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Bedding Application and Increasing Dosage of Trenbolone Acetate and Estradiol in Implants for Beef Steers: Influence on Growth Performance, Carcass Characteristics, and Circulating Metabolite Responses

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BEDDING APPLICATION AND INCREASING DOSAGE OF TRENBOLONE ACETATE AND ESTRADIOL IN IMPLANTS FOR BEEF STEERS: INFLUENCE ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, AND CIRCULATING METABOLITE RESPONSES

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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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ABBREVIATIONS

ADF	acid detergent fiber
ADG	average daily gain
AMPK-α	adenosine monophosphate-activated protein kinase-α
AOAC	Association of Official Analytical Chemist
BED	treatment, bedding applied
BMR	basal metabolic rate
BRSV	respiratory syncytial virus
BVD	bovine viral diarrhea
BW	body weight
°C	degrees Celsius
cAMP	cyclic adenosine monophosphate
C/EBPβ	CCAAT-enhancer-binding protein-β
CH	treatment, Synovex Choice
CH_4	methane
CNES	California net energy system
CO_2	carbon dioxide
CP	crude protein
d	day
DAG	diacylglycerol
dL	deciliter
DM	dry matter
DMI	dry matter intake

DOF	days on feed
DP	dressing percentage
EB	estradiol benzoate
EBF	empty body fat
E_2	estradiol-17β
EE	ether extract
EG	daily energy gain
EM	maintenance energy
FBW	final BW
FFG	feed available for gain
FFM	feed available for maintenance
FHP	fasting heat production
g	grams
G:F	gain to feed
GH	growth hormone
GH	grass hay (appendix A)
GHRH	growth hormone-releasing hormone
GPER-1	G protein-coupled estrogen receptor 1
GPR	G protein-coupled receptors
GPR41	G protein-coupled receptor 41
GPR43	G protein-coupled receptor 43
h	hour
HCW	hot carcass weight

HCl	hydrochloric acid
HE	heat energy
IBR	bovine rhinotracheitis
IGFBP	insulin-like growth factor binding proteins
IGF-I	insulin-like growth factor I
IP3	inositol trisphosphate
K	degrees Kelvin
Kg	kilogram
k_g	partial efficiency of energy for gain
k_m	partial efficiency of energy for maintenance
LTc	lower critical temperature
LWG	live weight gain
M	molarity
Mcal	megacalorie
ME	metabolizable energy
mEq	milliequivalents per liter
mg	milligram
ml	milliliter
mm	millimeter
MQ	estimated maintenance coefficient
mRNA	messenger ribonucleic acid
N	nitrogen
NASEM	National Academies of Sciences, Engineering, and Medicine

NDF	neutral detergent fiber
NEFA	non-esterified fatty acid
NE	net energy
NE_g	net energy for gain
NE_m	net energy for maintenance
NI	treatment, no implant
nm	nanometer
NO	treatment, no bedding applied
O_2	oxygen
pa	performance adjusted
pg	picogram
PI_3	parainfluenza-3
PL	treatment, Synovex Plus
PPARγ	peroxisome proliferator-activated receptor gamma
PSPS	Penn State Particle Separator
RE	retained energy
REA	ribeye area
RF	ribfat
RNC	Ruminant Nutrition Center in Brookings, South Dakota
RY	retail yield
SCD	stearoyl CoA desaturase
SUN	sera urea nitrogen
TBA	trenbolone acetate

TbOH	trenbolone
TMR	total mixed ration
USDA	United States Department of Agriculture
UTc	upper critical temperature
W	weight (kg)
YG	calculated yield grade
µL	microliter

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ABSTRACT

BEDDING APPLICATION AND INCREASING DOSAGE OF TRENBOLONE ACETATE AND ESTRADIOL IN IMPLANTS FOR BEEF STEERS: INFLUENCE ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, AND CIRCULATING METABOLITE RESPONSES

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Three randomized complete block design feedlot experiments were conducted over the course of two years. Two experiments were conducted to investigate the effect of bedding use in confined beef steers. The third experiment evaluated the effects of implants containing increasing doses of trenbolone acetate (TBA) and estradiol benzoate (EB) in confined beef steers. Experiment 1 used Simmental × Angus steers (n = 240; initial body weight (BW) = 365 ± 22.5 kg). Experiment 2 used newly weaned Charolais × Red Angus steers (n = 162; initial BW = 278 ± 13.4 kg). Steers were allotted to 1 of 2 treatments: 1) no bedding (NO), or 2) 1.8 kg (Exp. 1) or 1.0 kg (Exp. 2) of wheat straw (as-is basis) bedding/steer·d^{-1} (BED). In Exp. 1 and Exp. 2 data were analyzed as a randomized complete block design with pen serving as the experimental unit for all analyses. In Exp.1, applying bedding improved ($P \leq 0.01$) dry matter intake (DMI), gain:feed (G:F), and average daily gain (ADG). Bedding reduced ($P = 0.01$) the estimated maintenance coefficient (MQ). Dressing percentage, rib fat, marbling, and yield grade were increased ($P \leq 0.03$) in NO. Bedding resulted in an increase ($P = 0.01$) in serum insulin-like growth factor I (IGF-I). In Exp. 2, a tendency ($P = 0.06$) for increased DMI for NO was noted. Bedding improved G:F ($P = 0.01$). MQ was elevated ($P = 0.03$) for

NO and NO had an increase ($P = 0.02$) in serum concentration of urea-N (SUN). An increase ($P = 0.01$) in serum non-esterified fatty acid was noted for NO. These data indicate that bedding application should be considered to improve growth performance and feed efficiency by reducing maintenance energy requirements in beef steers during the feedlot receiving and finishing phase. In experiment 3, yearling Simmental × Angus crossbred beef steers (n = 240; allotment BW = 365 ± 22.5 kg) from a South Dakota auction facility were transported 117 km to Brookings, SD and used in a randomized complete block design feedlot study to evaluate the effects of implants (both from Zoetis, Parsippany, NJ) containing increasing doses of TBA and EB administered 124 d prior to harvest have on finishing phase growth performance, carcass characteristics, and serum concentrations of urea-N (SUN) and insulin-like growth factor I (IGF-I). Thirty pens (10 pens/treatment) were assigned to 1 of 3 treatments: 1) negative control given no implant (NI); 2) a steroidal implant containing 100 mg TBA and 14 mg EB administered subcutaneously in the center one-third of the ear on d 1 (Synovex Choice, Zoetis, Parsippany, NJ; CH); 3) a steroidal implant containing 200 mg TBA and 28 mg EB administered subcutaneously in the center one-third of the ear on d 1 (Synovex Plus, Zoetis; PL). Cattle were fed for 124 d post-implantation. Steers were fed a common diet throughout the study. Treatment effects were evaluated by the use of orthogonal polynomials. Pen was the experimental unit for all analyses; an α of 0.05 determined significance. There was a quadratic effect ($P = 0.01$) on carcass adjusted final BW. Increasing doses of TBA and EB resulted in a linear increase for both ADG ($P = 0.01$) and DMI ($P = 0.02$). A quadratic effect on G:F was observed ($P = 0.01$). No quadratic ($P \geq 0.40$) or linear ($P \geq 0.14$) effects were observed for dressing percentage, rib fat (RF),

calculated yield grade, or marbling scores. A quadratic increase ($P = 0.01$) in hot carcass weight (HCW) and a linear increase ($P = 0.01$) in ribeye area (REA) was detected. No significant implant × day interaction ($P \geq 0.09$) was noted for serum concentrations of urea-N or IGF-I. Implants decreased ($P = 0.01$) circulating SUN compared to NI. Serum concentration of IGF-I was increased ($P = 0.04$) in implanted steers compared to NI steers. In yearling crossbred beef steers the use of steroidal implants containing a combination of 100 mg TBA + 14 mg EB or 200 mg TBA + 28 mg EB increases growth performance, HCW, and REA at equal RF accumulation without detriment to marbling score compared to non-implanted steers.

Key words: bedding, estradiol, implant, maintenance coefficient, trenbolone acetate

CHAPTER I: REVIEW OF LITERATURE

INTRODUCTION

A sizeable portion of cattle on feed in the United States are fed in the Upper Midwest and Northern Plains region where temperatures routinely fall below freezing during late fall, winter, and early spring. The persistent cold temperatures coupled with snow accumulation, wind, moisture and ice can cause undesirable pen conditions for confined cattle, ultimately resulting in decreased insulative capacity of cattle hair coat as a result of dampness and mud or manure accumulation. For cattle, the insulative capacity of the haircoat is an important factor related to their lower critical temperature (LTc) threshold. The LTc for homeotherms is the temperature below which the organism's metabolic rate must increase in order to maintain homeostasis (Young, 1983). Using bedding to improve cattle comfort and growth performance is a common practice used in livestock production. However, the exact degree to which bedding improves growth performance is difficult to quantify. Previous work related to effects of bedding application and housing techniques (Birkelo and Lounsberry, 1992; Stanton et al., 1994b; Anderson et al., 2006; Mader and Colgan, 2007) on beef cattle performance has provided inconclusive with regard to animal growth performance and carcass characteristics. Thus, during winter months, understanding the amelioration in maintenance requirement as a result of bedding application is crucial as it may allow for more accurate tracking and growth performance prediction in beef cattle.

Steroidal implants containing trenbolone acetate (TBA) and estradiol-17β (E_2) have been used in commercial beef production in the United States to capture economic advantages, when compared to non-implanted cattle, for over 63 yr and remain one of the

most cost-effective technologies that can be used in beef production systems. Steroidal implants can be expected to improve average daily gain (ADG) 10 to 30%, feed efficiency 5 to 15%, and carcass leanness 5 to 8% (Preston, 1999). Combination TBA + E_2 implants of differing doses are commonly used in beef cattle production. Hermesmeyer et al. (2000) found that steers implanted with either an implant containing 120 mg TBA + 24 mg E_2 or an implant containing 200 mg TBA + 20 mg E_2 and fed to a target rib fat depth of 1.4 cm had improved live weight gains, heavier hot carcass weights (HCW), and greater ribeye area (REA) compared to non-implanted steers. However, along with large improvements in growth performance, the effect of steroidal implant on marbling score has often been shown to be negative (Herschler et al., 1995; Duckett et al., 1997; Johnson and Beckett, 2014; Smith et al., 2018). Bruns et al. (2005) suggested that combination TBA + E_2 steroidal implants administered during early periods of growth may adversely impact the development of marbling in steers. The safety and efficacy of combination TBA + E_2 implants has been proven (Preston, 1999) and further investigation into the effects of combination TBA + E_2 implant dose on beef cattle growth performance and effects on carcass performance is warranted.

BEDDING APPLICATION

Brief history of nutritional energetics

Nutritional energetics relating to animals and man can be traced back to Lavosier during the 1700's, who determined that life is essentially a complex combustion reaction and also established the early relationships between O_2 and CO_2 in the combustion process (Kleiber, 1961). Researchers such as Henry Armsby at Pennsylvania State University, Wilbur Atwater who was the director of the first United States Agricultural

Experiment Station at Wesleyan University, Oskar Kellner of the German Agricultural Experiment Station, Max Rubner at the University of Marburg and the University of Berlin, Samual Brody at University of Missouri, Max Kleiber, William Garrett, and Glen Lofgreen also at the University of California - Davis, and Sir Kenneth Blaxter of Great Britain continued to provide novel insights and concepts that would eventually evolve into the modern net energy system currently used in beef cattle production.

The laws of thermodynamics, discovered in the 1840's, are the foundation on which the structure of nutritional energetics reside. The first law of thermodynamics is known as the law of conservation of energy. This law states that energy can neither be created nor destroyed. This law is of vital importance when making calculations related to animal nutrition. This law undergirds the assumption that ME = RE + HE, where ME = metabolizable energy, this is energy available to the animal not excreted in gas, urine, or feces; RE = retained energy, energy retained in animal tissue or product; HE = heat energy, heat energy released by the animal (NASEM, 2016). Heat energy can be divided into basal metabolism, heat of activity, formation of products and waste, digestion and absorption, and body temperature regulation (Ferrell and Oltjen, 2008). The complexity pertaining to partitioning these subcategories of heat production into meaningful metabolic processes provides great difficulty. The second law of thermodynamics, better known as the law of Hess, states that the total amount of heat released or produced is independent of the path by which this chemical change is brought about. For example, the law of Hess holds that the amount of heat generated from 1-g of carbohydrate being oxidized completely in an adiabatic bomb calorimeter, is the same as the total heat generated from 1-g of carbohydrate being oxidized completely after being consumed by

an animal. The final law of thermodynamics holds that a system's entropy approaches a constant value as the temperature approaches absolute zero (0°K). The law of conservation of energy and the law of Hess are fundamental for nearly all calculations related to animal energetics. Direct calorimetry, through the principles of the laws of thermodynamics, allowed for researchers such as Atwater, Armsby, Blaxter, and others to directly measure heat produced by the animal (Ferrell and Oltjen, 2008). Other researchers such as Armsby, Atwater, Kellner and Rubner used open and closed-circuit calorimeters to measure heat or gas production. Perhaps the largest development made in calorimetry occurred upon the development of the Brouwer equation in 1965 (Brouwer, 1965) which allowed researchers to calculate heat production from O_2 consumption, CO_2 and CH_4 production, and urinary N.

Researchers developed energy systems by investigating the effect of different feeds on energy expenditure to better quantify energy values of feedstuffs. Among these early systems were Kellner's starch equivalent system (Kellner and Goodwin, 1909), Atwater's physiological fuel values system (Atwater, 1900), and Armsby-Forbes net energy system. Ultimately building upon the body of calorimetry and net energy work conducted in the past, as well as the principles of the laws of thermodynamics, the California Net Energy System (CNES) was developed by Lofgreen and Garrett (1968). The CNES is currently the basis for systems included in the modern revisions of the NRC (NRC, 1984, 1996, 2016). The CNES was the first system based on RE in the carcass. Lofgreen and Garrett (1968) measured RE in the carcass using the comparative slaughter method and HE was estimated by deducting energy retained from ME intake.

