018). One of the main characteristics of this vitamin is the presence of Co in the compound at a concentration between 4.4 to 5.8% (Bridwell-Rabb and Drennan, 2017; Rizzo and Laganà, 2020).

Vitamin B12 acts as either a cofactor or a coenzyme in a number of essential biochemical processes involving methylation, isomerization, reductive dehalogenation, and radical S-adenosylmethionine processes (Bridwell-Rabb and Drennan, 2017). Vitamin B12 can be synthetized by ruminal microflora. However, is necessary that ruminal Co concentration be greater than 0.5 mg/mL, if this level is not reached, the ruminal production of B12 is limited or inhibited and therefore reduces the supply of vitamin B12 available to the host (Stemme et al., 2008).

The generation of B12 by ruminal bacteria is commonly acknowledged to be sufficient to prevent vitamin B12 deficiency symptoms in ruminants (Girard et al., 2001), although it has been observed that ruminal microbiota significantly consumes dietary folic acid and vitamin B12 (Zinn et al., 1987; Girard et al., 2001). The amount of cobalamin synthesized depends on multiple factors, including the composition of the diet (i.e., the forage:concentrate ratio), DMI, and Co in the diet (González-Montaña et al., 2020).

The efficiency with which Co is used by the ruminal microorganisms that produce B12 is quite low; the quantity of Co in the food transformed into vitamin B12 in the

rumen ranges from 3 to 13% of consumption. The ruminal synthesis of vitamin B12 decreases rapidly once Co is not present in the diet (Smith, 1987; Girard et al., 2001). When fed a Co-deficient diet, the vitamin B12 stored in the liver of mature ruminants is generally adequate to endure for several months (Goff, 2000).

Nonspecific clinical signs of vitamin B12 insufficiency include decreased food intake, delayed development, muscular atrophy, rough coat, and skin thickening (Albers et al., 2002; González-Montaa et al., 2020). Ruminants appear to be more susceptible to vitamin B12 deficiency than non-ruminants, due mainly to their reliance on gluconeogenesis to meet tissue glucose needs (González-Montaa et al., 2020). A decompensation in propionate metabolism at the point where methylmalonyl CoA is converted to succinyl-CoA may be a core issue resulting from a vitamin B12 deficit (Goff, 2000).

5. Conclusions

Beef cattle in United States are becoming bigger and more efficient over the years in terms of weight gain and final body weight. To support this improvement, it is essential to supply or supplement adequate amounts of trace minerals and vitamins to the diet. However, with the change in frame size and targeting to the optimize performance, the exact requirement remains unknown, leading ruminant nutritionist to overfeed trace minerals and vitamins (Samuelson et al., 2016). Additionally, better understanding of the delivery method of feed additives is necessary to allow for more accurate predict performance output and prevent diseases in beef cattle.

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Chapter 2 : SUPPLEMENTAL ORGANIC TRACE MINERALS AND A YEAST CULTURE PRODUCT "STRESS PACK" IN NEWLY WEANED STEER CALVES: EFFECTS OF USE AND DELIVERY METHOD ON GROWTH PERFORMANCE, EFFICIENCY, AND HEPATIC TRACE MINERAL CONTENT

2.1. ABSTRACT

The objective of this study was to determine if delivery method of organic trace minerals and *Saccharomyces cerevisiae* yeast culture product influence growth performance, feed efficiency, and hepatic trace mineral measures in newly-weaned steers. Crossbred steers (n = 192; 256 ± 14 kg) were used in a 49-d receiving phase experiment. Within 36 h of weaning, steers were weighed, allotted to 24 pens (n = 8 steers/pen; 8 pens/treatment) and randomly assigned to treatments: 1) traditional receiving diet (Con); 2) traditional receiving diet plus the "stress-pack" directly in the diet (Force); 3) traditional receiving diet plus a low-moisture, cooked molasses block fortified with the "stress-pack" (Tub). The "stress-pack" was offered the first 28 d of the 49-d. Biopsy samples were collected from a subsample of steers (n = 14 steers) on the day of weaning and subsequent samples were collected from the same steer (n = 1 steer/pen) on d 14, 28 and 49 for hepatic trace mineral concentration determination. A treatment × day interaction ($P \leq 0.01$) for hepatic Cu concentration was noted. Force had greater hepatic Cu ($P \leq 0.05$) compared to Tub and Con for the entire period. Tub had greater hepatic Cu compared to Con on d 14 and 28 ($P < 0.05$), but was similar to Con on d 49 ($P > 0.10$). Force tended ($P = 0.08$) to have greater DMI compared to Tub from d 1 to 14. From d 15 to 28, steers offered "stress-pack" had greater DMI ($P = 0.01$) and tended ($P = 0.07$) to have greater ADG compared to Con by 12.5%. From d 29 to 49, "stress-pack" steers had greater DMI ($P = 0.01$) and

Force consumed 6.9% more DM compared to Tub (P = 0.01). Cumulative DMI (P = 0.01) and ADG (P = 0.05) was greater for Force compared to Tub by 5.4% and 9.4%, respectively. Application of a “stress-pack” in diets offered to newly-weaned cattle enhanced production responses, but delivery method influences DMI and daily gain.

Key words: Feedlot, receiving period, trace mineral, yeast culture product

2.2. INTRODUCTION

Weaning and transportation are typically the most stressful events in the life of a beef calf. Additionally, road transportation entails the loading of animals at their origin, confinement on a moving vehicle, unloading, and penning at their end destination where they are exposed to a different source of feed and water, and co-mingled, resulting in immune, hormonal, physiological and nutritional changes and challenges (Loerch and Fluharty, 1999, Earley et al., 2012;). In addition, stress factors will affect TM status of the cattle (NRC, 1996) by mobilizing tissue reserves of Co, Cu, Mn, and Zn for enhancement of immune function, particularly in newly received animals (Duff and Galyean, 2007).

Saccharomyces cerevisiae yeast culture products have long been used in domestic animal diets, and has been shown to positively influence the ruminal environment and stimulate the growth of beneficial microorganisms (Geng et al, 2016; Callaway and Martin, 1997, Miller-Webster et al, 2002). *Saccharomyces cerevisiae* yeast culture products are composed of byproducts of the fermentation process from the species of yeast called *Saccharomyces cerevisiae*, including B - vitamins, amino acids, and nucleic acids that affect the development and growth of various ruminal microorganisms (Wagner et al., 2016; Callaway and Martin, 1997; Mohammed et al, 2018). Studies suggested that yeast culture products (YCP) have positive effects on growth performance such as ADG and FBW, greater DMI, improved G:F, rumen and small intestine health improvement and better immune responses (Geng et al., 2015, Alugongo et al., 2017; Mohammed et al, 2018). The total ruminal anaerobic and cellulolytic bacteria increase during the period that animals are fed YCP improved fiber digestibility, mineral retention

and flow of microbial protein to the small intestine (Cole et al., 1992; Newbold et al., 1996; Mohammed et al., 2018).

The supplementation of TM and YCP together have led to greater performance and also a greater hepatic TM content compared to non-treated animals (Hamilton et al., 2021), however different delivery methods of these products combined have not been evaluated in a feedlot setting.

The objective of this study was to determine if use and method of delivery of added organic TM (Availa 4, ZINPRO, Eden Prairie, MN) and a *Saccharomyces cerevisiae* YCP (Diamond V XPC, Diamond V, Cedar Rapids, IA) fed in combination (stress-pack) affect growth performance, measures of applied energetics, and hepatic trace mineral concentration upon feedlot introduction in newly-weaned beef steer calves.

2.3. METHODS AND MATERIALS

2.3.1. Use of Animal Subjects

All procedures involving the use of animals in this experiment were approved by the South Dakota State University Institutional Animal Care and Use Committee (Approval #2108-049A).

2.3.2. Animal Description and Initial Processing

Charolais x Angus steers calves (n = 192; 256 ± 14.0 kg) from a single source, were transported to the Ruminant Nutrition Center in Brookings, SD after being transported 515 km. Upon arrival (6.5 h transport from the ranch), steers were offered long-stem grass hay and ad libitum access to water. The following day, steers were weighed (for

allotment purposes), tagged, and vaccinated against infectious bovine rhinotracheitis virus, bovine viral diarrhea Types 1 and 2 viruses, parainfluenza 3 virus, and bovine respiratory syncytial virus (Bovi-Shield GOLD 5, Zoetis) and clostridial species; *Clostridium chauvoei*, septicum, novyi, sordellii and perfringens types C and D (Ultrabac 7/Somubac, Zoetis), administered a dose of pour-on moxidectin (Cydectin, Bayer Animal Health, Shawnee Mission, KS), and sorted into study pens the afternoon following initial processing (n = 8 steers per pen; 8 pens/treatment). Steers were individually weighed again on d 1. The initial BW was the average of the two BW measures collected on d-1 and 1. Live BW measures, excluding initial BW, were shrunk 4% to account for digestive tract fill.

2.3.3. Experimental Design and Treatments

This study used 8 replicate pens per treatment and each pen contained 8 steers (n = 64 steers/treatment). Each pen was assigned to one of three dietary treatments in a randomized complete block design, where location in the feedlot was considered the blocking factor. Dietary treatments included: 1) traditional receiving diet (Con); 2) traditional receiving diet plus the “stress-pack” directly in the diet (Force); 3) traditional receiving diet plus a low-moisture, cooked molasses block fortified with the “stress-pack” (Tub).

2.3.4. Dietary Management

The traditional receiving diet (Table 2.1) consisted of 40% wheat silage, 10% oat hay, 36% soybean hulls, 9% DDGS and 5% liquid supplement on DM basis (15% CP; 59% NDF; NEm; NEg; 1.73 and 1.04 Mcal/kg, respectively).

The liquid supplement was included at 5% of diet and provided to the diet (DM basis) 25 g/t monensin sodium and in-organic trace minerals: 0.20 mg/kg Co, 10 mg/kg Cu, 20 mg/kg Mn, and 35 mg/kg Zn to meet the nutrient requirements for growing beef steers (NASEM, 2016).

All steers were fed twice daily (0800 h and 1400 h) in equal proportions. On d 1, 2 kg/hd of the diet (DM basis) was offered to the steers and increased approximately 0.5 kg/hd (DM basis) until d 7. Bunks were managed using a slick bunk management approach from d 8 to 49 such that bunks were managed to be devoid of feed by 07:30 h most mornings. Pens were 7.6 × 7.6 m concrete surface pens with 7.6 m of linear bunk space and equipped with a heated, continuous flow concrete waterer.

The low cooked molasses “stress tubs" were introduced to the Tub treatment pens approximately 36 h following arrival to the RNC. Tubs were cleaned and weighed daily, and consumption was determined from daily disappearance. Individual consumption was calculated by dividing daily tub disappearance by the number of steers /in the pen. Steers were provided access to the tubs through d 28 of the experiment. Stress tub nutritional values are presented in Table 2.2.

Force supplement was provided at a rate of 0.22 kg/hd/d, it was added in the diet using a soybean hull carrier to facilitate the mixing process; supplement composition is included in Table 2.3. The Force supplement was designed so that TM and YCP intake would be equivalent to Tub assuming a targeted Tub intake of 0.22 kg per day. The Force supplements were included in the diet from d 1 to d 28.

Weekly ingredient samples were dried in a forced air oven at 60 °C until no further weight change occurred. Individual commodity ingredients collected each week

were ground to 1 mm and composited into a single individual sample for nutrient analyses at a commercial laboratory using AOAC procedures (Servi-Tech, Hastings, NE, USA). Actual diet formulation based upon weekly DM determination and feed batching record along with tabular energy content (Preston, 2016) is presented in Table 2.1, and tabular energy values according to Preston, 2016 were used for determination of tabular dietary net energy (NE) content. Feed intake and ingredient inclusion (DM basis) were summarized at weekly intervals.

2.3.5. Growth Performance Calculations

Steers were weighed prior to feeding, and on d -1, 1, 14, 28, and 49. Growth performance data were summarized from initial to d 28 (treatment phase), d 29 to d 49 (non-treatment phase), and from initial to d 49 (cumulative). Initial BW was not shrunk, while all other BW measures were shrunk 4% to account for digestive tract fill. Average daily gain was determined using the difference between each beginning and ending period weight divided by days on feed. Efficiency of weight gain was calculated by dividing the period ADG by the period daily DMI. Body weight at 28% empty body fatness for these steers was estimated to be 625 kg (Smith, 2020). Observed dietary NE was calculated from daily energy gain (EG; Mcal/d): EG = (ADG from initial to d 49) $^{1.097}$ × 0.0557W$^{0.75}$, where W is the mean equivalent BW [average trial BW × (478/625)], [kg; (NRC, 1996)]. Maintenance energy required (EM; Mcal/d) was calculated by the following equation: EM = 0.077BW$^{0.75}$, where BW is the mean trial BW (kg). Using the estimates required for maintenance and gain, the observed dietary NEm and Neg values of the diet were generated using the quadratic formula: x=(-b±√(b^2-4ac))/2c, where x = Nem, Mcal/kg, a = −0.41EM, b=0.877EM+0.41DMI +EG, c=−0.877DMI (Zinn and

Shen, 1998) and Neg was determined from: 0.877Nem−0.41 (Zinn, 1987). The ratio of observed-to-expected NE was determined from observed dietary NE for maintenance or gain tabular NE for maintenance or gain (Preston, 2016).

2.3.6. Hepatic Biopsies

For the determination of hepatic trace mineral content, liver biopsies were collected on d 14, 28 and 49 following the process below. Briefly, steers were secured in a hydraulic squeeze chute with mild squeeze pressure. The biopsy was collected through an incision in the 11^{th} intercostal space (between the 11^{th} and 12^{th} ribs) on a line from the hook bone (tubercoxae) to the point of the shoulder (scapula-humoral joint). Hair was clipped from an area approximately 10.16 cm × 10.16 cm around the biopsy site. The surgical site was prepared with iodine and 70% isopropyl alcohol. A solution composed of Lidocaine hydrochloride and bicarbonate (90%/10%, v/v) was applied by inserting the needle through the muscle layer and release the solution (1 mL) as the needle was withdrawn from the biopsy location, after 15 seconds, the incision was made. A 6.35 mm incision was made by inserting a scalpel blade through the skin and intercostal muscle tissue, perpendicular to the body wall. Surgical tubing was applied to the biopsy needle (DJ-series Jamshidi bone marrow needle 8 ga and 10.16 cm long; Cardinal Health catalog number DJ4008X 13), which was inserted into the incision site then into the liver and was used to collect hepatic tissue by using back pressure on a 10 mL syringe. Contents were emptied from the syringe and surgical tubing into a wire mesh screen, and excess blood was rinsed-off using 0.01M of phosphate buffered saline (PBS), and the PBS rinse was repeated as necessary to remove excess blood (Hamilton et al., 2021). Wounds were sprayed with iodine, and biopsy sites were checked for swelling and complications at 24

h and 48 h post-biopsy. No complications were noted due to the hepatic biopsy procedure in the present study. Hepatic samples were shipped to Michigan State University Diagnostic Center for Population and Animal Health (Lansing, MI, USA) for analysis of hepatic mineral content. Concentrations of Co, Cu, Mn, and Zn were measured using an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) via procedures reported by (Wahlen et al., 2005).

2.3.7. Statistical Analysis

Data were analyzed as a RCBD with pen as an experimental unit. Treatment was included as a fixed effect and block (location) was considered a random effect in the statistical model. Hepatic trace mineral content was analyzed via repeated measures and included the fixed effects of treatment, day, and their interaction, the covariance structure with the best fit (lowest Akaike information criterion) was autoregressive 1 (AR-1). Pre-planned orthogonal contrasts were overall, non-treated vs. treated, and Force vs. Tub (delivery method).

2.4. RESULTS AND DISCUSSION

2.4.1. Results and Discussion

Tub disappearance and DMI are depicted in Figure 2.1. The stress tub label indicated that cattle should consume between 0.15 and 0.22 kg/d. Average daily disappearance from the tubs was 0.115 ± 0.06 kg/d, meaning that the animals under-consumed the supplement, a common issue of a free-choice supplement (Ranches et al., 2021). During the first 9d cattle consumed more of the Tub, after that the consumption

dropped. Additionally, on d9 they reached 2% of DMI equivalent to BW. Meaning in theory that they were full and therefore, stopped consuming the supplement.

Force steers tended ($P = 0.08$) to have greater DMI compared to Tub from d 1 to 14. Steers offered additional TM and YCP from either Force or Tub the "stress-pack" from d 15 to 28, had greater DMI by 4.15% ($P = 0.01$) and tended ($P = 0.07$) to have 12.5% greater ADG compared to Con from d 15 to 28. In addition, treated steers had greater DMI from d 29 to 49 ($P = 0.01$, Table 2.4). Hamilton et al., (2021) reported a tendency for 22.8% increased ADG by for steers supplemented with a stress tub; however, DMI did not differ. In this trial, steers from Force consumed 6.9% more DM compared to Tub ($P = 0.01$). Cumulative DMI ($P = 0.01$) and ADG ($P = 0.05$) were greater for steers from Force compared to Tub by 5.4% and 9.4%, respectively (Table 2.7). However, no difference was found on net energy utilization. Berrett et al. (2015) found that feeding no additional TM or additional TM in different concentrations did not result in improved feedlot performance.