The CNES was the first energy system that quantified the partial efficiency of ME use for maintenance functions (k_m) and the partial efficiency of ME use for gain or productive functions (k_g). The relationship for these partial efficiencies allows n et energy for maintenance (NE_m) and net energy for gain (NE_g) to be quantified; $NE_m = k_m \times ME$, $NE_g = k_g \times ME$ (Ferrell and Oltjen, 2008). The CNES was the first system to assign two net energy values to each feedstuff and in doing so overcame limitations of previously mentioned earlier systems such as Kellner's starch equivalent system (Kellner and Goodwin, 1909), Atwater's physiological fuel values system (Atwater, 1900), and Armsby-Forbes net energy system. Kellner's starch equivalent system that was based on the NE values of feeds for fattening, was the most widely used example of an early system based on NE concepts. The principle limitation being that the CNES overcame was the differing relative efficiencies of feedstuffs when used for maintenance or for gain. In previous systems, forage was undervalued relative to corn or starch when used for maintenance purposes. Suleiman and Mathison (1979), demonstrated that steers appeared to use the digestible energy from wheat straw with efficiencies comparable to that from all-concentrate diets when energy intakes were slightly greater than maintenance.

Cold environment effect on maintenance energy requirements

Maintenance can be defined as the state in which there is no net gain nor loss of energy from the body. Within this, the maintenance energy requirement of the animal can be further defined to the amounts of energy necessary to achieve and maintain an equilibrium state (Young, 1983). This would include the cost of any minimal muscular activities necessary to consume and process the required number of calories. Lofgreen

and Garrett (1968), determined the maintenance energy requirement of beef animals to be $0.077W^{0.75}$ where NE_m is in Mcal per day and W = bodyweight in kg. However, the CNES was developed in a thermoneutral environment and so the system itself was not initially created to be dynamic in terms of adaptation to adverse environmental conditions and other potential factors affecting input variables. Although cattle were not actually fed at zero feed intake, to determine the NEm requirements for growing and finishing beef cattle, Lofgreen and Garrett (1968), assumed that at zero feed intake, heat increment, which is associated with digestion of feedstuffs and absorption of resulting substrate, is equal to zero and thus the remaining components of heat production are simply basal metabolism and heat associated with activity which can then be considered to be equal to the NE_m.

Basal metabolism or basal metabolic rate (BMR) can be defined as the minimal rate of heat production from the fasted and rested animal when the environmental ambient temperature is within the range of upper critical temperature (UTc) and LTc (Kleiber, 1961; Blaxter, 1989). The LTc can vary based on a number of factors related to insulative capacity of hair coat and intake level. The LTc for cattle with 8 mm hair and and ad libitum feed intake is -1°C, while a cow with the same hair coat in a fasted state has an LTc of 18°C (NRC, 1981). Basal metabolic rate, when determined in man, is measured when the subject is in a post-absorptive state (~12-hr fast), laying down in complete muscular relaxation, and in a thermoneutral environment. Animals provide difficulty when attempting to accurately determine BMR as they cannot be made to stay completely still in a fasted state for measurement. As such, the fasting metabolic rate, or fasting heat production (FHP), is what is usually measured in animals (Blaxter, 1989).

Fasting heat production includes heat from voluntary activity of the animal that would be mostly mitigated by muscular relaxation. Basal metabolic rate and FHP will be treated as interchangeable from herein. Basal metabolic rate can be affected by several factors such as previous plane of nutrition, sex, age, body condition score, genetics, stage of production, and environmental conditions. If the ambient temperature is below the LTc for a homeotherms, then the organism's metabolic rate must increase in order to maintain homeothermy (Young, 1983). Prolonged exposure to cold environments can have a marked impact on the energy required for maintenance in beef cattle. This increase in maintenance energy required by the beef animal is a result of increased basal metabolic intensity to manage increasing heat production demands to maintain homeothermy during prolonged exposure to temperatures below the animal's LTc. This is not simply an acute response in basal metabolism but is instead indicative of metabolic adaptation to cold (Young, 1981). Robinson et al. (1986) conducted a study in which treatment groups of four Hereford × Red Angus yearling steers were adapted to a different environmental temperature for a period of 4 months and then heat production and other measures were assessed for a 2 month period. The three temperature treatments that cattle were acclimated to included cold (3°C), thermoneutrality (20°C), and heat (35°C). Robinson et al. (1986) concluded that heat production for cattle adapted to the colder temperature (3 C°) was greater than the heat production of cattle adapted in the thermoneutral temperature. In a similar study, Boyles et al. (1991), housed crossbred steers with an initial weight of 257 kg in environmental chambers that were acclimated to three temperature treatments (0°C, 5°C, and 15°C) for a 7 day period and then a subsequent 28 day experimental period followed. Heat production for cattle exposed to 0°C and 5°C

treatments had increases in heat production of 15 and 23%, respectively, compared to 15°C treatment. It is of interest that a linear increase in heat production did not occur as temperature decreased. Instead, a tendency was noted for cattle exposed to 5°C to have greater heat production when compared to the 0°C treatment. In this study, two of the treatment groups were exposed to the 5°C treatment and were then rotated to the 0°C. The reduced heat production for the 0°C group indicates that acclimation occurred when exposed to 5°C. Researchers Delfino and Mathison (1991) conducted a an experiment where Hereford and Hereford-cross yearling steers with initial body weight (BW) of 340 kg were fed all concentrate diets in either an indoor temperature controlled environment with no bedding, or outdoors with wood shavings for bedding from January to April. The mean temperatures for indoor and outdoor locations were 16.9 ± 2.7°C and -7.6 ± 6.8°C. It was reported that steers housed outdoors retained 65% less energy and had an 18% increase in FHP. Housing steers outdoors resulted in a 41% increase in ME use for maintenance compared to steers housed indoors.

Effect of bedding application on cattle performance

The geographical location of a cattle feeding operation dictates the environmental conditions and challenges that will be encountered. Cattle fed in the southern United States and High Plains region deal with persistent high temperatures and dry, dusty pen conditions. Cattle fed in the upper Midwest experience mild temperatures during late spring and summer months, however during late fall, winter, and early spring, persistent cold temperatures coupled with snow accumulation, wind, and ice can cause undesirable pen conditions for cattle. Undesirable pen conditions can result in decreased insulative capacity of the cattle hair coat as the result of dampness and mud or manure

accumulation. For cattle, the insulative capacity of the haircoat is an important factor related to their LTc threshold (Wagner et al., 2008). Total insulation can be described as a function of tissue insulation (subcutaneous fat and hide), coat insulation (hair coat), and air insulation (Blaxter, 1989). Mud, moisture, and wind can compromise the insulative capacity of the hair coat thus allowing for both acute and persistent increases in heat loss.

A limited amount of work has been done to directly investigate the effects of bedding application on feedlot cattle growth performance and, specifically, the resulting alterations in energetic demand. Results have been variable with regards to feedlot growth performance and carcass characteristics. The observed inconsistency in performance response to bedding application is likely related to several external factors that play a crucial role in the outcome of performance results. These factors include ambient temperature, wind, precipitation, pen size, stocking density, condition of hair coat, and age of animal among other things. This is of importance, as modern tracking systems used to predict cattle performance rely on two previously discussed requirements of the beef animal, NE_m and NE_g (Lofgreen and Garrett, 1968) Thus, during winter months, understanding the alteration in basal metabolic rate and thus net energy required for maintenance is crucial as it is directly correlated to feed available for gain (FFG) and may allow for more accurate tracking and performance prediction. This principle has been demonstrated in several previous studies dealing with bedding application and cold environments.

Following severe winter storms in Colorado, Wagner et al. (2008) conducted a post-hoc analysis that investigated the effect of severe winter weather on net energy for maintenance required by yearling steers. The average temperature experienced by steers

included in the post-hoc analysis ranging from December 26, 2006, through February 22, 2007, was -8.43°C. Average temperature was calculated from the average of the daily high and daily low temperatures during the period. Data indicated that NE_m required by cattle during and in the aftermath of a major winter weather event may be 2.5 times higher than NE_m required under standard thermoneutral feeding conditions. Pastoor et al. (2012) found that metabolic requirements were reduced, and comfort was likely improved in cattle fed in bedded confinement housing compared to open lots.

Anderson et al. (2006), using preconditioned steer calves with an initial BW of 329 kg, investigated the effects of bedding level on beef steer growth performance and carcass characteristics. Wheat straw bedding level treatments included no bedding, modest bedding, and generous bedding, which was simply 2× the amount of the "modest" bedding treatment. The modest bedding treatment was applied on a subjective judgment basis to keep bedding available for steers to lay on. It was reported that during winter months both modest and generous amounts of bedding applied during the initial phase of the feeding period resulted in an approximately 20% increase in ADG. Birkelo and Lounsberry (1992) used crossbred beef steers with an initial BW of 265 kg to evaluate the effect of oat straw and newspaper bedding as well as housing system in a trial ranging from November through May where the average temperature was approximately 1°C. Bedding was applied every 3 to 10 days to maintain a dry spot large enough for all steers to lay down at one time. The reported improvement in ADG as a result of bedding application regardless of bedding type was 8.3%. Stanton et al. (1994b) used both steers and heifers with an initial BW of 370 kg to evaluate the effects of wheat straw bedding application on cattle growth performance and carcass characteristics. The study began in

January, bedding was applied 10 times throughout the study at a rate of 2.1 kg/steer·d^{-1}, and the average temperature during the study was approximately 5.5°C. Stanton et al. (1994b) reported a 5.3% increase in ADG as a result of bedding application. Mader and Colgan (2007) conducted two trials beginning in mid-December using crossbred beef steers to evaluate the effect of oat straw bedding application, pen stocking density, and facility type. In both trials, bedding was applied at a rate of approximately 1 kg/steer·d^{-1}. Trials 1 and 2 used crossbred beef steers with initial BW of 373 and 400 kg, respectively, and average temperature during both trials was approximately 0°C. However, in contrast to the previously discussed studies, it was reported that bedding application, in both trials 1 and 2, did not cause a significant response in ADG. In some previous work, during winter and spring months, final BW was increased in bedded treatments compared to non-bedded controls when cattle were marketed at equal days on feed (Birkelo and Lounsberry, 1992; Anderson et al., 2006). This is attributed to the mathematical relationship between dietary intake energy, energy required for maintenance, and the resulting proportion of intake energy that is ultimately available to be used for gain or productive function. Bedded steers, due to decreased maintenance energy requirements, likely had a greater proportion of intake energy available for gain, thus when cattle were harvested at equal days, bedded cattle had greater final BW.

Cold temperatures are known to stimulate appetite as a mechanism to cope with the concurrent increase in metabolic demand of the animal (NRC, 1987). Interestingly, previous work conducted regarding the effects of bedding on feedlot growth performance during winter months did not report any differences in DMI as a result of bedding application (Birkelo and Lounsberry, 1992; Stanton et al., 1994b; Anderson et al., 2006;

Mader and Colgan, 2007). A common physiological reaction of ruminants, in addition to increased intake when exposed to cold stress, has been shown to be increased reticulorumen motility and rate of passage of digesta (Westra and Christopherson, 1976). Westra and Christopherson (1976) exposed shorn lambs to treatment temperatures of 21.2 and 1.3°C for 4 to 6 weeks and observed that the mean number of reticulum contractions per hour was increased 21% for sheep exposed to 1.3 C°. The physiological response of increased digesta flow, along with increased rate of basal metabolism, may account for the observed disparity in feed efficiency observed in some previous publications. Several previous studies have reported improved feed efficiency as a result of bedding application (Birkelo and Lounsberry, 1992; Anderson et al., 2006; Mader and Colgan, 2007). The degree to which bedding application affects feed efficiency may be largely dependent on numerous environmental factors.

Effects of bedding application on Carcass Characteristics

As cattle are subjected to cold stress, dietary energy is diverted towards maintenance function. Bedding application, shelterbelts, wind fence, and sheltered housing facilities have been shown to mitigate negative effects of a cold environment that are responsible for increases in required energy for maintenance. It can be expected that in addition to bedding application altering live growth performance, it may impact carcass characteristics as well. Anderson et al. (2006), evaluated effects of bedding level on feedlot cattle performance, reported that “generous” bedding level improved HCW in bedded pens for cattle fed for equal days. However, in previous work, other authors (Stanton et al., 1994b; Mader and Colgan, 2007) reported no effect on HCW for beef cattle fed for equal days. Anderson et al. (2006) reported a 5% increase in REA for

bedded steers compared to non-bedded steers fed for equal days. Limited additional data is available reporting the effect of bedding application on REA in beef steers. Mader and Colgan (2007) reported that bedding did not cause a significant response in dressing percentage in either of their two trials. However, other studies (Stanton et al., 1994b; Anderson et al., 2006) reported that bedded treatments had improved dressing percentages compared to non-bedded cattle. Anderson et al. (2006) reported no difference in RF as a result of bedding application. Mader and Colgan (2007) reported no difference in marbling score as a result of bedding application in both bedding trials. In an initial trial, Anderson et al. (2006) reported an improvement in marbling score favoring bedded cattle, however, in the following trial, no effect on marbling score was observed. Differences in USDA marbling score in bedded vs. non-bedded cattle could potentially be related to the relationship between NE_m and NE_g; as maintenance requirements increase, feed available for gain subsequently decreases unless this disparity is compensated for in the form of increased intake. Garrett (1980) also stated that the composition of gain appears to an important factor affecting k_g, thus, differences in growth rates resulting from bedding application would likely affect composition of gain.

STEROIDAL IMPLANTS

Steroidal implant history and performance responses

Steroidal implants have been used in U.S. commercial beef production to capture economic advantages over non-implanted cattle for over 63 y and remain one of the most cost-effective technologies that can be used in beef production systems. A steroidal implant is administered subcutaneously in the back of the ear in cattle using an implant needle and applicator; most implants consist of small compressed pellets containing a

high concentration of steroid compound and other non-active ingredient that acts as a carrier like lactose, cholesterol, silastic rubber, or polyethylene-glycol polymers. After administration, the implanted pellets will begin to dissolve slowly, thus releasing steroid hormones that are then released into the blood stream and transported to economically relevant target tissues such as skeletal muscle and adipose tissue, and other target tissues including the liver and bone (Johnson and Beckett, 2014). The three major categories of steroid hormone included in the implant are: androgens, estrogens, and progestins. The active compound in a first generation, non-coated, steroidal implant is released from the carrier over a period of approximately 60 to 120 days (Mader, 1998; Smith et al., 2018). This period of release is often referred to as implant "payout". Effectively, steroidal implants increase the frame size of the beef animal, thus increasing the body weight of the animal at a given level of chemical maturity (i.e. delay fattening) by way of promoting deposition of lean tissue rather than fat compared to non-implanted cattle (Preston, 1999; Guiroy et al., 2002). Implanting during the feedlot phase on average increases growth rate 10 to 30%, feed efficiency 5 to 15%, and carcass leanness 5 to 8% compared with non-implanted cattle (Preston, 1999). Additionally, Duckett and Pratt (2014) reported that administration of a steroidal implant during the finishing phase increases feed intake 6%, carcass weight 5%, and ribeye area 4% when compared with non-implanted cattle. Use of a high-potency steroidal implant can improve the final weight of an animal by 70-kg compared to a non-implanted animal (NASEM, 2016).

Postnatal skeletal muscle growth

Skeletal muscle tissue is one of the key economically relevant tissues when discussing livestock production. The number of muscle fibers in an animal is fixed at

birth and that total number cannot be changed during post-natal growth. Thus, post-natal skeletal muscle growth does not occur by way of hyperplastic growth, which would involve an increase in the number of muscle cells via proliferation. As such, post-natal increase in lean tissue mass occurs via hypertrophy, which is the enlargement of existing muscle fibers. In mammals, the muscle fiber unit in the body is a large multinucleated cell. Mammalian hypertrophic growth of skeletal muscle is supported by the addition of new nuclei to the multinucleated muscle fiber (Moss and Leblond, 1971). Accumulation of lean tissue relies on an increase in protein synthesis and a decrease in protein catabolism, thus increasing net protein synthesis. Skeletal muscle is a dynamic tissue in that it is constantly in flux as protein is constantly being synthesized and degraded. The synthesis and degradation of peptide bonds accounts for a substantial amount of maintenance energy requirements in animals. McCarthy et al. (1983) demonstrated that fractional synthesis and fractional breakdown of muscle protein does not differ between cattle of different mature sizes even from very different genetic bases. McCarthy et al. (1983) also determined that muscle tissue growth relies more heavily on rate of synthesis under normal conditions, and that with age synthesis decreases more rapidly than protein breakdown.

Biological response to steroidal implant

The estrogenic constituent of steroidal implants is thought to exert its effect on lean tissue accretion in an indirect manner via the somatotropic axis. This results in increased release of hepatic somatotropin and insulin-like growth factor I (IGF-I) (Johnson et al., 1996b; Reinhardt, 2007). These resulting secondary hormones promote muscle protein accretion. Circulating growth hormone (GH) acts on the liver to promote

expression of the IGF-I, substantially increasing the circulating concentration of IGF-I (Florini et al., 1996). The androgenic constituent of steroidal implants acts directly on muscle tissue local production of IGF-I in skeletal muscle, stimulating protein synthesis and reducing muscle catabolism. Increased local IGF-I production was noted in steers implanted with a combination TBA + E_2 implant through measurement of concentration of IGF-I mRNA in the longissimus muscle of (Johnson et al., 1998; Parr et al., 2014). Local IGF-I is critical for the recruitment of satellite cells needed in order to support postnatal skeletal muscle hypertrophy. Skeletal muscle hypertrophy requires an increase in the number of myonuclei present in the individual fibers. However, the nuclei in muscle fibers are unable to divide and so the additional nuclei must be recruited from an outside source. Bovine satellite cells provide the additional nuclei needed to support postnatal muscle fiber hypertrophy and are critical in determining the extent of muscle growth (Dayton and White, 2013).

Following implantation, the steroid hormones contained in the implant are released from the compressed pellet carrier into the bloodstream during the payout period. Once in circulation, the hormones are converted into their biologically active form. Estradiol benzoate (EB), which has approximately 71% the biological activity of E_2, is converted into E_2 and TBA is converted into trenbolone-17β (TbOH). Once converted into their biologically active form, the insoluble steroid then binds to specific carrier proteins in the blood, such as steroid binding globulins and albumin, for delivery to target tissues such as economically relevant target tissues such as skeletal muscle and adipose tissue, as well as other target tissues including the liver and bone (Johnson and Beckett, 2014). Currently, no conclusively proven mode of action for steroidal implants

is available. However, some mechanisms believed to be related to muscle tissue accretion following exposure to steroid hormones have been reported. Responses of steroid hormones on target tissues occur following ligand binding to a hormone receptor located in the cytosol of the cell with high affinity. Once ligand binding occurs, the ligand-receptor complex activates transcriptional activity in the nucleus of the target cell (Smith and Johnson, 2020). Transcription factors are instrumental in the growth processes of important tissues. For example the estrogen response element located on the growth hormone-releasing hormone (GHRH) gene in the hypothalamus and in skeletal muscle, an example is the androgen response element on the promoter region of the IGF-I gene (Smith and Johnson, 2020). The impact on bovine satellite cell recruitment and protein synthesis due to exposure to steroid hormones is also thought to be mediated through the nongenomic mechanisms of G protein-coupled receptors (GPR). Nearly all membrane bound steroid hormone receptors are members of this receptor super family. G protein-coupled receptors span the plasma membrane of the cell, and use secondary messenger systems to exert their influence, namely through cyclic adenosine monophosphate (cAMP) or inositol trisphosphate (IP3) and diacylglycerol (DAG). The secondary messengers are then capable of altering physiological responses in the target tissue. This occurs very rapidly, in a matter of seconds, compared to traditional nuclear hormone responses. The G- protein-coupled estrogen receptor 1 (GPER-1) has been identified in the endoplasmic reticulum of skeletal muscle and reportedly regulates the actions of E_2 in some cell types (Revankar et al., 2005). Work needs to be done to further elucidate specific mechanisms that underlie the effect steroidal implants have on tissue growth

Effect of steroidal implant dose

One may expect that as dose of steroidal implant increases, so does growth performance response. It has been shown in previous work that this relationship between dose and resulting performance response is not always correlated to steroidal dose. The relative growth performance responses when comparing differing implant doses may perhaps be attributable to other factors such as environment, bunk management, genetics, timing of implant, and duration of feeding, among many other things. Herschler et al., (1995) investigated single implants containing a combination of TBA and EB at two different ratios each at three different doses. No difference in cumulative ADG was noted in steers treated with a 5:1 TBA + E_2 ratio for all three TBA/EB treatment doses; 70:20, 140:40, or 210:60. For steers treated with a 10:1 TBA + E_2 ratio, similar cumulative ADG responses were noted at TBA/EB doses of 100:14 or 200:28; the 300:42 dose treatment had the greatest cumulative ADG and was similar to 200:28. In a meta-analysis, Reinhardt and Wagner (2014), noted that implanting with 200 mg TBA + 28 mg EB or 200 mg TBA + 20 mg E_2 did not result in a significant response for ADG, F:G, or HCW when compared to 120 mg TBA + 24 mg E_2. However, in another comparison from the same meta-analysis, ADG and HCW tended to be increased for the higher dose of 200 mg TBA + 20 mg E_2 (10:1 TBA + E_2 ratio) vs. 120 mg TBA + 24 mg E_2 (5:1 E_2 + TBA ratio). Parr et al. (2011) investigated dose of TBA and E_2 with doses of no implant applied, 120 mg of TBA + 24 mg of E_2 , or a partially coated implant containing 80 mg TBA + 16 mg E_2 (noncoated) and 120 mg TBA + 24 mg E_2 (coated) for a total of 200 mg of TBA + 40 mg of E_2. Implanting with the higher dose of E_2 resulted in a 6.0% increase in ADG and an 18 kg increase in final BW.

Steroidal implant effect on carcass characteristics

Steroidal implants delay fattening and increase lean tissue deposition and as such decrease the percentage of adipose tissue in the carcass when fed for an equal number of days. Steroidal implants increase HCW and REA compared to non-implanted cattle when harvested at equal RF thickness (Guiroy et al., 2002; Reinhardt, 2007; Parr et al., 2011). While steroidal implants consistently provide positive improvements in growth performance and feed efficiency, a long-standing concern regarding the use of high-potency combination TBA + E_2 implants on USDA quality grade remains. In several previous publications, the use of combination TBA + E_2 implants has been shown to decrease marbling score (Duckett et al., 1999; Pritchard, 2000; Bruns et al., 2005; Smith et al., 2018). Keeping in mind the negative effects on marbling score associated with steroidal implants, it is important to note that implants promote a greater proportion of lean tissue deposition relative to fat at a given bodyweight when compared to non-implanted cattle. Therefore, the resulting beef carcasses tend to be leaner, with less marbling when harvested at similar days-on-feed (DOF) as animals that have not received a steroidal implant. Therefore, in order to achieve the same degree of marbling, implanted cattle must be fed to a heavier body weight (Johnson and Beckett, 2014). While days spent on feed, relative to non-implanted cattle, is certainly an important factor related to disparities in quality grade as a result of implant, it has been shown that implanting at particular time points during growth can dictate the effect of steroidal implants on marbling score. Bruns et al. (2005), conducted a study where serial slaughter treatments were used to evaluate deposition of intramuscular fat relative to changes in body composition in steers implanted with a combination TBA + E_2 implant (containing 120 mg TBA + 24 mg of E_2) at two different points in the finishing phase growth curve.

Treatments included: 1) no implant administered; 2) early implant on d 1 (BW = 309 kg); or 3) delayed implant on d 57 (BW = 385 kg)]. Steers implanted early had increased ADG up to d 56, however, from d 57 to d 112 and on a cumulative basis ADG (d 140) did not differ from controls or the delayed implant treatment. It was also observed that early implant application resulted in an adverse response in marbling score while delayed implant application did not effect marbling score. Steroidal implants administered during early periods of growth adversely affect the development of marbling in steers. While improper timing of implant and level of caloric intake at time of implant application have been shown to adversely influence marbling score, other factors have been shown to perhaps play a role as well. Smith et al. (2017), evaluated the dose and payout pattern of TBA + E_2 on adenosine monophosphate-activated protein kinase-α (*AMPK-a*), *C/EBPβ*, G protein-coupled receptor 41(*GPR41*), G protein-coupled receptor 43 (*GPR43*), *PPARγ*, and stearoyl CoA desaturase (*SCD*) expression in the longissimus muscle in beef steers. These genes can be used as indicators of adipogenesis and marbling development in beef steers. Treatments included: 1) no implant (NI), 2) 120 mg TBA + 24 mg E_2 (REV-S) , or 3) delayed release implant containing 80 mg TBA + 16 mg E_2 [uncoated], 120 mg TBA + 24 mg E_2 [coated] (200 mg TBA + 40 mg E_2 [total]) (REV-X). Marbling scores were numerically lower for REV- S and REV-X but did not differ from NI. The REV-X treatment had the greatest expression of genes associated with marbling development. Smith et al. (2017) suggested that the delayed release rate of TBA + E_2 for REV-X might have mitigated the decreases in marbling generally attributed to multiple short acting TBA + E_2 implants. Duckett et al. (1999) attributed the observed decrease in intramuscular fat deposition and composition to a dilution effect caused by increased

REA due to implantation with a combination TBA + E_2 implant. Effect of steroidal implant on marbling score and quality grade can be attributed to several interrelated factors and results investigating these factors have been relatively inconclusive.

Steroidal implant effect on serum hormone and metabolite concentration

Use of a combination TBA + E_2 steroidal implant has been shown to increase circulating serum concentration of IGF-I in beef cattle (Johnson et al., 1996a; Bryant et al., 2010; Smith et al., 2018). Increases of serum concentration of IGF-I in beef cattle implanted with a combination TBA + E_2 are related to the effect they elicit on the hypothalamus, as well as increase the size of acidophilic cells in the anterior pituitary (Smith and Johnson, 2020). Additionally, the androgens and estrogens binding directly to skeletal muscle and this increases local IGF-I production as evidenced by increased gene expression of IGF-I in longissimus muscle following implantation with TBA + E_2 (Johnson et al., 1998; Johnson and Beckett, 2014). Bryant et al. (2010), noted increased serum concentration of IGF-I by d 42 for heifers implanted with a combination TBA + E_2 implant containing 200 mg of TBA and 20 mg E_2. Smith et al. (2018) observed that implantation with TBA + E_2 increased circulating concentrations of sera IGF-I in the present study. Serum concentration of IGF-I by d 35 was observed in steers implanted initially with a partially uncoated or uncoated TBA + E_2 implant containing either 80 mg TBA + 16 mg E_2 (noncoated) + 120 mg TBA + 24 mg E_2 (coated) for a total dose of 200 mg TBA + 40 mg E_2 or 200 mg TBA + 20 mg E_2 (noncoated).

A decrease in serum concentration of urea-N is a useful biological marker of anabolism when cattle are consuming similar amounts of dry matter and rumen degradable protein is constant across diets (Smith and Johnson, 2020). It has been well

documented that use of steroidal implants in beef cattle results in decreased serum concentration of urea-N (Bryant et al., 2010; Parr et al., 2014; Smith et al., 2018). This has been well demonstrated by, Lobley et al. (1985), where improvements in nitrogen retention based on changes in tissue metabolism as a result of implantation with a combination TBA + E_2 indicated a net decrease in protein turnover in skeletal muscle tissue by way of decreased degradation, increased synthesis, or both.