In the present study, a treatment × day interaction ($P \leq 0.01$), for hepatic concentrations of Co and Cu was noted (Figure 2 and 3). Force had greater hepatic Co ($P \leq 0.05$) compared to Tub and Con on d 14, 28, and 49. Similar results were found by Hamilton et al. (2021) and Ranches et al. (2021) where TM were supplemented using molasses blocks. Hamilton et al., (2021) noted that supplemented steers had greater hepatic Co during the treatment period; however, from d 21 to d 42, Co hepatic concentration did not differ from control. Similar effects were observed in this experiment where steers from Tub had greater hepatic Co compared to Con on d 14 ($P < 0.05$), but hepatic Co content was similar to Con on d 28 and 49 ($P > 0.10$).

Steers from Force treatment had greater hepatic Cu ($P \leq 0.05$) compared to Tub and Con on d 14, 28, and 49. Feeding an organic source of TM, Rhoads et al. (2003) and Marques et al. (2016), showed that hepatic Cu stores can be increased with increased dietary concentrations of Cu. In the present study, steers from Tub had greater hepatic Cu compared to Con on d 14 and 28 ($P < 0.05$), but hepatic Cu content was similar to Con on d 49 ($P > 0.10$). However, Hamilton et al. (2021) observed that during the initial period, treated cattle had a greater hepatic Cu, but on d 42 the non-treated group hepatic Cu levels were similar compared with the treated group.

In the present study a tendency for treatment x day interaction was found ($P = 0.07$; $P = 0.09$) of Mn and Zn hepatic concentration, respectively. Additionally, a day effect was found ($P= 0.04$; $P = 0.01$) on Mn and Zn hepatic concentration, respectively. Differing from Hamilton et al. (2021) where a treatment x day interaction was found on d7 for Zn hepatic concentration, being 27% higher when compared to non-supplemented steers. However, on d21 and d42 hepatic concentrations of Zn did not differ. Additionally, the hepatic Cu levels of the steers of the present study was considerably higher when compared with Hamilton et al. (2021), leading to a lower availability of Zn considering that Cu is a strong antagonist (Radwińska and Żarczyńska, 2014). However, hepatic Zn did not differ due to treatment.

Providing TM supplementation for calves through limit-fed creep feeding, injectable trace minerals or low moisture cooked molasses blocks improves trace mineral status of cattle (Caramalac et al., 2017; Arthington et al., 2014; Ranches et al., 2021, Hamilton et al., 2021). Additionally, YCP increases mineral retention by the animal (Cole et al., 1992).

2.5. CONCLUSION

The main point to use the lick tub is the lower labor comparing to other supplementation methods. However, a major issue with free choice supplementation is over or under consumption, on this experiment steers from tub under consumed the supplement leading to a poor performance comparing to Force. The Force treatment resulted in the best performance and greater Co and Cu hepatic concentration compared to the other treatments. Tub treatment had a superior performance compared to Con and greater Co and Cu hepatic concentrations on d 14, and 14 and 28, respectively. In summary, the application of a “stress-pack” in diets offered to newly weaned cattle enhanced Co and Cu hepatic concentration and production responses, but method of delivery influences DMI and daily gain.

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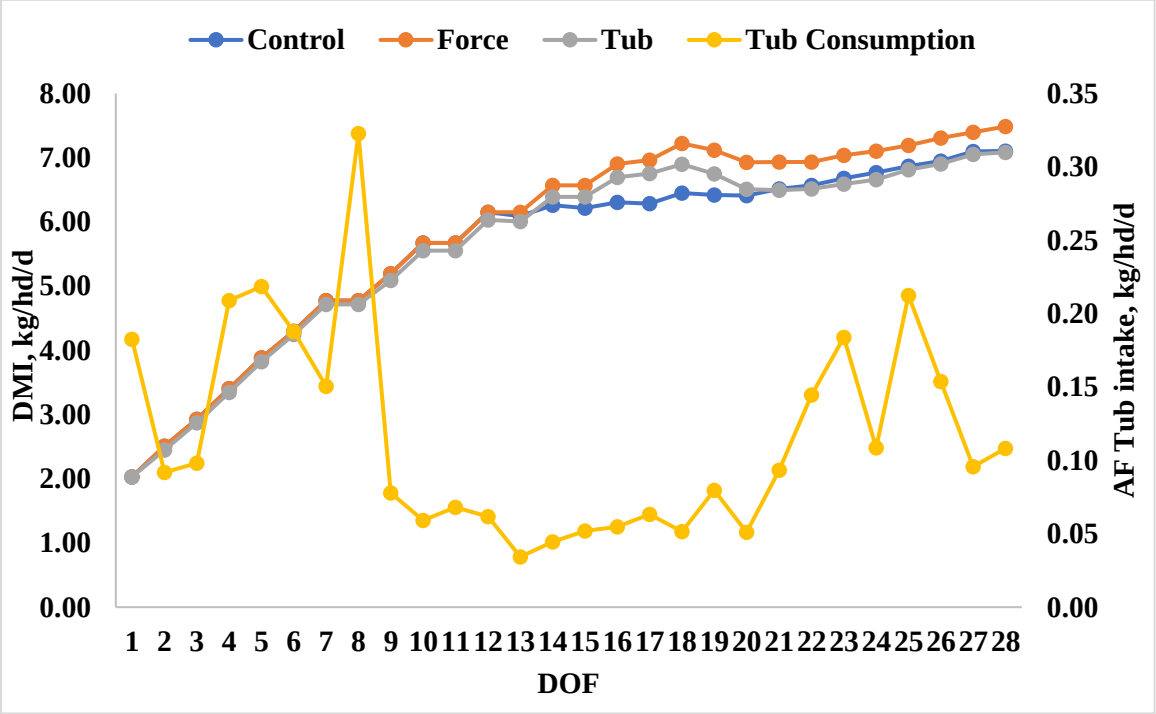

Figure 2.1. Dry matter intake and Stress Tub intake of newly weaned steers initial to 28-d post weaning. Daily (n = 8 pens) consumption (kg/steer·d −1) of the cooked molasses stress tub (Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA) during the 28-d period, offered to newly weaned steer calves. Dry matter intake (kg/steer·d^{-1}) Con; Force; Tub.

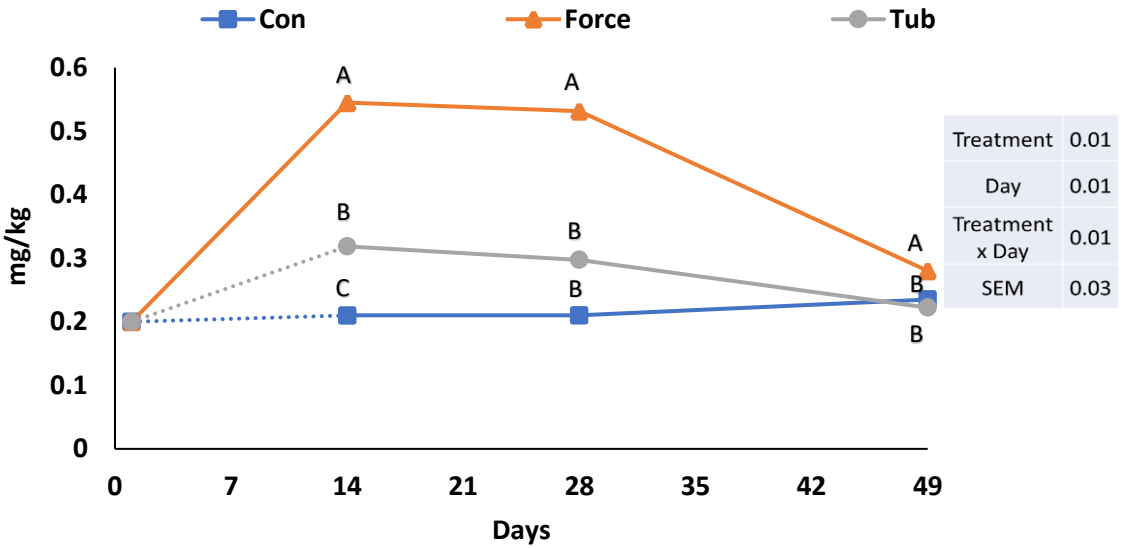

Figure 2.2. Hepatic Concentration of Cobalt in Newly Weaned Steers Initial to 49-d post Weaning. No "stress pack" (Con), "stress-pack" directly fed in the diet (Force; Organic trace minerals (Availa 4, ZINPRO, Eden Prairie, MN) and a *Saccharomyces cerevisiae* yeast culture product (Diamond V XPC, Diamond V, Cedar Rapids), or cooked molasses stress tub (Tub; Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA). A, B means within a day lacking a common superscript differ ($P \leq 0.05$).