CONCLUSIONS TO REVIEW OF LITERATURE

Exposure to cold environments below the LTc increases the energy required for maintenance in homeotherms and beef cattle are no exception (Young, 1983). Bedding confined cattle during winter months in regions where snow accumulation, wind, moisture, and ice are highly prevalent has been shown to be of value when considering growth performance and carcass characteristics (Birkelo and Lounsberry, 1992; Stanton et al., 1994a; Anderson et al., 2006; Mader and Colgan, 2007). Cattle growth performance improvements observed during previous work evaluating the effects of bedding applications are indicative that bedding application ameliorates energy required for maintenance through mechanisms such as reduced conductive heat loss to the pen surface and improved insulative capacity of the hair coat. Thus, during winter months, understanding the maintenance requirement is crucial due to the mathematical relationship maintenance energy has with intake energy and consequently energy available to gain. Better understanding of the effects of bedding application on maintenance requirements will allow for more accurate tracking and growth performance prediction in beef cattle.

Steroidal implants increase the frame size of the beef animal, thus increasing the body weight of the animal at a given level of chemical maturity (i.e. delay fattening) by way of promoting deposition of lean tissue rather than fat compared to non-implanted cattle (Preston, 1999; Guiroy et al., 2002). Steroidal implants can be expected to improve growth rate 10 to 30%, feed efficiency 5 to 15%, and carcass leanness 5 to 8% (Preston, 1999). However, it has been well documented that use of combination TBA + E_2 implants has been shown to decrease marbling score when cattle are fed for equal days (Duckett et al., 1999; Pritchard, 2000; Bruns et al., 2005; Smith et al., 2018). It has been demonstrated that combination TBA + E_2 implants administered during early periods of growth can adversely affect the development of marbling in steers. Timing of implant administration and level of caloric intake at time of implant seem to be of importance relative to marbling development. Furthermore, it has been shown that dose and payout pattern of TBA + E_2 have an effect on the expression of genes associated with marbling development (Smith et al., 2017). Smith et al. (2017) found that the delayed release implant treatment which contained an initial uncoated portion (80 mg TBA + 16 mg E_2) and a coated portion (120 mg TBA + 24 mg E_2 [coated]) had the greatest expression of genes associated with marbling development and as such may have mitigated the decreases in marbling generally attributed to multiple TBA+ E_2 implants with shorter payout periods. Additional investigation into the effects of combination TBA + E_2 implant dose on beef cattle growth performance and effects on carcass performance is warranted.

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CHAPTER II: BEDDING APPLICATION TO FEEDLOT STEERS: INFLUENCE ON GROWTH PERFORMANCE, ESTIMATED MAINTENANCE COEFFICIENT, CARCASS CHARACTERISTICS, AND CIRCULATING METABOLITES IN BEEF STEERS

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ABSTRACT

Two randomized complete block design experiments were conducted to evaluate the effect of bedding use in confined beef steers. Experiment 1, used Simmental × Angus steers (n = 240; initial body weight (BW) = 365 ± 22.5 kg). Experiment 2, used newly weaned Charolais × Red Angus steers (n = 162; initial BW = 278 ± 13.4 kg). Steers were allotted to 1 of 2 treatments: 1) no bedding (NO), or 2) 1.8 kg (Exp. 1) or 1.0 kg (Exp. 2) of wheat straw (as-is basis) bedding/steer·d^{-1} (BED). In Exp.1, applying bedding improved ($P \leq 0.01$) dry matter intake (DMI), kg of gain to kg of feed (G:F), and average daily gain (ADG). Bedding reduced (° 0.01) the estimated maintenance coefficient (MQ). Dressing percentage, rib fat, marbling, and yield grade were increased ($P \leq 0.03$) in NO. Bedding resulted in an increase ($P = 0.01$) in serum insulin-like growth factor I (IGF-I). In Exp. 2, a tendency ($P = 0.06$) for increased DMI for NO was noted. Bedding improved G:F ($P = 0.01$). MQ was elevated ($P = 0.03$) for NO and NO had an increase ($P = 0.02$) in serum concentration of urea-N (SUN). An increase ($P = 0.01$) in serum non-esterified fatty acid was noted for NO. These data indicate that bedding application should be considered to improve growth performance and feed efficiency by reducing maintenance energy requirements in beef steers during the feedlot receiving and finishing phase.

Keywords: bedding, feedlot, maintenance coefficient, steers

INTRODUCTION

Feeding cattle in the upper Midwest can pose a unique set of environmental challenges. During late fall, winter, and early spring, persistent cold temperatures coupled with snow accumulation, wind, and ice can cause undesirable pen conditions for cattle. These undesirable pen conditions can negatively impact the insulative capacity of cattle hair coat as a result of dampness and mud or manure accumulation. For cattle, the insulative capacity of the hair coat is a contributing factor to their lower critical temperature (LTc) threshold. The LTc for all homeotherms is the temperature below which the organism's metabolic rate must increase in order to maintain homeothermy (Young, 1983). The maintenance requirement of an animal is an estimate of the amount of energy necessary to keep an animal in an equilibrium state (Garrett, 1980).Temperatures falling below the lower critical temperature for cattle with a dry, heavy winter coat (~-7.8°C) will result in a subsequent increase in maintenance requirements and due to this diversion of energy towards maintenance function, a resulting decrease in feed available for gain and productive function is likely to be observed through decreased performance.

Previous work has been done related to effects of bedding application and housing techniques on beef cattle performance, however, results have been variable with regards to feedlot growth performance and carcass trait responses (Birkelo et al., 1991; Stanton et al., 1994; Anderson et al., 2006; Mader and Colgan, 2007). Modern performance tracking systems currently used to predict cattle performance rely on two specific requirements of the beef animal, net energy required for maintenance and net energy for gain (Lofgreen and Garrett, 1968). Thus, during winter months, understanding the alteration in

maintenance requirement is crucial as it may allow for more accurate tracking and performance prediction.

Little work has been done directly investigating the effects of bedding on receiving phase growth performance in beef steers. The receiving phase is a critical time in beef cattle production that involves a variety of potential stressors. A newly received calf may be exposed to a wide array of stressors including but not limited to: environmental conditions, weaning, transportation, lack of feed and water, and introduction to unfamiliar feed resources (Blom, 2019). Therefore, mitigating stress by applying bedding may prove valuable when considering newly weaned calf performance in the feedlot.

The objective of these experiments were to evaluate the effect of bedding use on growth performance (Exp. 1 and 2), carcass characteristics (Exp. 1), estimated maintenance requirement (Exp. 1 and 2), and sera metabolite responses (Exp. 1 and 2) in beef steers of differing ages and during different phases of feedlot production. The hypothesis was that bedding application would increase growth performance and lower estimated maintenance requirement compared to non-bedded steers regardless of stage of production.

MATERIALS AND METHODS

Use of Animal Subjects

Animal care and handling procedures used in this study were approved by the South Dakota State University Institutional Animal Care and Use Committee (Approval numbers: 18-096A and 19-054E).

Animal Description and Initial Processing

In Exp. 1, Simmental x Angus crossbred beef steers (n = 240; initial BW = 365 ± 22.5 kg) were transported (1.5 hours) from a cattle auction facility in eastern South Dakota and received in January of 2019. Steers were allotted to 30 concrete surface pens (7.25 m^2/steer; 94.5 cm of bunk space/steer; n = 8 steers/pen) at the Ruminant Nutrition Center (RNC) in Brookings, SD and provided ad libitum access to long-stem grass hay and water upon arrival.

Initial processing included an individual body weight measurement (scale readability 0.454 kg), application of a unique identification ear tag, and a rectal temperature measurement along with vaccination for bovine respiratory syncytial virus (BRSV), bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD) Types 1 and 2, parainfluenza-3 (PI_3), Mannheimia haemolytica (pasteurella), and clostridium perfringens type A; and administered pour-on moxidectin according to label instructions. Any steer with a rectal temperature of greater than 39.4°C was administered tulathromycin according to label instructions. On day 36, cattle were implanted with a trenbolone acetate and estradiol benzoate implant and re-vaccinated for clostridium perfringens type A and were poured with an anti-parasitic to control for lice.

In Exp. 2, newly weaned Charolais x Red Angus crossbred beef steers (n = 162; initial BW = 278 ± 13.4 kg) were transported (6.0 hours) from a sale barn in western South Dakota to the RNC in October of 2019. Upon arrival to the RNC, steers were housed in 18 concrete surface pens (6.45 m^2/steer; 84.7 cm of bunk space/steer; n = 9 steers/pen) with 7.62 m of linear bunk space and provided ad libitum access to long-stem grass hay and water upon arrival.

The following day (day −1), all steers were individually weighed (readability 0.454 kg), applied a unique identification ear tag, vaccinated for viral respiratory pathogens: IBR, BVD 1 and 2, PI_3, and BRSV as well as clostridials. The afternoon following initial processing, all steers were allotted to their study pens (n = 9 steers/pen and 9 pens/treatment). The following morning (day 1) all steers were again individually weighed as well as administered pour-on moxidectin according to label directions. On study day 14, all steers were implanted with 200 mg progesterone and 20 mg estradiol benzoate. The initial BW was the average of processing BW (day −1 BW) and day 1 BW. Steers were used to evaluate the effect of bedding application on growth performance and maintenance energy requirements during the feedlot receiving phase. Diets were offered on top of long-stem grass hay (GH) for the first 2 d of the receiving period. There was no morbidity or mortality noted Exp. 2. Diets presented in tables 2.1 and 2.2 and are composed of actual DM (dry matter) diet composition, actual nutrient concentrations, and tabular energy values (Preston, 2016).

Experimental Design and Treatments

In both experiments, bedding was applied as was necessary with the goal of maintaining a dry, bedded area large enough for all steers within the particular bedded pen to lay down. Amount of bedding applied is presented kg per steer per day (as-is basis) of wheat straw and was calculated as an average based on total kg of bedding applied to the bedded pens throughout the study divided by days on feed and number of head per pen.

In Exp. 1, pens were assigned to 1 of 2 bedding treatments (n = 15 pens/treatment): No bedding applied (NO); 1.8 kg (as-is basis) of wheat straw

bedding/steer·d^{-1} (BED). The first 9 pen replicates began on test 14 d prior to the last 6 pen replicates for each treatment due to timing of acquisition of sufficient cattle to enroll in the experiment. In Exp. 2, pens were assigned to one of two treatments (n = 9 pens/treatment): No bedding (NO); 1.0 kg (as-is basis) of wheat straw bedding/steer·d^{-1} (BED). The goal of bedding application, in both experiments, was to maintain a dry, bedded area large enough for all steers to lay down in BED treatment pens at all times during the study.

Dietary Management

In both Exp. 1 and 2, fresh feed was manufactured twice daily at 0800h and 1400h in a stationary mixer (2.35 m^3; scale readability 0.454 kg) and bunks were managed according to slick bunk management approach. Orts were collected, weighed, and dried in a forced air oven at 100°C for 24 h to determine DM content if carryover feed spoiled or was present on weigh days. If carryover feed was present on weigh days, the residual feed was removed prior to the collection of BW measurements. 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. Actual diet formulation and nutrient composition was determined based upon weekly feed analyses [Crude protein (CP), AOAC (1984); neutral detergent fiber (NDF) and acid detergent fiber (ADF), (Goering and Soest, 1970); ash and DM, (AOAC, 1990)] and corresponding feed batching records were generated.

In Exp. 1, upon arrival cattle were stepped up from a 50% to 90% concentrate diet. All pens were on the final high-concentrate diet by d 18. A common diet (Table 2.1) consisting of dry-rolled corn, dried distillers grains, and oatlage or grass hay was fed that

contained 14.2% crude protein, 2.10 Mcal/kg of net energy for maintenance NE_m and 1.40 Mcal/kg of net energy for gain NE_g A liquid supplement was provided to add 33 mg/kg of monensin sodium to diet DM along with supplemental vitamins and minerals to meet NASEM (2016) requirements. Cattle from BED and NO were on feed 143 and 178 d, respectively, prior to being harvested at a commercial abattoir when the population reached sufficient fat cover to grade United States Department of Agriculture (USDA) Choice.

Diets in Exp. 2, consisted of corn silage, dried distillers grains plus solubles, grass hay, and a pelleted supplement (Table 2.2). The diet was fortified with vitamins and minerals to meet nutrient requirements and provided monensin sodium (DM-basis) at 27.6 mg/kg (NASEM, 2016).

Growth Performance Calculations and Carcass Data Collection

In both Exp. 1 and 2, the following equation was used to calculate estimated maintenance coefficient (MQ) based upon intake, dietary net energy content and retained energy (RE) required for the observed ADG (NRC, 1984, 1996).

In Exp. 1, steers were individually weighed on d −1, 1, 36, 64, 92, and 120 relative to study initiation. Cattle from BED were removed from the experiment where they were then marketed and harvested on d 148 and 134, respectively. The remaining cattle from the group that started 14 d earlier were weighed on d 162 and 183; steers from the group that started 14 d later were weighed on d 148 and 169. Weight gain was based upon initial un-shrunk BW (average of days −1 and 1 BW) and final BW was calculated from HCW/0.625 (a common dressing percentage).

In Exp. 2, all steers were weighed on d -1, 1, 14, 28, 42, and 56. Weight gain was based upon initial un-shrunk on test BW (average of days −1 and 1 BW) and final BW that was pencil shrunk 4% to account for gastrointestinal tract fill.