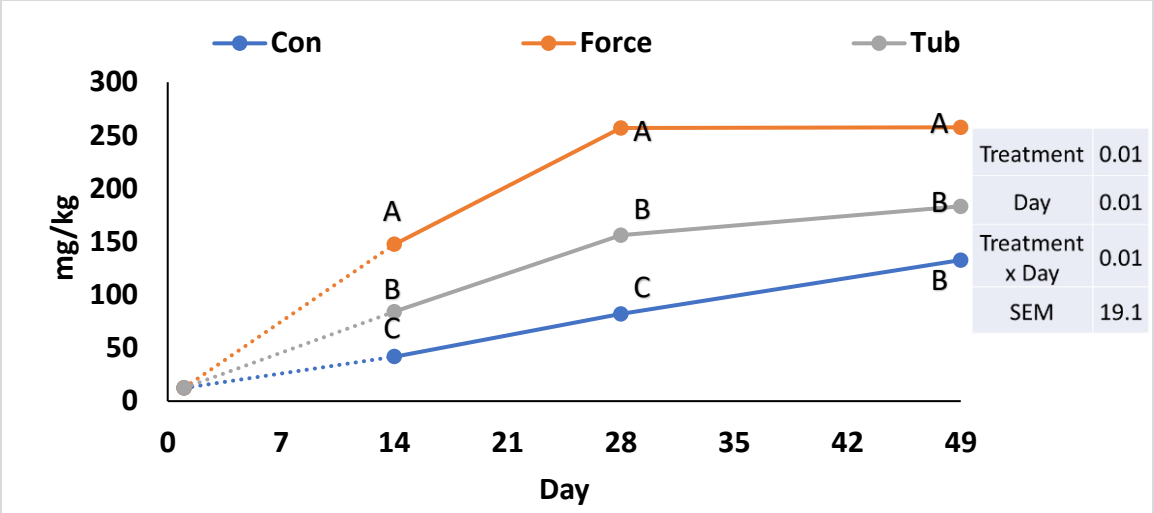

Figure 2.3. Hepatic concentration of copper in newly weaned steers initial to 49-d post weaning. No "stress pack" (Con), "stress-pack" directly fed in the diet (Force; Organic trace minerals (Availa 4, ZINPRO, Eden Prairie, MN) and a *Saccharomyces cerevisiae* yeast culture product (Diamond V XPC, Diamond V, Cedar Rapids), or cooked molasses stress tub (Tub; Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA). A, B means within a day lacking a common superscript differ ($P \leq 0.05$).

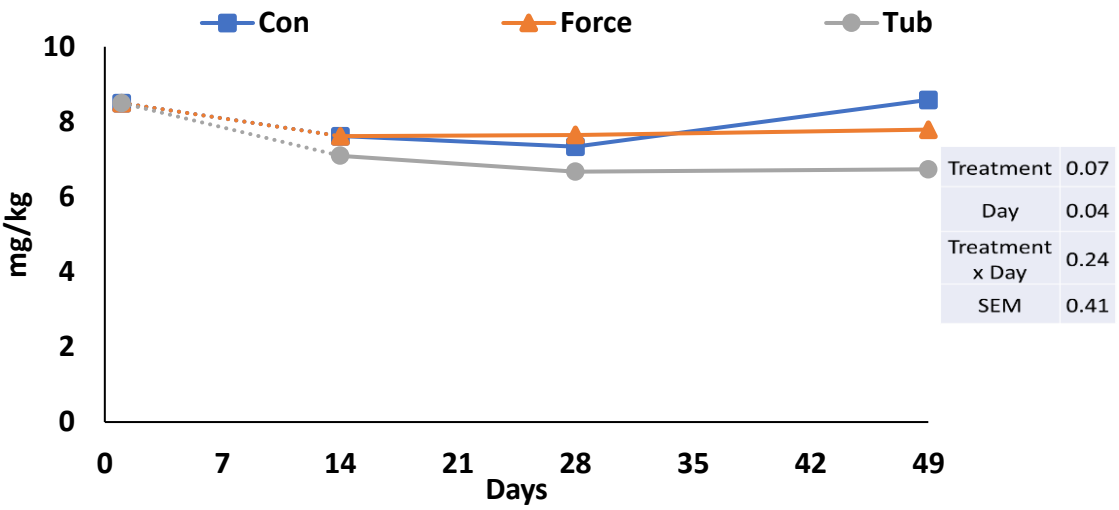

Figure 2.4. Hepatic concentration of manganese in newly weaned steers initial to 49-d post weaning. No "stress pack" (Con), "stress-pack" directly fed in the diet (Force; Organic trace minerals (Availa 4, ZINPRO, Eden Prairie, MN) and a *Saccharomyces cerevisiae* yeast culture product (Diamond V XPC, Diamond V, Cedar Rapids), or cooked molasses stress tub (Tub; Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA). A, B means within a day lacking a common superscript differ ($P \leq 0.05$).

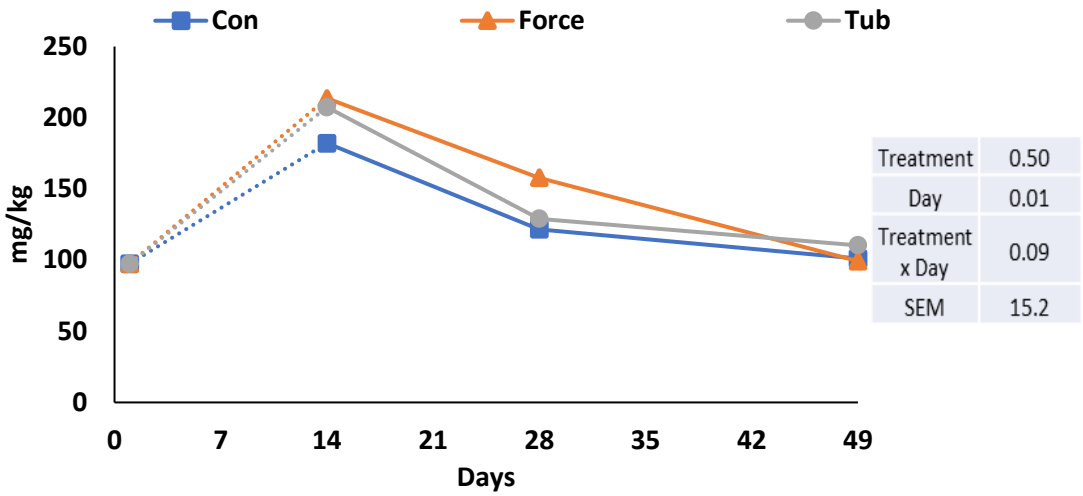

Figure 2.5. Hepatic concentration of zinc in newly weaned steers initial to 49-d post weaning. No "stress pack" (Con), "stress-pack" directly fed in the diet (Force; Organic trace minerals (Availa 4, ZINPRO, Eden Prairie, MN) and a *Saccharomyces cerevisiae* yeast culture product (Diamond V XPC, Diamond V, Cedar Rapids), or cooked molasses stress tub (Tub; Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA). A, B means within a day lacking a common superscript differ ($P \leq 0.05$).

Table 2.1. Actual diet formulation based upon weekly DM determinations of ingredients and nutrient composition based upon 49-d period composites of weekly ingredients and reconstructed diet composition (DM basis) for nutrient content determination[1].

Item	D1 to 49
Wheatlage, %	39.62
DDGS[2], %	9.39
Oat Hay, %	10.18
Pelleted Soybean Hulls, %	35.69
Liquid Supplement [3], %	5.12
DM, %	51.37
CP [4], %	12.92
NDF [5], %	56.49
ADF [6], %	38.64
Ash, %	6.99
EE [7], %	2.62
NEm [8], Mcal/kg	1.73
NEg [9], Mcal/kg	1.04

[1] All values except for dry matter (DM) on a DM basis.
[2] Dried distillers grains plus solubles.
[3] Contained (DM basis) 36.27% crude protein, 28.00% nonprotein nitrogen, 0.74 Mcal/kg of net energy for maintenance, 0.50 Mcal/kg of net energy for gain, 1.62% crude fat, 1.06% crude fiber, 4.62% calcium, 0.43% P, 2.28% K, 0.47% Mg, 5.00% NaCl, 3.38% Na, 0.54% S, 4.00 ppm Co, 200.00 ppm Cu, 20.00 ppm I, 11.41mg/lb. of ethylenediamine dihydroiodide 150.29 ppm Fe, 400.00 ppm Mn, 3.08 ppm Se, 700.00 ppm Zn, 20,000 IU/kg of vitamin A, 200.00 IU/kg of vitamin E, and 500.00 g/Mg of monensin sodium (Rumensin, Elanco, Indianapolis, IN, USA).
[4] Crude protein.
[5] Neutral detergent fiber.
[6] Acid detergent fiber.
[7] Ether Extract.
[8] Net energy for maintenance.
[9] Net energy for gain.

Table 2.2. Minimum guaranteed analysis (as-is basis) for the low-moisture, molasses-based block (Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA)[1].