In Exp. 1, steers were harvested at a commercial abattoir when the population reached sufficient fat cover to grade USDA Choice. Carcass data including ribeye area, hot carcass weight, 12th rib fat, kidney, pelvic, and heart fat percent, and USDA marbling score were collected by the camera grading system at the abattoir. Yield grade was calculated by using the USDA regression equation (USDA, 1997). Estimated empty body fat (EBF) from carcass traits was calculated according to Guiroy et al. (2002). Retail yield (RY) as a percentage of HCW was calculated according to Murphey et al. (1960) Carcass data were not collected in experiment 2. Average daily gain was calculated from initial BW subtracted by final BW and divided by the days on feed. Gain to feed ratio was calculated from average daily gain divided by dry matter intake.

Blood Sample Collection

In both experiments whole blood samples were collected from sentinel steers (n = 2 steers/pen) into 10 mL non-additive tubes during the interim weighing process prior to feeding. For Exp. 1, whole blood was collected on days 36, 64, 92, and 120 (relative to study initiation). For Exp. 2, whole blood was collected in on days 1, 14, 28, 42, and 56 (relative to study initiation). In both experiments, once collected, whole blood was transported from the RNC to the Ruminant Nutrition Lab and allowed to clot for 24 h at 4 °C and were subsequently centrifuged at 1250 × g at 4°C in order to harvest sera.

Serum Hormone and Metabolite Quantification

In Exp. 1, serum concentration of urea-N (SUN) were determined by a method described by Fawcett and Scott (1960) using sodium phenate and sodium hypochlorite. The determination of SUN is measured based on the reaction of ammonia with sodium phenate and hypochlorite to yield a blue color to be measured in a spectrophotometer. The SUN assay was performed using serum from each individual steer (n = 2 steers/pen) and these values were averaged together prior to statistical analysis. The standard curve constructed for the SUN assay was between 0 and 25.0 mg/dL. Absorbance for reactions of standards and samples were read at 625 nm. Samples were considered for re-runs if the coefficient of variation (CV) was greater than 10% among triplicate determinations. Intra- and inter-assay CV were 6.3% and 10.9%, respectively.

In Exp. 1, serum concentrations of insulin-like growth factor I (IGF-I) were determined in duplicate via radioimmunoassay procedure (Echternkamp et al., 1990; Funston et al., 1995). Insulin-like growth factor binding proteins (IGFBP) in sera were extracted using a 1:17 ratio of sample to acidified ethanol (12.5% 2 N HCl: 87.5% absolute ethanol) (Daughaday et al., 1980). Extracted samples were centrifuged (12,000 × g at 4°C) to separate IGFBP. A portion of the resulting supernatant was removed and neutralized with 0.855 M Tris base, incubated for an additional 4 h at 4°C, and then centrifuged at 12,000 × g at 4°C to remove any additional IGFBP. When samples of this extract, equivalent to the original serum sample, were subjected to Western ligand blot analysis and subsequent phosphoimagery, no detected binding of I-IGF-I to IGFBP was observed. Inhibition curves of the neutralized extracted serum ranging from 12.5 to 50 µL were parallel to the standard curve. Recombinant human IGF-I (GF-050; Austral Biological, San Ramon, CA, USA) was used as the standard and radioiodinated antigen.

Antisera AFP 4892898 (National Hormone and Peptide Program, National Institutes of Diabetes, Digestive and Kidney Diseases, Bethesda, MD, USA) was used at a dilution of 1:62,500. Sensitivity of the assay was 14.7 pg/tube. No samples were considered for re-runs and the assay was completed in a single run. The intraassay CV was 7.7%.

In Exp. 2, the quantification of circulating SUN concentration was determined on a microplate spectrophotometer in triplicate 5 µL determinations, using diacetylmonoxime via a commercially available kit (STANBIO Urea Nitrogen-0580; STANBIO Laboratory, Boerne, TX). The SUN assay was performed using serum from each individual steer (n = 2 steers/pen) and these values were averaged together prior to statistical analysis. The standard curve constructed for the SUN assay was between 0 and 25.0 mg/dL. Absorbance for reactions of standards and samples were read at 520 nm. Samples were considered for re-runs if the coefficient of variation among the absorbance values for triplicate determinations was greater than 5%. For the SUN analysis in Exp. 2, the intra-assay CV was 6.6% and the inter-assay CV was 10.4%.

In Exp. 2, quantification of serum concentration of non-esterified fatty acids (NEFA) was determined using triplicate 5 µL determinations via colorimetric assay using a commercially available kit that involved acyl-CoA synthetase, acyl-CoA oxidase, and peroxidase in 96 well microtiter plates (NEFA-HR; Wako Diagnostics, Richmond, VA). The NEFA assay was performed using sera from each individual steer (n = 2 steers/ pen) and these values were averaged together prior to statistical analysis. The standard curve constructed for the NEFA assay was between 0 and 1.0 mEq/L. Samples were considered for re-runs if the coefficient of variation among the absorbance values for triplicate

determinations was greater than 5%. For the NEFA analysis, the intra-assay and inter-assay CV were 3.6% and 3.7%, respectively.

Management of pulls and removals

All steers that were pulled from their home pen for health evaluation were then monitored in individual hospital pens prior to being returned to their home pens. When a steer was moved to a hospital pen the appropriate amount of feed from the home pen was removed and transferred to the hospital pen. If the steer in the hospital returned to their home pen, this feed remained credited to the home pen. If the steer did not return to their home pen, all feed that was delivered to the hospital pen was deducted from the feed intake record for that particular pen back to the date the steer was hospitalized. Eight steers were removed during the course of experiment 1 for reasons determined to be health anomalies not related to treatment. Six steers from NO were removed due pneumonia (1), bloat (1), identified as a bull (1) and musculoskeletal issues (3). Two steers from BED were removed due to being identified as bulls.

Statistical Analysis

Data were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). Experiments 1 and 2 were both randomized complete block designs. Fixed effects included in the model for Experiment 1 were bedding treatment and block (pen location). Fixed effects in Experiment 2 included in the model were bedding treatment and block (pen location). The pen served as an experimental unit for all analyses in both studies; a *P*-value of less than 0.05 ($\alpha = 5\%$) determined significance and a *P*-value between 6% and 10% was considered a tendency.

Serum metabolite data were analyzed according to a randomized complete block design appropriate for repeated measures using the MIXED procedure of SAS 9.4 (SAS Inst. Inc.). The model included the fixed effects of bedding, day, and their interaction. Day was included as the repeated variable and pen served as the experimental unit. The covariance structure with the lowest Akaike information criterion was used. All results were reported as least squares means. A *P*-value of 0.05 ($\alpha = 5\%$) determined significance and a *P*-value between 6% and 10% was considered a tendency.

RESULTS

Weather - Experiment 1 + 2

Experiment 1 was conducted from January 15 to July 17, 2019. Daily ambient temperature (Figure 2.1) averaged 4.4 ± 14.6°C with an average wind chill of 2.9 ± 15.8°C during the course of the study. Experiment 2 was conducted from October to December of 2019. Daily ambient temperature (Figure 2.2) averaged -3.0 ± 5.5°C and wind chill averaged -5.1 ± 6.1°C during the 56 d receiving study.

Growth Performance day 1 to day 36 - Experiment 1

Growth performance and carcass data from Exp. 1, are located in Table 2.3. During the receiving phase of Exp. 1 (d 1 to 36), weather was more severe than the remainder of the study. Initial BW did not differ (*P* = 0.95) between NO and BED. Dry matter intake was not affected (*P* = 0.57) by bedding treatment and d 36 BW was greater for BED (*P* = 0.01; 419 vs. 402 ± 1.1 kg) compared to NO. A 48.0% increase (*P* = 0.01) in receiving phase ADG and a 49.2% increase in receiving phase G:F (*P* = 0.01) was observed in BED compared to NO. An increase (*P* = 0.01) in MQ was noted for NO (0.146 vs. 0.104 ± 0.0032 Mcal/ $BW^{0.75}$, kg) relative to BED.

Cumulative Growth Performance - Experiment 1

In Exp. 1, final BW tended to differ ($P = 0.07$) between NO and BED. Dry matter intake was increased ($P = 0.01$) by 5.8% in BED compared to NO. Cumulative ADG ($P = 0.01$) and G:F were improved ($P = 0.01$) in BED by 21.0% and 15.0%, respectively. The cumulative estimated maintenance coefficient was elevated ($P = 0.01$; 0.109 vs. 0.098 ± 0.010 Mcal/$BW^{0.75}$, kg), for NO compared to BED steers.

Carcass Characteristics - Experiment 1

Hot carcass weight tended to differ ($P = 0.07$) between NO and BED. Cattle from NO required an additional 35 days to achieve similar final live-basis BW. Rib eye area ($P = 0.69$) did not differ between NO and BED. Dressing percentage, rib fat, marbling, and yield grade were increased ($P \leq 0.03$) in NO steers compared to BED.

Serum Hormones and Metabolites - Experiment 1

No bed × day interaction ($P = 0.66$) was detected for SUN concentration in Exp. 1 (Figure 3.). The main effect of bedding treatment did not cause a significant response ($P = 0.75$) in SUN between treatments, however, SUN did differ over time ($P = 0.01$).

Growth Performance - Experiment 2

Growth performance responses for Exp. 2 are located in Table 4. Initial BW did not differ ($P = 0.69$) between treatments at study initiation. Bedding application did not influence ($P \geq 0.67$) final BW or ADG. Dry matter intake tended to increase ($P = 0.06$) in NO steers relative to the BED. Gain to feed was increased ($P = 0.01$) by 5.6% for cattle in bedded pens relative to NO. Estimated MQ was elevated ($P = 0.03$; 0.052 vs. 0.044 ± 0.0022 Mcal/$BW^{0.75}$, kg), for NO steers compared to BED steers.

Serum Metabolites - Experiment 2

No bed × day interaction ($P = 0.67$) was detected for SUN concentration in Exp. 2 (Figure 5.). The main effect of bedding treatment resulted in a 13% increase ($P = 0.02$) in SUN for NO compared to BED. Additionally, SUN differed over time ($P = 0.01$).

No bed × day interaction ($P = 0.52$) was detected for serum NEFA concentration (Figure 6). Bedding treatment resulted in a 22% increase ($P = 0.01$) in serum concentration of NEFA in NO compared to BED steers. Serum concentration of NEFA also differed over time ($P = 0.01$).

DISCUSSION

Growth Performance day 1 to day 36 - Experiment 1

Little work has been done to directly investigate the effects of bedding application on feedlot growth performance, and specifically, the resulting alterations in energetic demand. Interim performance data from the initial 36-day receiving period of Exp. 1 has been included to better illustrate the effects of the severe environmental conditions (Figure 1) on receiving phase growth performance. This is of importance because earlier work (Lofgreen et al., 1975; Galyean et al., 1993) determined that growth performance improvements observed during the receiving phase can often be maintained during subsequent feeding periods.

At the conclusion of the initial 36-day receiving period, a 4.0% increase in d 36 BW was observed for BED steers, which amounted to approximately 17 kg of additional BW gain during the initial 36-day period. A 48.0% increase in ADG was noted for the BED treatment during the 36-day receiving period relative to the NO steers. Interim performance data for BW and ADG, as a result of bedding application, have been reported in previous work, but results have varied. In a study that investigated the effects

of bedding level on cattle performance, Anderson et al. (2006) reported that during winter months, both modest and generous amounts of bedding applied during the start of the feeding period resulted in an approximately 20% increase in ADG. Alternatively, in a study that investigated both bedding and shelter effects, Mader and Colgan (2007) reported that bedding application during winter months did not result in any appreciable response in BW or ADG at the conclusion of the initial 36-day period. The variation in effects on performance due to bedding application can likely be explained by the large number of external factors that play a pivotal role in the occurrence and magnitude of performance results. These factors may include geographical location, temperature, wind, precipitation, time of year, pen size, stocking density, hair coat condition of animals included in the study, age of animal, and many other possible factors. Performance results from the present study, specifically the initial feedlot receiving period of days 1 to 36, are likely of greater magnitude due to the persistent exposure of the cattle to abnormally low ambient temperatures and severe wind chill.

Bedding application had no effect on DMI in the initial 36-day period as both treatments consumed similar amounts of dry matter. Intakes were controlled by the feedlot manager as cattle were being stepped up to the high concentrate finishing diet. With no difference in DMI between treatments and significant responses in both d 36 BW and ADG favoring the BED treatment during the initial 36-day period, a 49.2% improvement in G:F ratio was observed in BED steers. It has been well documented that cold temperatures cause an increase in metabolic demand of beef cattle (Young, 1983; Birkelo et al., 1991; Mader and Colgan, 2007; Wagner et al., 2008), and so if cattle are

not able to compensate by consuming more DMI, a resulting decrease in feed efficiency will likely be observed.

It was during the initial 36-day period that the magnitude of difference in MQ was largest between treatment groups. As a response to winter weather conditions such as sustained cold temperatures, snow accumulation, and wind, beef cattle are well known to have increased maintenance requirements in order to maintain homeothermy (Young, 1981, 1983). This principle has been demonstrated in a number of previous studies dealing with bedding application and cold stress (Birkelo et al., 1991; Anderson et al., 2006; Mader and Colgan, 2007). During the initial 36-day period of Exp. 1, relative to the BED treatment, NO had an MQ that was elevated 40.4%. It should be noted that the severe environmental conditions during the initial 36-day period experienced by all cattle on test, regardless of treatment, caused an increase in their maintenance energy requirements relative to the standard NE_m requirement value for beef cattle of 0.077 $Mcal/BW^{0.75}$ (Lofgreen and Garrett, 1968). The increases in MQ for NO and BED relative to the standard value of 0.077 $Mcal/BW^{0.75}$ were 90% and 35%, respectively. In a case study by Wagner et al. (2008), data indicated that NE_m required by cattle during and in the aftermath of a major winter weather event may be 2.5 times higher than NE_m required under standard thermoneutral conditions. These results indicate that, regardless of bedding application and pen surface condition, severe weather events can cause alterations in the energetic demand of beef cattle and thus an increase in feed required for maintenance.

Cumulative Growth Performance - Experiment 1

In Exp. 1, there was a tendency for final BW to differ between NO and BED, however it should be noted that steers from NO remained on feed for an additional 35 d to achieve a similar compositional endpoint as BED steers. It is probable that, had cattle been marketed at equal days on feed, final BW would have favored the BED treatment. In some previous work, during winter and spring months, final BW was increased in bedded treatments compared to non-bedded controls when cattle were marketed at equal days on feed (Birkelo and Lounsberry, 1992; Anderson et al., 2006). Steer ADG was improved in BED by 21.0% compared to the NO control steers. Mader (2003), along with a number of other studies (Birkelo and Lounsberry, 1992; Stanton et al., 1994; Anderson et al., 2006) reported increases in ADG as a result of bedding application. However, other work previously reported did not observe increases in ADG as a result of bedding application (Mader and Colgan, 2007). As it relates to feedlot cattle, cold temperatures are well known to increase energy required for maintenance, increase rate of passage, and stimulate appetite in cattle as a response to the increased metabolic demands (Young, 1983). In the present study, cattle from BED treatment consumed 5.8% more DMI than cattle from NO. Previous work conducted regarding the effects of bedding application on feedlot growth performance during winter months did not report any differences in DMI as a result of bedding application (Birkelo and Lounsberry, 1992; Stanton et al., 1994; Anderson et al., 2006; Mader and Colgan, 2007). The difference observed in DMI that favored the BED treatment could be a lasting effect resulting from increased growth performance captured during the initial 36-day period of the study. As stated previously, growth performance improvements observed during the receiving phase can often be maintained during subsequent feeding periods (Lofgreen et al., 1975; Galyean et al.,

1993). Overall G:F was improved in BED cattle by 15.0% compared to NO. A common physiological reaction of ruminants, when exposed to cold stress, has been shown to be increased reticulorumen motility and rate of passage of digesta (Westra and Christopherson, 1976). This physiological response may, in part, account for the observed disparity in feed efficiency. The improvement in feed efficiency for the BED treatment observed in the present study as a result of bedding treatment is consistent with previous work (Birkelo and Lounsberry, 1992; Anderson et al., 2006; Mader and Colgan, 2007). Although, the degree to which feed efficiency improved in these previous studies varied, likely because of geographical location and weather conditions.

Estimated maintenance coefficient was elevated 11.2% for NO compared to BED which is similar to previous findings (Anderson et al., 2006; Mader and Colgan, 2007). The estimated maintenance coefficient for steers in BED pens compared to NO can likely be explained as a function of the performance results previously reported and discussed for Exp. 1 where BED cattle required fewer days on feed (DOF), consumed more dry matter, and had improved ADG and G:F. Bedding application appears to have decreased the proportion of metabolizable energy (ME) intake partitioned to maintenance functions, when compared to NO , which allowed a greater proportion of ME intake to be used for productive function and stored as retained energy (RE) rather than heat production to maintain homeothermy. Both NO and BED treatments had increased MQ relative to the 0.077 Mcal/$BW^{0.75}$ value from (Lofgreen and Garrett, 1968).

The effects of bedding on beef cattle feedlot performance are inherently linked to the environmental conditions experienced by the cattle being evaluated. The unavoidable variation in pen condition, geographical location, and weather conditions pose

considerable challenges when attempting to compare performance results from previous work. Additionally, potential long-term effects on growth performance as a result of exposure to extreme winter temperatures, like those environmental conditions experienced by steers during the first 36 days of Exp. 1, in a non-bedded versus bedded pen environment, requires further investigation.

Carcass Characteristics - Experiment 1

There was a tendency for NO steers to have heavier HCW compared to and the BED steers. Anderson et al. (2006), in a study evaluating effects of bedding level on feedlot cattle performance, reported that "generous" bedding level improved HCW in bedded pens for cattle fed for equal days. However, in previous work, other authors (Stanton et al., 1994; Mader and Colgan, 2007) reported no effect on HCW for beef cattle fed for equal days. In the present study, had cattle been harvested at an equal number of days on feed, it is likely that a response in HCW favoring BED cattle would have been noted given cattle from NO required an additional 35 d to achieve final live BW similar to that of the BED treatment. Conversely, perhaps an explanation to oppose that idea is that during this experiment an inadvertent increase in frame size occurred in NO treatment due to a decreased amount of feed available for gain as a result of the increased calculated maintenance coefficient during the early periods of this experiment. Rib eye area did not differ between NO and BED. This result is inconsistent with Anderson et al. (2006) that reported a significant increase in REA for bedded steers compared to non-bedded controls fed for equal days. Limited additional data is available reporting the effect of bedding on REA in beef steers. Dressing percentage was increased for the NO treatment, differences in manure tag load, frame size, gut fill, and days on feed may

explain this response. The dressing percentage response favoring the NO treatment, in the present study, is inconsistent with previous work where bedded treatments had improved dressing percentages compared to non-bedded control treatments (Stanton et al., 1994; Anderson et al., 2006). Mader and Colgan (2007) reported that bedding did not cause a significant response in dressing percentage in either of their two trials.

In the present study, rib fat was increased for NO steers compared to BED. This is inconsistent with findings from Anderson et al. (2006) that reported no difference in rib fat as a result of bedding application. Additional work reporting the effect of bedding on rib fat in beef steers is currently limited. Marbling score was also improved in NO steers compared to BED. Mader and Colgan (2007) reported no difference in marbling score as a result of bedding application in both bedding trials. Anderson et al. (2006) reported a significant response in marbling score favoring bedded cattle. In another experiment, Anderson et al. (2006) did not observe an effect on marbling score. In the present study, a response was noted where NO steers had increased calculated yield grade compared to BED steers. This is likely a function of increased rib fat and estimated empty body fat (Guiroy et al., 2002). Anderson et al. (2006) reported increased calculated yield grade for bedded cattle compared to non-bedded controls. Other workers reported no effect of bedding on calculated yield grade (Stanton et al., 1994; Mader and Colgan, 2007).

Serum Hormones and Metabolites - Experiment 1

Serum concentration of urea-N was not affected by bedding treatment in Exp. 1. However, SUN did differ over time. The SUN concentration was at its lowest point from d 36 and 64 and then increased on d 92 and 120. The observed decrease from d 36 and 64 for serum concentration of urea-N, indicative of anabolism, may have been caused by the

additive effects of increased intakes, implantation on d 36, and perhaps improved weather conditions as the study progressed.

Bedding treatment, in Exp. 1, resulted in a 17% increase in the serum concentration IGF-I. Insulin-like growth factor I is a somatotropin-dependent anabolic peptide that stimulates proliferation and differentiation of many cell types, including muscle (Florini et al., 1991). Therefore, changes in serum concentration of IGF-I, were likely a factor that improved growth rate in BED steers and caused them to reach harvest 35 d sooner than NO steers. Serum concentration of IGF-I differed over time, perhaps a function of improving weather conditions where bedding treatment became less important.

Growth Performance - Experiment 2

Previous receiving phase growth performance data investigating effects of bedding application is limited. In the present study, bedding application did not influence final BW. Previous studies have reported interim data that can be used to compare receiving phase performance results seen in the present study. In two bedding related research trials using cattle with initial BW of 329 kg and 296 kg, respectively, Anderson et al. (2006) reported no difference in d 56 BW. Mader and Colgan (2007), also conducted a pair of trials related to effect of bedding on feedlot performance. Body weights were reported for d 35 and d 34 respectively for trials 1 and 2. In trial 1, where the initial BW of cattle was 373 kg, a significant response in BW was not reported. In trial two, cattle (initial BW = 400 kg) from the bedded treatment had a significantly increased d 34 BW. No improved response was observed for ADG in the present study. With equal initial BW and no change in final BW a response in ADG was not expected.

This response is inconsistent with some previous work (Stanton et al., 1994; Anderson et al., 2006; Mader and Colgan, 2007) where enhanced responses for ADG were observed during the early periods of the respective trial. There was a tendency for NO steers to have a 4.5% increase in dry matter intake (DMI) compared to BED steers in Exp. 2. This agrees with results reported from trial 2 by Anderson et al. (2006), where non-bedded cattle consumed a greater amount of DMI. However, other work reported no effect on DMI (Stanton et al., 1994; Mader and Colgan, 2007). The decrease in DMI for BED steers compared to NO may, in part, be attributable to consumption of the bedding material. However, it may also be due to decreased maintenance requirements for the BED steers as a result of bedding application.

Overall G:F was increased 5.6% for BED steers relative to NO steers in Exp. 2. Steers from the BED treatment tended to consume less DMI throughout the 56 d receiving period but had equal final BW and ADG, subsequently, allowing for greater G:F. Anderson, Wiederholt and Schoonmaker (Anderson et al., 2006) did not report a difference in d 56 G:F in trial 1, however, G:F was significantly increased for the bedded treatment in trial 2. Mader and Colgan (2007) reported no improvements in G:F during the initial periods of trial 1 and 2. The MQ in Exp. 2, was elevated by 18% for NO compared to BED. Daily ambient temperature averaged -3.0 ± 5.5°C and windchill averaged -5.1 ± 6.1°C during the 56-day receiving study. Temperatures during Exp. 2 were not as severe as the initial 36-day period in Exp. 1. However, an 18% cumulative increase in MQ was still noted for NO steers compared to BED. Cold temperatures are well known to increase the maintenance requirement of beef cattle (Young, 1981, 1983), and this has been demonstrated in a number of previous studies (Birkelo et al., 1991;

Anderson et al., 2006; Mader and Colgan, 2007). In the present study, steers from NO had increased maintenance requirements relative to BED. Bedding application likely lessened the increase in maintenance energy costs in BED steers by providing improved comfort and insulative protection to conserve body heat as well as mitigating some of the stress commonly experienced by cattle during the receiving phase.

Serum Metabolites - Experiment 2

A 13% decrease in SUN concentration was noted for BED steers compared to NO. Concentration of SUN is often used as an indicator of metabolic status in beef cattle with regards to anabolism or catabolism of lean tissue. The observed decrease in SUN may be attributable to the bedding application which, perhaps, aided in stress mitigation via improved comfort and lowered the calculated maintenance coefficient for BED steers, thus, more energy was available for anabolism of lean tissue. Additionally, SUN differed over time. This is perhaps a result of lower temperatures later in the receiving period. Elevated serum NEFA are an indicator of adipose tissue catabolism. Not applying bedding during the 56-d receiving study resulted in a 22% increase in serum concentration of NEFA for NO steers compared to BED. The increase in serum concentration of NEFA for NO steers is likely further indication that BED cattle, due to their lower calculated maintenance coefficient, spent less time in a negative energy balance, and thus did not catabolize adipose tissue in a manner as the NO steers. Serum concentration of NEFA also decreased over time for both treatments. This decrease over time is expected as even healthy newly received calves, during the first week post-arrival, consume approximately 1.6% of BW. In addition to relatively low intakes, newly received calves encounter a large variety of stressors during this period such as weaning,

adverse environmental conditions, transportation, lack of feed and water, and introduction to unfamiliar feed resources (Blom, 2019). Therefore, these stressors are a likely explanation for serum concentration of NEFA initially being elevated for both treatments and subsequently decreasing throughout the 56-d receiving study.

CONCLUSIONS

In Experiment 1, applying wheat straw bedding to yearling crossbred beef steers at a rate of 1.8 kg/steer·d^{-1} increased DMI, G:F, and ADG. Bedding cattle also reduced the estimated MQ during the entirety of the trial by 11.2%. In Experiment 2, newly weaned receiving calves bedded with 1.0 kg of wheat straw bedding/steer·d^{-1} tended to consume 4.5% less dry matter, and had a 5.6% improvement in G:F. Additionally, MQ was elevated 18% in the non-bedded treatment. These data indicate that, depending on geographical location, cost of bedding, and weather conditions, bedding application should be considered to improve growth performance and feed efficiency in beef steers by reducing maintenance energy requirements during the feedlot receiving and finishing phases.

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Table 2.1. Experiment 1 – Diet composition (DM basis)[a]

Item	Finisher 1	Finisher 2[b]
Dry-rolled corn, %	69.70	70.33
Dried distillers grains, %	17.00	16.85
Oatlage, %	8.37	-
Grass hay, %	-	7.89
Liquid supplement[c], %	4.93	4.93
Nutrient Composition[d]		
Dry matter, %	77.50	85.26
Crude protein, %	14.20	12.88
Neutral detergent fiber, %	16.60	17.76
Acid detergent fiber, %	6.84	7.14
Ash, %	5.25	5.30
NEm[e], Mcal/kg	2.10	2.10
NEg[f], Mcal/kg	1.40	1.40

[a]All values except dry matter or a DM basis.
[b]Diet fed for final 12-d of the study when oatlage supply was depleted
[c]Liquid supplement: formulated to add 30 g/t of monensin to diet DM and vitamins and minerals to meet or exceed NASEM (2016) requirements.
[d]Tabular NE from (Preston, 2016) and actual nutrient compositions from weekly assay of individual dietary ingredients and feed batching records
[e]Net energy for maintenance
[f]Net energy for gain

Table 2.2. Experiment 2 – Diet composition (DM basis)[a]

Item	
Corn silage[b]	63.69
Dried distillers grains plus solubles	20.31
Grass hay	10.00
Pelleted supplement[c]	6.00
Soybean meal	*(3.777)*
Soybean hulls	*(0.353)*
Trace mineralized salt	*(0.30)*
Calcium carbonate	*(1.11)*
Premix[d]	*(0.072)*
Nutrient Composition[d]	
Dry matter, %	41.99
Crude protein, %	13.09
Neutral detergent fiber, %	40.00
Acid detergent fiber, %	28.17
Ash, %	6.29
NE_M, Mcal/kg	1.74
NE_G, Mcal/kg	1.12

[a]All values except dry matter on a DM basis.
[b]Corn silage (n = 9 samples) contained (DM basis, except for dry matter): 31.50% dry matter, 6.18% crude protein, 39.50% NDF, 30.22% ADF, and 4.58% ash.
[c]Inclusion to total diet DM included in parentheses.
[d]Tabular NE from (Preston, 2016) and actual nutrient compositions from weekly assay of individual dietary ingredients and feed batching records.