Item	Value
Crude protein, %	12.00
Crude fat, %	4.00
Crude fiber, %	4.00
Calcium, %	2.00
Phosphorus, %	1.00
Salt, %	1.50
Magnesium, %	1.00
Potassium, %	2.00
Manganese, ppm	1300.00
Cobalt, ppm	60.00
Copper, ppm	785.00
Iodine, ppm	40.00
Selenium, ppm	13.00
Zinc, ppm	2500.00
Vitamin A, IU/kg	440,920.00
Vitamin D3, IU/kg	49,603.50
Vitamin E, IU/kg	1763.68

[1]Each 0.227 kg (as-is) an organic trace mineral product that provided: 200.2 mg of manganese, 12.6 mg of cobalt, 126.0 mg of copper, and 360.5 mg of zinc in each in each 0.227 kg (Availa 4; Zinpro, Eden Prairie, MN) and 14 g of Saccharomyces cerevisiae fermentation product (Diamond V Original XPC; Cedar Rapids, IA).

Table 2.3. Force fed supplement formulation (as-is basis) [1]

Ingredient Name	% of as-fed
Soybean hulls	63.94
Dry distillers grains plus solubles	16.90
Saccharomyces cerevisiae fermentation product [2]	6.25
Beet molasses	5.00
Organic trace mineral product [3]	3.10
Salt	1.75
Potassium chloride	1.29
Selenium .16%	0.81
Vitamin E 50	0.35
Zinc sulfate	0.25
Manganese sulfate	0.14
Copper sulfate	0.09
Vitamin A 650	0.07
EDDI 9.2%	0.05
Vitamin D3 500	0.01

[1]Force fed supplement was included in the diet at 0.23g/head/day (as-fed basis);
[2]Diamond V Original XPC; Cedar Rapids, IA;
[3]Availa 4; Zinpro, Eden Prairie, MN.

Table 2.4. Effects of use and delivery method of supplemental organic trace minerals and a yeast culture product "stress pack" in newly weaned steer calves on growth performance and efficiency of energy utilization from day 1 to 14.

	Treatments[a]				*P* – value		
Item	Control (1)	Force (2)	Tub (3)	SEM	Overall	1 vs. 2 and 3	2 vs. 3
Pens, n	8	8	8	-	-	-	-
Steers, n	64	64	64	-	-	-	-
Start BW[1], kg	257	256	257	1	0.17	0.94	0.06
d 14 BW[2], kg	269	270	269	2.8	0.72	0.53	0.62
ADG[3], kg	0.88	0.98	0.88	0.225	0.5	0.56	0.31
DMI[4], kg	4.44	4.47	4.39	0.097	0.19	0.68	0.08
DMI, % BW[5]	1.69	1.70	1.67	0.015	0.12	0.54	0.05
AF tub intake[6], kg/d				0.13	-	-	-
G:F[7]	0.197	0.22	0.199	0.0222	0.53	0.54	0.35
F:G	5.08	4.55	5.03	-	-	-	-
DMI, x NEm[8]	1.53	1.53	1.51	0.014	0.16	0.58	0.06

[1] No shrink was applied to initial BW.
[2] 4% shrink applied to d 14 to account for digestive tract fill.
[3] Average daily gain
[4] Dry matter intake
[5] Dry matter intake as a percentage of body weight
[6] As-fed tub intake
[7] Feed for gain (G:F) = ADG, kg divided by DMI, kg.
[8] Tabular dietary NEm was 1.73 Mcal/kg and NEg was 1.04 Mcal/kg.
[a] No "stress pack" (Con), "stress-pack" directly fed in the diet (Force; Organic trace minerals (Availa 4, ZINPRO, Eden Prairie, MN) and a *Saccharomyces cerevisiae* yeast culture product (Diamond V XPC, Diamond V, Cedar Rapids), or cooked molasses stress tub (Tub; Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA).

Table 2.5. Effects of use and delivery method of supplemental organic trace minerals and a yeast culture product "stress pack" in newly weaned steer calves on growth performance and efficiency of energy utilization from day 15 to 28.

	Treatments[a]				*P* - value		
Item	Control (1)	Force (2)	Tub (3)	SEM	Overall	1 vs. 2 and 3	2 vs. 3
Pens, n	8	8	8	-	-		
Steers, n	64	64	64	-	-		
d 28 BW[1], kg	283	286	285	3.3	0.19	0.09	0.53
ADG[2], kg	1.00	1.13	1.11	0.163	0.18	0.07	0.76
DMI[3], kg	6.23	6.67	6.33	0.237	0.01	0.01	0.01
DMI, % BW[4]	2.26	2.40	2.29	0.035	0.01	0.01	0.01
AF tub intake[5], kg/d			0.10	-	-	-	-
G:F[6]	0.160	0.170	0.175	0.0105	0.34	0.17	0.64
F:G	6.25	5.88	5.71	-	-	-	-
DMI, x NEm[7]	2.07	2.20	2.09	0.032	0.01	0.01	0.01

[1] 4% shrink applied to d 28 to account for digestive tract fill.
[2] Average daily gain
[3] Dry matter intake
[4] Dry matter intake as a percentage of body weight
[5] As-fed tub intake
[6] Feed for gain (G:F) = ADG, kg divided by DMI, kg.
[7] Tabular dietary NEm was 1.73 Mcal/kg and NEg was 1.04 Mcal/kg.
[a] No "stress pack" (Con), "stress-pack" directly fed in the diet (Force; Organic trace minerals (Availa 4, ZINPRO, Eden Prairie, MN) and a *Saccharomyces cerevisiae* yeast culture product (Diamond V XPC, Diamond V, Cedar Rapids), or cooked molasses stress tub (Tub; Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA).

Table 2.6. Effects of use and delivery method of supplemental organic trace minerals and a yeast culture product "stress pack" in newly weaned steer calves on growth performance and efficiency of energy utilization from day 29 to 49.

	Treatments[a]				*P* - value		
Item	Control (1)	Force (2)	Tub (3)	SEM	Overall	1 vs. 2 and 3	2 vs. 3
Pens, n	8	8	8	-	-		
Steers, n	64	64	64	-	-		
d 49 BW[1], kg	308	313	309	4.7	0.1	0.17	0.09
ADG[2], kg	1.20	1.28	1.14	0.219	0.37	0.89	0.17
DMI[3], kg	6.79	7.10	6.64	0.279	0.01	0.45	0.01
DMI, % BW[4]	2.30	2.37	2.24	0.037	0.01	0.79	0.01
G:F[5]	0.177	0.180	0.171	0.0088	0.74	0.9	0.45
F:G	5.65	5.56	5.85	-	-	-	-
DMI, x NEm[6]	2.14	2.21	2.08	0.035	0.01	0.68	0.01

[1] 4% shrink applied to d 49 to account for digestive tract fill.
[2] Average daily gain
[3] Dry matter intake
[4] Dry matter intake as a percentage of body weight
[5] Feed for gain (G:F) = ADG, kg divided by DMI, kg.
[6] Tabular dietary NEm was 1.73 Mcal/kg and NEg was 1.04 Mcal/kg.
[a] No "stress pack" (Con), "stress-pack" directly fed in the diet (Force; Organic trace minerals (Availa 4, ZINPRO, Eden Prairie, MN) and a *Saccharomyces cerevisiae* yeast culture product (Diamond V XPC, Diamond V, Cedar Rapids), or cooked molasses stress tub (Tub; Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA).

Table 2.7. Effects of use and delivery method of supplemental organic trace minerals and a yeast culture product "stress pack" in newly weaned steer calves on growth performance and efficiency of energy utilization cumulative from day 1 to 28.

	Treatments[a]				*P* - value		
Item	Control (1)	Force (2)	Tub (3)	SEM	Overall	1 vs. 2 and 3	2 vs. 3
Pens, n	8	8	8	-	-		
Steers, n	64	64	64	-	-		
Initial to d 28							
ADG[1], kg	0.94	1.06	0.99	0.114	0.10	0.07	0.23
DMI[2], kg	5.34	5.57	5.36	0.132	0.01	0.02	0.01
DMI, % BW[3]	1.98	2.06	1.98	0.019	0.01	0.03	0.01
AF tub intake[4], kg/d			0.12	-	-	-	-
G:F[5]	0.18	0.19	0.19	0.0085	0.23	0.11	0.57
F:G	5.71	5.26	5.41	-	-	-	-
DMI, x NEm[6]	0.82	0.85	0.82	0.0179	0.01	0.03	0.01

[1] Average daily gain
[2] Dry matter intake
[3] Dry matter intake as a percentage of body weight
[4] As-fed tub intake
[5] Feed for gain (G:F) = ADG, kg divided by DMI, kg.
[6] Tabular dietary NEm was 1.73 Mcal/kg and NEg was 1.04 Mcal/kg.
[a] No "stress pack" (Con), "stress-pack" directly fed in the diet (Force; Organic trace minerals (Availa 4, ZINPRO, Eden Prairie, MN) and a *Saccharomyces cerevisiae* yeast culture product (Diamond V XPC, Diamond V, Cedar Rapids), or cooked molasses stress tub (Tub; Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA).

Table 2.8. Effects of use and delivery method of supplemental organic trace minerals and a yeast culture product "stress pack" in newly weaned steer calves on growth performance and efficiency of energy utilization cumulative from day 1 to 49.