Table 2.3. Experiment 1: Effect of bedding on cattle growth performance and carcass characteristics[a]

	Bedding Treatment[a]			
Item	NO	BED	SEM	*P*-values
Pens, *n*	15	15	-	-
Initial Growth Performance (d 1 – 36)				
Initial body weight, kg	365	365	0.4	0.95
d 36 BW	402	419	1.5	0.01
Average daily gain, kg/d	1.02	1.51	0.044	0.01
Dry matter intake, kg/d	8.19	8.22	0.047	0.57
ADG/DMI, kg/kg	0.124	0.185	0.0047	0.01
Maintenance coefficient, Mcal/$W^{0.75}$	0.146	0.104	0.003	0.01
Cumulative Growth Performance (d 1 – harvest)				
Days on Feed	178	143	-	-
Final Shrunk BW, kg [b]	575	569	2.0	0.07
Average daily gain (ADG), kg/d	1.18	1.43	0.019	0.01
Dry matter intake (DMI), kg/d	9.30	9.84	0.124	0.01
ADG/DMI, kg/kg	0.127	0.146	0.002	0.01
Maintenance Coefficient, Mcal/$W^{0.75}$	0.109	0.098	0.010	0.01
Carcass Characteristics				
Dressing percentage, %[c]	63.29	62.30	0.140	0.01
Hot carcass weight (HCW), kg	359	356	1.3	0.07
Ribeye area, cm^2	83.16	82.71	0.76	0.69
Rib fat, cm	1.20	1.09	0.02	0.01
Marbling[d]	475	437	6.6	0.01
Estimated empty body fat, %[e]	28.95	28.29	0.140	0.01
Calculated yield grade	2.95	2.81	0.045	0.03
Retail yield, %[f]	50.53	50.92	0.100	0.01

[a]Treatments: No bedding applied (NO), 1.8 kg (as-is basis) of wheat straw/steer·d^{-1} (BED).
[b]Calculated from HCW/0.625.
[c]HCW/final BW (shrunk 4%).
[d]400 = $Small^{00}$ (USDA Low Choice).
[e]According to Guiroy et al. (2002).
[f]As a percentage of HCW according to Murphey et al. (1960).

Table 2.4. Experiment 2 - Effect of bedding on cattle growth performance

	Bedding Treatment[a]			
Item	NO	BED	SEM	*P*-values
Pens, *n*	9	9	-	-
Days on feed	56	56	-	-
Growth Performance (d 1 – 56)				
Initial body weight, kg	278	278	0.22	0.69
Final shrunk BW[b]	353	355	2.2	0.70
Average daily gain, kg/d	1.36	1.38	0.04	0.67
Dry matter intake, kg/d	6.9	6.6	0.09	0.06
ADG/DMI, kg/kg	0.198	0.209	0.005	0.03
Maintenance coefficient, Mcal/$W^{0.75}$	0.052	0.044	0.002	0.03

[a] Treatments: No bedding applied (NO), 1.0 kg (as-is basis) of wheat straw bedding/steer·d^{-1} (BED).

[b] Final BW was BW from day 56 that was pencil shrunk 4% to account for gastrointestinal tract fill.

FIGURE CAPTIONS

Figure 2.1. Experiment 1: Cumulative average daily ambient temperature (C°) and average wind chill temperature (C°) during the study (January 15, 2019 to July 17, 2019).

Figure 2.2. Experiment 2: Cumulative average daily ambient temperature (C°) and average wind chill temperature (C°) during the study (October 24, 2019 to December 19, 2019).

Figure 2.3. Experiment 1: Effect of bedding treatment on serum concentration of urea-N (SUN) in finishing steers (n = 15 pens/treatment; pooled bed × day; SEM = 0.23). Treatments were: No bedding applied (NO); 1.8 kg (as-is basis) of wheat straw bedding/steer·d^{-1} (BED). Blood collected and harvest as sera on d 36, 64, 92, and 120.

Figure 2.4. Experiment 1: Effect of bedding treatment on serum concentration of insulin-like growth factor I (IGF-I) in finishing steers (n = 15 pens/treatment; pooled bed × day; SEM = 25.71). Treatments were: No bedding applied (NO); 1.8 kg (as-is basis) of wheat straw bedding/steer·d^{-1} (BED). Blood collected and harvest as sera on d 36, 64, 92, and 120.

Figure 2.5. Experiment 2: Effect of bedding treatment on serum concentration of urea-N (SUN) in finishing steers (n = 9 pens/treatment; pooled bed × day; SEM = 0.82). Treatments were: 1) no bedding (NO), or 2) 1.0 kg (as-is basis) of wheat straw bedding/steer·d^{-1} (BED). Blood collected and harvest as sera on d 1, 14, 28, 42, and 56.

Figure 2.6. Experiment 2: Serum concentration of non-esterified fatty acids (NEFA) in finishing steers (n = 9 pens/treatment; pooled bed × day; SEM = 0.038). Treatments were: 1) no bedding (NO), or 2) 1.0 kg (as-is basis) of wheat straw bedding/steer· d^{-1} (BED). Blood collected and harvest as sera on d 1, 14, 28, 42, and 56.

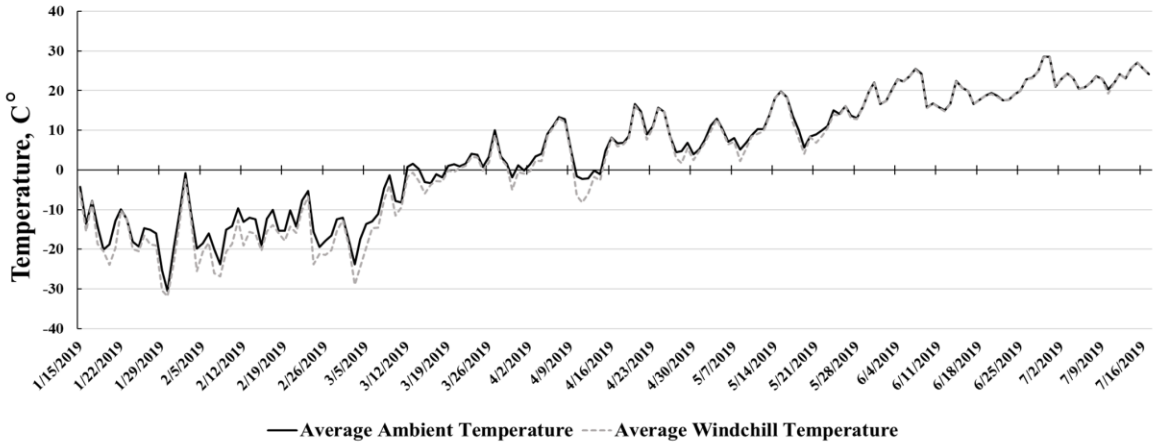

Figure 2.1. Experiment 1: Cumulative average daily ambient temperature (°C) and average wind chill temperature (°C).

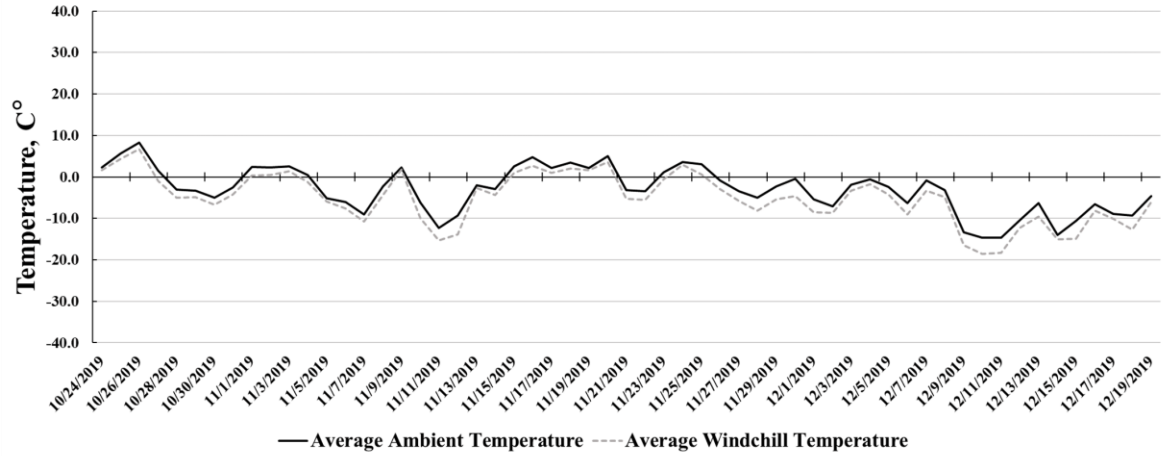

Figure 2.2. Experiment 2: Cumulative average daily ambient temperature (°C) and average wind chill temperature (°C).

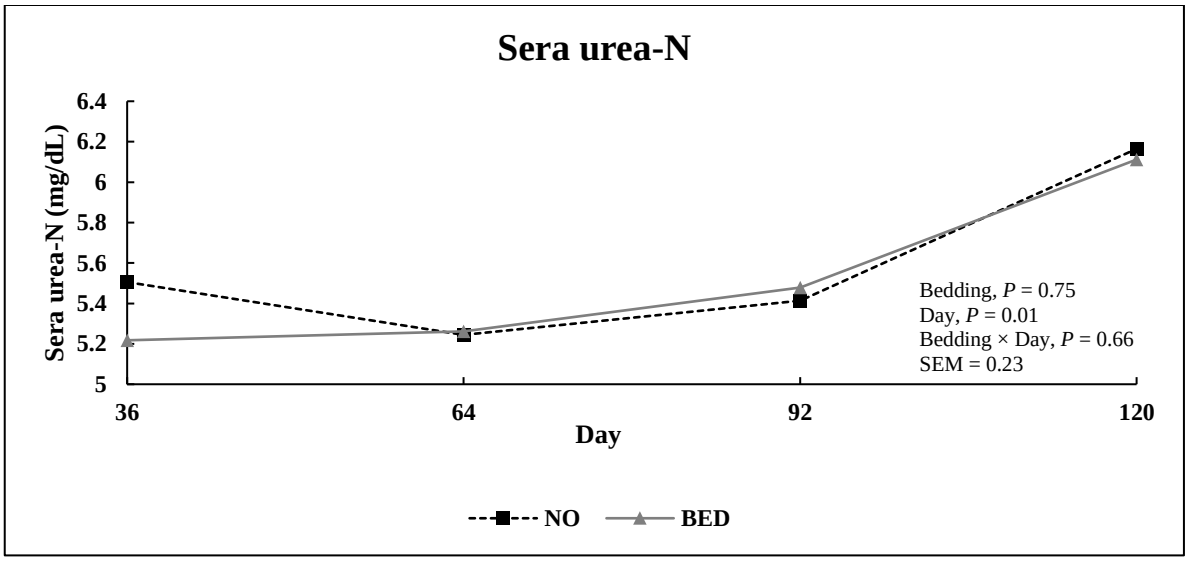

Figure 2.3. Experiment 1: Effect of bedding treatment on serum concentration of urea-N (SUN) in finishing steers.

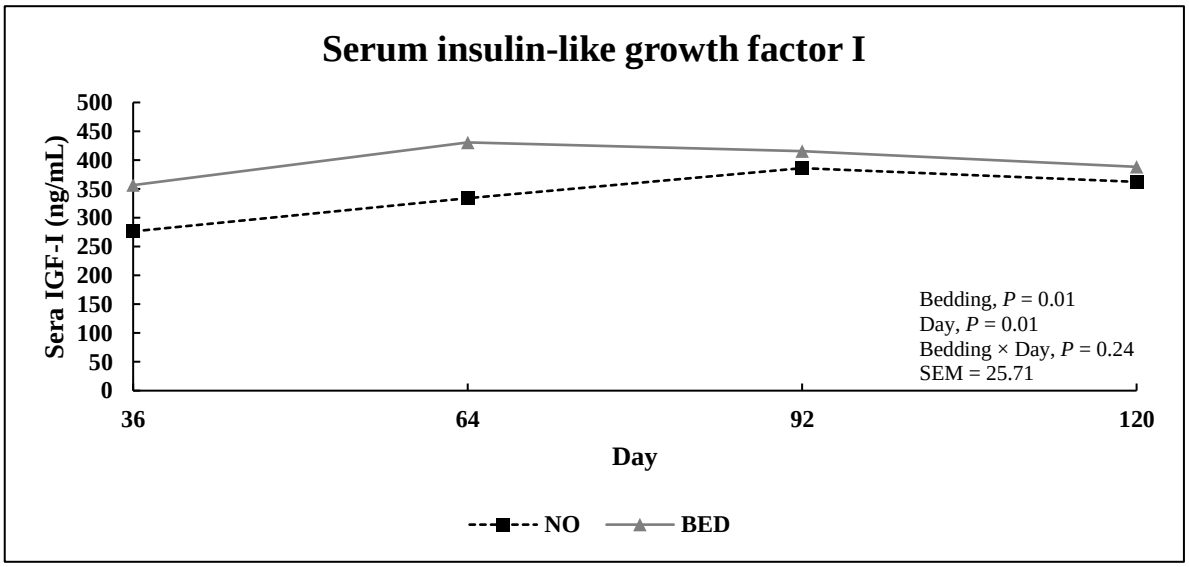

Figure 2.4. Experiment 1: Effect of bedding treatment on serum concentration of insulin-like growth factor I (IGF-I) in finishing steers.