	Treatments[a]				*P* - value		
Item	Control (1)	Force (2)	Tub (3)	SEM	Overall	1 vs. 2 and 3	2 vs. 3
Pens, n	8	8	8	-	-		
Steers, n	64	64	64	-	-		
Initial to d 49 BW, kg							
ADG[1], kg	1.05	1.16	1.06	0.1	0.07	0.18	0.05
DMI[2], kg	5.96	6.22	5.91	0.158	0.01	0.10	0.01
DMI, % BW[3]	2.11	2.19	2.09	0.019	0.01	0.11	0.01
G:F[4]	0.176	0.185	0.179	0.0058	0.28	0.26	0.26
F:G	5.68	5.41	5.59	-	-	-	-
DMI, x NEm[5]	1.94	2.02	1.92	0.019	0.01	0.10	0.01
O/E DMI[6]	0.98	0.97	0.97	0.0167	0.61	0.34	0.83
O/E ADG[7]	1.04	1.07	1.07	0.0314	0.64	0.35	1.00
O/E NEm[8]	1.02	1.03	1.03	0.0137	0.46	0.23	0.86
O/E Neg[9]	1.03	1.04	1.04	0.0194	0.67	0.39	0.90

[1] Average daily gain
[2] Dry matter intake
[3] Dry matter intake as a percentage of body weight
[4] Feed for gain (G:F) = ADG, kg divided by DMI, kg.
[5] Tabular dietary NEm was 1.73 Mcal/kg and NEg was 1.04 Mcal/kg.
[6] Observed x expected dry matter intake
[7] Observed x expected average daily gain
[8] Observed x expected net energy for maintenance
[9] Observed x expected net energy for gain
[a] No "stress pack" (Con), "stress-pack" directly fed in the diet (Force; Organic trace minerals (Availa 4, ZINPRO, Eden Prairie, MN) and a *Saccharomyces cerevisiae* yeast culture product (Diamond V XPC, Diamond V, Cedar Rapids), or cooked molasses stress tub (Tub; Stress Tub; Purina Animal Nutrition, St. Louis, MO, USA).

Chapter 3: EVALUATION OF A RUMEN PROTECTED B-VITAMIN BLEND ON FINISHING STEER GROWTH PERFORMANCE, EFFICIENCY OF DIETARY NET ENERGY UTILIZATION, CARCASS TRAIT RESPONSES, AND LIVER ABSCESS PREVALENCE AND SEVERITY

3.1 ABSTRACT

The objective of this study was to determine the influence that a rumen-protected B-vitamin (RPBV) blend (containing B5, B6, B7, B9, and B12) had on growth performance, efficiency of dietary net energy utilization, carcass trait responses, and liver abscess severity and prevalence in beef steers fed a finishing diet. Steers (n = 246; initial shrunk BW = 411 ± 25.8 kg) from two sources, were used in a 126-d RCBD experiment. Within 48 h after arrival, steers were individually weighed and allotted to one of 24 pens (n = 10 to 12 steers; 8 pens/treatment) and randomly assigned to 1 of 3 treatments: 1) No RPBV; 2) RPBV1 at 1 g/hd/d; 3) RPBV2 at 2 g/hd/d. During the first 14 d cattle received two transition diets. From d 15 to d 126 cattle were fed the final diet containing 53% dry-rolled corn; 23% corn silage; 20% MDGS; and 4% suspended supplement. No differences ($P \geq 0.13$) amongst treatments were found for DMI, live final BW, ADG, or G: F. Carcass-adjusted final BW, ADG, and G:F were not influenced by treatment ($P \geq 0.59$). How carcass weight, dressing percentage, marbling score, kidney-pelvic-heart fat, or BW at 28% empty body fat did not differ among treatments ($P \geq 0.11$). Ribeye area (REA) was altered (quadratic effect, $P = 0.02$) by treatment; steers from RPBV1 had decreased REA compared to others. Additionally, calculated yield grade (YG) and calculated retail yield (RY) were altered (quadratic effect, $P \leq 0.01$) by treatment; steers from RPBV1 had increased YG and decreased RY compared to others. Estimated empty body fatness tended ($P = 0.06$) to be greater from steers fed RPBV compared to control.

Overall USDA YG distribution was altered by dietary treatment (P = 0.01). The proportions of YG1 and YG5 carcasses were unaffected by treatment, but there was a shift in the proportion of carcasses that graded YG2, YG3, and YG4 among treatments. Distribution of USDA Quality Grade was not altered by treatment (P = 0.53). No treatment differences in liver abscess incidence or severity were observed (P = 0.13), The use of RPBV altered carcass muscularity and rib fat accumulation impacting the overall YG distribution. However, RPBV did not appreciably influence any cumulative growth performance measures or liver abscess outcome.

Key words: B-vitamin, beef, finishing diet.

3.2. INTRODUCTION

The United States beef production system has substantially increased the efficiency of beef production in terms production per unit of feed input in feedlots over the years. Improvements in genetics, management and technologies resulted in faster growing, heavier animals resulting in changes in animal requirements (Capper, 2011). Additionally, to optimize production cost and support animal performance feed additives must be used to supplement the amount of nutrients in the diet (e.g., trace minerals and B vitamins).

B - complex vitamins are necessary for a variety of metabolic process in the animal, including energy, carbohydrate, lipid and AA metabolism. However, knowledge regarding B vitamin requirements for feedlot beef cattle is limited. Because B complex are synthetized by ruminal microbials, it is thought that this production is enough to satisfy the requirement of the host (Zinn et al., 1987). However, changes in the forage to concentrate ratio of the diet are known to alter the microbial activity of the rumen (Allison, 1965) and are therefore likely to affect the amount of vitamins produced.

Additionally, more studies are necessary to understand the requirement of the modern ruminant genetic potential and variation of B vitamin synthesis according to the feed ingested.

3.3 MATERIAL AND METHODS

3.3.1 Use of Animal Subjects

All procedures involving the use of animals in this experiment were approved by the South Dakota State University Institutional Animal Care and Use Committee (Approval #3958-01).

3.3.2 Animal Description and Initial Processing

Crossbred beef steers (n =292) were sourced as two separate consignments from local South Dakota auction facilities approximately 5 d prior to the initiation of the experiment. This experiment was conducted at the Southeast Research Farm (SERF) of the South Dakota Agricultural Experiment Station located near Beresford, SD. Steers from the first consignment were used in replicate pens 1 to 7, and steers from the second consignment were used in replicate pens 8 to 24. Approximately 48 h after arrival (d -3), steers were tagged, vaccinated against viral respiratory (Bovi-Shield Gold 5, Zoetis, Parsippany NJ) and clostridia pathogens (Ultrabac 7/Somubac, Zoetis) and administered pour-on moxidectin (Cydectin, Bayer, Shawnee Mission, KS) according to label instructions. The BW collected on d -3 was used for allotment purposes. Steers were selected for study enrollment based upon uniformity of BW and temperament on d -3. Steers were weighed on d 1 and allotted to their respective pen. The average of the BW measurements collected on d -3 and d 1 were used as the initial BW (n = 246 steers; initial average shrunk BW = 411 ± 25.4 kg). This study used 8 replicate pens of 10 to 12 steers (10 steers per pen in replicate 1 to 7 and 12 steers per pen in replicate 8 to 24)

assigned to each of the 3 dietary treatments (24 pens total). All steers were implanted on d 28 (98 d before harvest) with a 200 mg trenbolone acetate and 28 mg estradiol benzoate implant (Synovex Plus, Zoetis, Parsippany NJ). All implants were checked on d 56 (28 d following implantation) and any implant abnormalities were noted, steers identified with missing, or abscessed-out implants were administered another implant. All steers were fed ractopamine HCl at a rate of 300 mg per steer daily for the final 28 d prior to harvest.

3.3.3. Experimental Design and Treatments

Pens were assigned to 1 of 3 treatments with 8 replicate pens assigned to each treatment: 1) fed no rumen-protected B-vitamin blend (RPBV0) 2) fed the rumen-protected B-vitamin blend at a rate of 1.0 g/steer·d-1 (RPBV1); 3) fed the rumen-protected B-vitamin blend at a rate of 2.0 g/steer·d-1 (RPBV2). The dietary treatment was included in a ground corn carrier and offered in replacement of dry-rolled corn at a rate of 0.10 lbs/ steer daily (As-fed basis).

3.3.4 Dietary Management

Cattle were fed once a day and bunks were managed for *ad libitum* access to feed with minimal day to day variation in the amount of feed not consumed being the primary target for feed calls. Cattle were transitioned to the 90% concentrate diet, over the course of 14 d (Table 3.1). The final diet (Table 3.2) was fed from d 15 until d 126 (d before harvest).

Feedstuff samples were taken weekly and analyzed for DM content. Weekly ingredient samples were composited monthly for CP, NDF, ADF, and ash determination

using AOAC procedures. A single TMR sample was collected and analyzed for Ca, P, Co, Cu, Mn, Mo, Se, and Zn according to AOAC procedures. Orts were collected, weighed, and dried in a forced air oven at 100°C for 24 h in order to determine DM content. The DMI of each pen was adjusted to reflect the total DM delivered to each pen after subtracting the quantity of dry orts for each interim period. Feed was manufactured in a mixer (4.16 m^3; 8 pens fed per batch) connected to a tractor, all ingredients were added into the mixer to the nearest 1-kg, and feed was delivered to each pen separately (weighed out of the mixer to the nearest 1-kg). Batching sequence was RPBV0 (8 pens), RPBV1 (8 pens), and RPBV2 (8 pens). Following each batch of feed, long stem grass hay (4.54 kg) was added to the mixer and used to flush out all residual feed remaining in the mixer. Mixing of the following batch did not occur until the scale head read 0 to 1 kg.

Weekly ingredient samples were stored in a freezer at -20° C until nutrient analyses were completed. After weekly DM determination (method no. 935.29), weekly samples from each ingredient were composited by month and analyzed for N (method no. 968.06; Rapid Max N Exceed; Elementar; Mt. Laurel, NJ), and ash (method no. 942.05) content (AOAC, 2012, 2016). Modified distillers grains plus solubles samples were analyzed for ether extract content using an Ankom Fat Extractor (XT10; Ankom Technology, Macedon, NY). Percentages of ADF and NDF were assumed to be 3 and 9 percent for dry-rolled corn (*P*reston, 2016). Analysis of ADF and NDF composition for all other feeds was conducted as described by (Goering and VanSoest, 1970). Diets presented in Table 3.1 and 3.2 are actual DM diet composition, actual nutrient concentrations, and tabular energy values (Preston, 2016).

3.3.5 Growth Performance and Carcass Data Collection

Steers were individually weighed on d -3, 1, 28, 56, 98 (start RH) and 126 (d before harvest). Cumulative growth performance was calculated on live and carcass-adjusted basis. Initial BW was the average of the d -3 and d 1 BW. All live BW measures used were pencil shrunk 4% to account for gastrointestinal tract fill and carcass-adjusted final BW was calculated from hot carcass weight (HCW) divided by 0.625. Average daily gain was determined as the difference between final and initial BW divided by days on feed (126 d). Dry matter intake was tabulated at weekly intervals and summarized by interim period. Feed conversion ratio was calculated using ADG divided by DMI.

Growth performance (live-basis) was used to calculate performance-based dietary NE in order to determine efficiency of dietary NE utilization. The performance-based dietary NE was calculated from daily energy gain (EG; Mcal/d): $EG = ADG^{1.097} \times 0.0557W^{0.75}$, where W is the mean equivalent shrunk BW [kg; (NRC, 1996)] from median feeding shrunk BW and final BW at 28% estimated empty body fatness (AFBW) calculated as: [median feeding shrunk BW × (478/AFBW), kg; (NRC, 1996)]. Maintenance energy (EM) was calculated by the equation: EM = 0.077 × median feeding shrunk $BW^{0.75}$. Dry matter intake is related to energy requirements and dietary NEm (Mcal/kg) according to the following equation: DMI = EG/(0.877NEm − 0.41), and can be resolved for estimation of dietary NEm by means of the quadratic formula x=(-b±√(b^2-4ac))/2c, where a = −0.41EM, b = 0.877EM + 0.41DMI + EG, and c = −0.877DMI (Zinn and Shen, 1998). Dietary NEg was derived from NEm using the following equation: NEg= 0.877NEm − 0.41 (Zinn, 1987).

Steers were marketed and harvested at a commercial abattoir with treatment blinded personnel determine that 60% of the population has sufficient fat cover to grade USDA Choice. Steers were loaded onto trucks, shipped 80 km, and harvested the following day at Tyson Fresh Meats in Dakota City, NE. Liver abscess prevalence and severity was determined by a trained technician using the Elanco system as Normal (no abscesses), A- (1 or 2 small abscesses or abscess scars), A (2 to 4 well organized abscesses less than 2.54 cm. diameter), or A+ (1 or more large active abscesses greater than 2.54 cm. diameter with inflammation of surrounding tissue). Video image data was obtained from the plant for rib eye area, rib fat, kidney-pelvic-heart fat, calculated USDA Yield Grade and USDA marbling scores. Dressing percentage was calculated as HCW/ (final BW × 0.96). Estimated empty body fat (EBF) percentage and AFBW were calculated from observed carcass traits (Guiroy et al., 2002), and proportion of closely trimmed boneless retail cuts from carcass round, loin, rib, and chuck was determined according to the equation described by (Murphey et al., 1960).

3.3.6 Management of Pulls and Removals

A total of five steers were removed from the experiment for reasons not related to dietary treatment. One steer was removed due to hardware disease (RPBV1), one steer was removed due to musculoskeletal issues (RPBV1), one steer was removed for overall poor gain (RPBV2), and two steers were found dead due to a perforated reticulo-rumen (both from RPBV2).

3.3.7 Statistical Analysis

Growth performance, carcass traits, and efficiency of dietary energy utilization was analyzed as a RCBD using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. The model included the fixed effect of dietary treatment and block was considered a random variable. Distribution of USDA Yield and Quality grade, as well as liver abscess severity and prevalence data were analyzed as multinomial distributions using the GLIMMIX procedure of SAS 9.4 to identify differences in the distributions among treatments. Individual steer served as the experimental unit for categorical outcome data and the same fixed and random effects in the model as described previously were used. The model specified a solution function for the multinomial response, with the number of animals slaughtered identified in the denominator. Least squares means were generated using the LSMEANS statement of SAS 9.4. Treatment effects were evaluated by the use of orthogonal polynomials (Steel and Torrie, 1960). An a of 0.05 was used to determine significance and an a of 0.06 to 0.15 was considered a tendency.

3.4 RESULTS AND DISCUSSION

Most B - vitamin supplementation research has been conducted with dairy cattle, where results have shown improved neutrophil function and improvements in general immune response, as well as more efficient glucose metabolism (Pinotti et al., 2005; Ghorbani et al., 2008), reduced heat stress (Wrinkle et al., 2012), improvements on the synthesis of keratin and reduced ulcers (Bergsten et al., 2003), and higher total milk, protein and fat yield (Graulet et al.,2007; Sacadura et al., 2008; Karkoodi and Tamizrad, 2009; Duplessis et al., 2014). In contrast to those positive responses, the current

experiment did not result in any performance benefit when offered to yearling feedlot steers.

Cumulative growth performance responses are shown in Table 3.3. Dry matter intake was not appreciably influenced by the addition of a rumen-protected B-vitamin blend ($P \geq 0.13$). Live-basis final BW, ADG, and G: F were not different among treatments ($P \geq 0.25$), agreeing with results in previous studies (Clifford et al., 1967, Zinn et al., 1987, Word et al., 2022). On the other hand, Leclerc et al. (2021) noted increased final BW, ADG, and G:F with similar DMI when 2 g/head·d^{-1} of a protected B - vitamin blend was fed. Deters et al. (2021) fed protected folic acid and observed improved ADG and G:F during the growing period with linear increases in DMI.

Carcass-adjusted final BW, ADG, nor G:F were not appreciably influenced by dietary treatment ($P \geq 0.59$). Word et al. (2022) observed that carcass-adjusted performance and HCW were numerically improved when supplementing a novel source of rumen-protected folate and Co; however, live growth performance was not affected by treatment. Observed dietary NE values for maintenance and gain based upon growth performance were not influenced by the addition of RPBV in the current experiment. The ratio of observed-to-expected dietary NE values were in close agreement with expectations (0.99) and were not influenced by dietary treatment ($P \geq 0.79$).

Carcass trait responses are located in Table 3.4. Hot carcass weight, dressing percentage, marbling, kidney-pelvic-heart fat, or BW at 28% EBF (AFBW) did not differ due to dietary treatment ($P \geq 0.11$). Word et al. (2022) observed that dressing percentage was improved the steers fed rumen-protected B-vitamins compared to the control with no differences in REA, marbling or EBF. In the current experiment, REA was altered by

treatment (quadratic effect, $P = 0.02$), with steers from RPBV1 having decreased REA compared to others. Hence, calculated yield grade and percentage of wholesale cuts obtained from the round, loin, rib and chuck (retail yield) were also altered by dietary treatment (quadratic effect, $P \leq 0.01$) with steers from RPBV1 having increased yield grade and decreased retail yield compared to steers fed RBPV0 or RPBV2. Estimated empty body fatness tended to be greater from steers fed RPBV compared to control ($P = 0.06$) presumably caused by numerically greater RF depth and lesser REA.

Categorical carcass outcomes are located in Table 3.5. The overall USDA Yield Grade distribution was altered by dietary treatment. There were similar levels of YG1 and YG5 carcasses among treatments, but a shift in distribution Yield Grades with Y greater proportions of YG 3 and 4 carcasses from RPBV0 and lesser YG2 compared to the other two treatments. Dietary treatment did not alter the overall distribution of USDA Quality Grade ($P = 0.53$), similar to results reported by Word et al. (2022).

The overall distribution of liver scores tended to be altered by dietary treatment. Steers from RPBV0 and RPBV1 had more livers classified as normal compared to RPBV2 steers. Deters et al. (2021) observed that folic acid supplementation at 30 or 60 mg/kg reduced the incidence of abscessed livers. Additionally, Word et al. (2022) trial, noted that the percentage of livers with abscesses tended to be reduced by B vitamin supplementation.

3.5 CONCLUSION

Use of RPBV had no appreciable influence on any cumulative growth performance responses when fed to finishing steers in this experiment. When RPBV is

used in finishing diets, carcass muscularity and rib fat accumulation were affected that in turn altered overall yield grade distribution. Additionally, RPBV application does not appear to reduce undesirable liver health outcomes.

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Table 3.1 Actual ingredient inclusion and nutrient content based upon feed batching records.

Item	Step 1 (d 1 to 7)	Step 2 (d 8 to 14)
Dry-rolled corn	35.13	46.24
Modified distillers grains plus solubles	17.83	17.92
Corn silage	24.00	21.89
Grass Hay	19.20	10.01
Suspended supplement[1]	3.84	3.94
Analyzed nutrient composition		
DM[2], % as fed	59.18	54.65
CP[3], %	13.36	13.49
ADF[4], %	18.87	14.15
NDF[5], %	31.34	24.61
Ash, %	6.91	6.07
EE[6], %	3.61	3.70
Neg[7], Mcal/kg	1.19	1.28

[1]Contained on a DM basis: 30, 844 IU of Vitamin A/kg, 170 IU of Vitamin E/kg, 827 g of Monensin/ metric ton (Elanco Animal Health, Greenfield, IN), and 165 g of Tylosin/metric ton (Elanco Animal Health).
[2] Dry Matter
[3] Crude protein
[4] Acid detergent fiber
[5] Neutral detergent fiber
[6] Ether extract
[7] Net energy for gain

Table 3.2 Composition of finishing diet (dry matter basis) §.

Ingredient	Finishing diet fed from d 15 to 126
Dry-rolled corn	53.18
Modified distillers grains plus solubles	19.93
Corn silage	23.10
Suspended supplement[1]	3.79
Analyzed nutrient composition	
DM[2, 9], % as fed	59.97
CP[3, 9], %	13.41
NPN[4], %	1.35
ADF[5,9], %	10.11
NDF[6,9], %	20.85
Ash[9], %	5.17
EE[7,9], %	3.91
Neg[8,10], Mcal/kg	1.37
Calcium, %	0.66
Magnesium, %	0.25
Phosphorus, %	0.46
Potassium, %	0.96
Sulfur, %	0.20
Cobalt, mg/kg	0.50
Copper, mg/kg	12.00
Iron, mg/kg	109
Manganese, mg/kg	40.00
Selenium, mg/kg	0.5
Zinc, mg/kg	106

[1]Contained on a DM basis: 30, 844 IU of Vitamin A/kg, 170 IU of Vitamin E/kg, 827 g of Monensin/ metric ton (Elanco Animal Health, Greenfield, IN), and 165 g of Tylosin/metric ton (Elanco Animal Health).
[2] Dry Matter

[3] Crude protein
[4] Non-protein nitrogen, CP equivalent.
[5] Acid detergent fiber
[6] Neutral detergent fiber
[7] Ether extract
[8] Net energy for gain
[9] Weekly ingredient samples were stored in a freezer at -20° C until nutrient analyses were completed. After weekly DM determination (method no. 935.29), weekly samples from each ingredient were composited by month and analyzed for N (method no. 968.06; Rapid Max N Exceed; Elementar; Mt. Laurel, NJ), and ash (method no. 942.05) content (AOAC, 2012, 2016). Modified distillers grains plus solubles samples were analyzed for ether extract content using an Ankom Fat Extractor (XT10; Ankom Technology, Macedon, NY). Percentages of ADF and NDF were assumed to be 3 and 9 percent for dry-rolled corn (Preston, 2016). Analysis of ADF and NDF composition for all other feeds was conducted as described by Goering and Van Soest et al. (1970). Diets presented her are actual DM diet composition, actual nutrient concentrations, and tabular energy values (*P*reston, 2016).
§All steers received 300 mg/hd/d ractopamine hydrochloride for the final 28 d on feed.

Table 3.3. Influence of RPBV on finishing steer growth performance, efficiency of dietary net energy utilization.

	RPBV, g/hd·d^{-1}				*P*-value		
Item	0	1	2	SEM	Con vs RPBV	Linear	Quadratic
Pens, n	8	8	8	-	-	-	-
Steers, n	82	80	79	-	-	-	-
Initial BW[1], kg	412	411	412	-	-	-	-
Final BW[1], kg	651	655	655	4.2	0.32	0.43	0.54
ADG, kg	1.90	1.94	1.92	0.034	0.31	0.52	0.34
DMI, kg	12.01	12.27	12.21	0.168	0.13	0.25	0.27
G:F	0.158	0.158	0.158	0.0022	0.78	0.72	0.94
Carcass Adjusted (HCW/0.625)							
Final BW, kg	675	677	677	6.2	0.64	0.68	0.84
ADG, kg	2.09	2.11	2.10	0.044	0.61	0.75	0.65
G:F	0.174	0.172	0.172	0.0030	0.59	0.64	0.79
Live-basis Performance-adjusted Net Energy (NE), Mcal/kg							
Maintenance	2.00	2.00	1.99	0.018	0.90	0.79	0.83
Gain	1.35	1.35	1.34	0.016	0.90	0.79	0.83
Observed-to-expected NE							
Maintenance	0.99	0.99	0.99	0.009	0.90	0.79	0.83
Gain	0.99	0.99	0.99	0.012	0.90	0.79	0.83

[1] Body weight (BW) was pencil shrunk 4% to account for digestive tract fill.

Table 3.4. Effects of RPBV on carcass traits of finishing beef steers.

	RPBV, g/hd·d^{-1}				*P*-value		
Item	0	1	2	SEM	Con vs RPBV	Linear	Quadratic
Pens, n	8	8	8	-	-	-	-
Steers, n	82	80	79	-	-	-	-
HCW, kg	422	423	424	3.9	0.64	0.68	0.84
DP	64.74	64.58	64.66	0.342	0.70	0.82	0.70
RF, cm	1.32	1.42	1.40	0.051	0.11	0.28	0.15
REA, cm^2	90.2	87.0	89.3	0.929	0.08	0.47	0.02
Marbling[3]	509	508	516	16.7	0.85	0.70	0.76
KPH, %	1.79	1.81	1.81	0.029	0.49	0.51	0.81
YG	3.22	3.50	3.35	0.087	0.02	0.19	0.01
Retail Yield	50.04	49.46	49.78	0.187	0.02	0.18	0.01
EBF[4], %	31.08	31.77	31.50	0.306	0.06	0.19	0.09
AFBW, kg	610	601	606	6.6	0.27	0.53	0.27

[1]Dressing percentage was calculated as HCW/(final BW × 0.96).

[2]Proportion of closely trimmed boneless retail cuts from carcass round, loin, rib, and chuck was be determined according to the equation described by (Murphey et al., 1960).

[3] 400=small^{00}

[4] Estimated empty body fat (EBF) percentage and AFBW was calculated from observed carcass traits (Guiroy et al., 2002).

Table 3.5. Effects of RPBV on overall distribution of yield and quality grade measurements and liver scores.

	RPBV, g/hd·d^{-1}				*P*-value		
Item	0	1	2	SEM	Con vs RPBV	Linear	Quadratic
Pens, n	8	8	8	-	-	-	-
Steers, n	82	80	79	-	-	-	-
Yield Grade, %							
1	1.2	1.3	1.3		0.01		
2	35.4	15.2	25.36				
3	50.0	60.8	59.5				
4	13.4	21.5	12.6				
5	0.0	1.3	1.3				
Quality Grade, %							
Select	11.0	14.29	9.0		0.53		
Choice	35.4	35.1	38.5				
Avg Choice	41.5	35.1	35.9				
Upper Choice	8.5	11.7	15.4				
Prime	3.7	3.9	1.3				
Liver Scores[1], %							
Normal	89.0	89.9	79.8		0.13		
A-	7.3	5.1	12.7				
A	0.0	0.0	1.3				
A+	3.7	5.1	6.3				

[1] Liver abscess prevalence and severity was be determined by a trained technician using the Elanco system as Normal (no abscesses), A- (1 or 2 small abscesses or abscess scars), A (2 to 4 well organized abscesses less than 2.54 cm. diameter), or A+ (1 or more large active abscesses greater than 2.54 cm. diameter with inflammation of surrounding tissue).