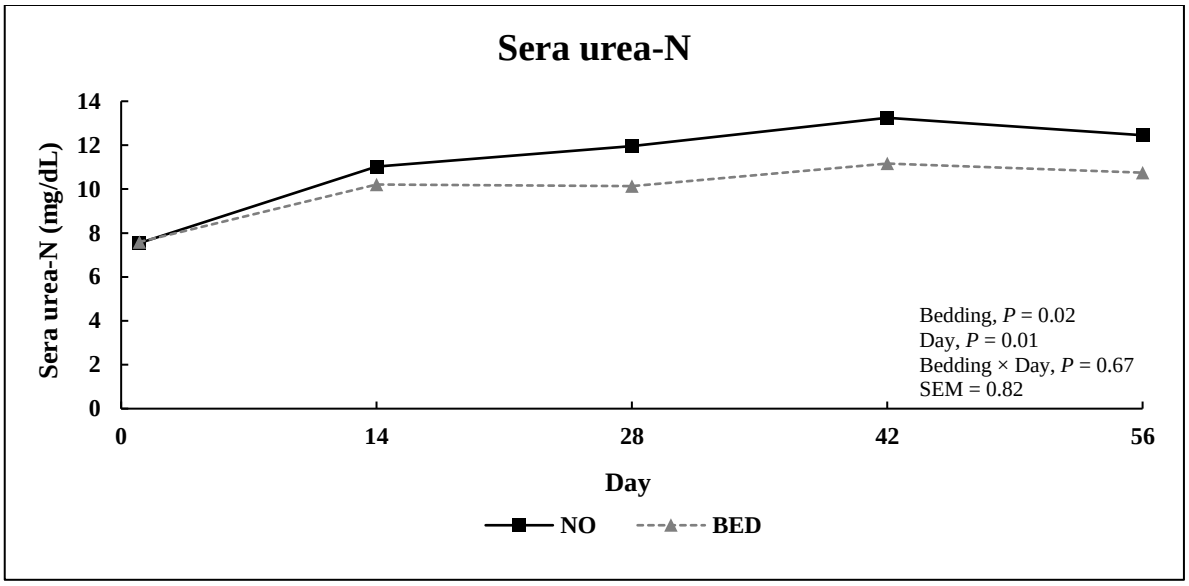

Figure 2.5. Experiment 2: Effect of bedding treatment on serum concentration of urea-N (SUN) in finishing steers.

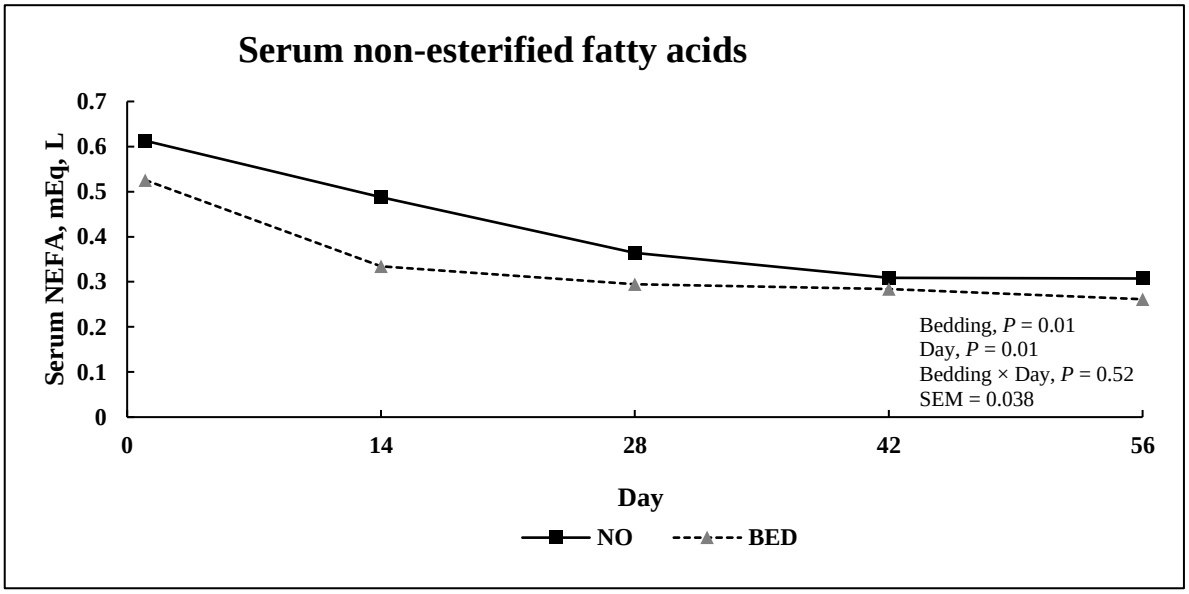

Figure 2.6. Experiment 2: Serum concentration of non-esterified fatty acids (NEFA) in finishing steers.

CHAPTER III: EFFECTS OF INCREASING DOSES OF TRENBOLONE ACETATE AND ESTRADIOL ON FINISHING PHASE GROWTH PERFORMANCE, CARCASS TRAIT RESPONSES, AND SERUM METABOLITES IN BEEF STEERS FOLLOWING IMPLANTATION

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ABSTRACT

Yearling Simmental × Angus crossbred beef steers (n = 240; allotment BW = 365 ± 22.5 kg) from a South Dakota auction facility were transported 117 km to Brookings, SD and used in a randomized complete block design feedlot study to evaluate the effects of implants (both from Zoetis, Parsippany, NJ) containing increasing doses of trenbolone acetate (TBA) and estradiol benzoate (EB) administered 124 d prior to harvest have on finishing phase growth performance, carcass characteristics, and serum concentrations of urea-N (SUN) and insulin-like growth factor I (IGF-I). Thirty pens (10 pens/treatment) were assigned to 1 of 3 treatments: 1) negative control given no implant (NI); 2) a steroidal implant containing 100 mg TBA and 14 mg EB administered subcutaneously in the center one-third of the ear on d 1 (Synovex Choice, Zoetis, Parsippany, NJ; CH); 3) a steroidal implant containing 200 mg TBA and 28 mg EB administered subcutaneously in the center one-third of the ear on d 1 (Synovex Plus, Zoetis; PL). Cattle were fed for 124 d post-implantation. Steers were fed a common diet throughout the study. Treatment effects were evaluated by the use of orthogonal polynomials. Pen was the experimental unit for all analyses; an α of 0.05 determined significance. There was a quadratic effect

(P = 0.01) on carcass adjusted final BW. Increasing doses of TBA and EB resulted in a linear increase for both average daily gain (P = 0.01) and dry matter intake (P = 0.02). A quadratic effect on gain to feed ratio was observed (P = 0.01). No quadratic ($P \geq 0.40$) or linear ($P \geq 0.14$) effects were observed for dressing percentage, rib fat (RF), calculated yield grade, or marbling scores. A quadratic increase (P = 0.01) in hot carcass weight (HCW) and a linear increase (P = 0.01) in ribeye area (REA) was detected. No significant implant × day interaction ($P \geq 0.09$) was noted for serum concentrations of urea-N or IGF-I. Implants decreased (P = 0.01) SUN compared to NI. Serum concentration of IGF-I was increased (P = 0.04) in implanted steers compared to NI steers. In yearling crossbred beef steers the use of steroidal implants containing a combination of 100 mg TBA + 14 mg EB or 200 mg TBA + 28 mg EB increases growth performance, HCW, and REA at equal RF accumulation without detriment to marbling score compared to non-implanted steers.

Key words: estradiol, growth performance, implant, trenbolone acetate

INTRODUCTION

Steroidal implants have been used in U.S. commercial beef production for over 63 years and can be expected to improve growth rate 10 to 30%, feed efficiency 5 to 15%, and carcass leanness 5 to 8% (Preston, 1999). A meta-analysis investigating feedlot steer implant programs found in a comparison that across all single-implant treatments, implants increase live weight gain, dry matter intake (DMI), dressing percentage (DP), hot carcass weight (HCW), ribeye area (REA), gain to feed ratio (G:F) and decrease the percentage of carcasses grading USDA Choice or greater, and USDA marbling score compared to non-implanted steers (Reinhardt and Wagner, 2014). Effect of steroidal implant on marbling score is often shown to be negative, however, it has been reported (Johnson et al., 1996a) that administration of a combination trenbolone acetate (TBA) and estradiol-17β (E_2) implant did not have deleterious effects on marbling score.

The androgenic constituent of steroidal implants, TBA, has a direct effect on skeletal muscle that increases muscle tissue anabolism while decreasing muscle tissue catabolism, thus increasing net protein synthesis (Smith and Johnson, 2020). Previous research has shown that the anabolic effect of steroidal implants results in decreased serum concentration of urea-N (SUN) concentrations after implantation with a combination TBA + E_2 implant (Smith et al., 2018b). The estrogenic constituent of steroidal implants, E_2, functions by increasing production and release of hepatic somatotropin and IGF-I (Reinhardt, 2007), and have been reported to increase local IGF-I production in steers through measurement of concentration of IGF-I mRNA in the longissimus muscle of steers implanted with a combination TBA + E_2 implant (Johnson et al., 1998). It has been previously reported that combination TBA + E_2 implants

increase circulating serum concentration of IGF-I (Johnson et al., 1996b; Smith et al., 2018a). It has been demonstrated that increasing the initial dosage of hormonal constituents does not increase cumulative live growth performance (Hilscher et al., 2016) when steers and heifers were administered the same terminal implant. Others have indicated in heifers that a greater total dose of steroidal hormones does not increase live-basis growth performance, and only moderately increases HCW as well as indicators of carcass muscularity and carcass leanness (Smith et al., 2020).

The objective of this study was to evaluate the effects of increasing doses of TBA and EB on finishing phase growth performance, carcass characteristics, and serum concentration of urea-N and IGF-I. The hypothesis was that increasing terminal implant dosage in steers would increase carcass-adjusted growth performance, HCW, and muscularity.

MATERIALS ANDS METHODS

Use of Animal Subjects

Animal care and handling procedures used in this study were approved by the South Dakota State University Animal Care and Use Committee (Approval number: 18-096A)

Animal Description and Initial Processing

Yearling Simmental × Angus crossbred beef steers (n = 240; allotment BW = 365 ± 22.5 kg) were transported 117 km from a South Dakota auction facility to the Ruminant Nutrition Center (RNC) in Brookings, SD for use in this experiment. Steers were allotted to 30 concrete surface pens (7.25 × 7.25 m; 6.57 m^2/steer; 90.6 cm of bunk space/steer; n = 8 steers/pen) 36 d prior to being implanted. The first 6 pen replicates began on test 14 d

prior to the last 4 pen replicates due to timing of acquisition of sufficient cattle needed in order to conduct the experiment.

Initial processing included an individual body weight measurement, application of a unique identification ear tag, and a rectal temperature measurement along with vaccination for respiratory syncytial virus (BRSV), bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD) Types 1 and 2, parainfluenza-3 (PI_3), Mannheimia haemolytica, and clostridium perfringens type A; and administered pour-on moxidectin. Cattle were re-vaccinated 36 d after initial processing for clostridium perfringens type A. Any steer with a rectal temperature of greater than 39.4°C was administered tulathromycin according to label instructions.

Experimental Design and Treatments

Pens were assigned to 1 of 3 implant treatments with ten replicate pens assigned to each treatment: 1) negative control given no implant (NI); 2) a steroidal implant containing 100 mg TBA and 14 mg estradiol benzoate administered subcutaneously in the center one-third of the ear on d 1 (Synovex Choice, Zoetis, Parsippany, NJ; CH); 3) a steroidal implant containing 200 mg TBA and 28 mg estradiol benzoate administered subcutaneously in the center one-third of the ear on d 1 (Synovex Plus, Zoetis; PL).

Dietary Management

Composition of the finishing diet fed from d 18 to harvest is presented in Table 1. Due to an evolving roughage inventory, a switch to grass hay from oatlage occurred with 12 d remaining in the experiment. The finishing diet consisted of dry-rolled corn, dried distillers grains plus solubles, and oatlage or grass hay was fed and contained 2.10 Mcal/kg of NE_m, and 1.40 Mcal/kg of NE_g. A liquid supplement was provided to add 30

g/907-kg of monensin sodium to diet DM along with supplemental vitamins and minerals to meet (NASEM, 2016) requirements.

All steers were fed twice daily at 0800h and 1400h; bunks were managed according to a slick bunk management approach. When necessary, orts were collected, weighed, and dried in a forced air oven at 100°C for 24 h to determine DM content if carryover feed went out of condition or was present on weigh days. If carryover feed was present on weigh days, the residual feed was removed prior to the collection of BW measurements. 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.

Diets presented in Table 3.1 are actual DM diet composition from weekly ingredient DM analysis, actual assayed nutrient concentrations from weekly commodity ingredient sampling of the dry rolled corn, dried distillers grains plus solubles and forage source for crude protein (CP), neutral detergent fiber (NDF; except for corn where the NDF was estimated to be 9%), acid detergent fiber (ADF; except for corn where the ADF was estimated to be 3%), ash, and ether extract (EE): method no. 968.06, (AOAC, 2016) for CP, using the Rapid Max N Exceed, Elementar, Mt. Laurel, NJ; NDF and ADF, (Goering and VanSoest, 1970); method no. 942.05; (AOAC, 2012) for ash; and EE using petroleum ether, method no. 2003.06; (AOAC, 2007), and tabular energy values according to Preston (2016) were used.

Blood Sample Collection

Whole blood samples were collected into 10 mL non-additive tubes during the weighing process prior to feeding on d 1, 28, 56, and 84 (relative to implantation) from sentinel steers (n = 2 steers/pen). Whole blood was allowed to clot for 24 h at 4°C and

was subsequently centrifuged at 1250 × g at 4°C for 20 min. A total of three aliquots were collected and stored at -20°C until subsequent analyses to quantify serum concentrations of urea-N and IGF-I.

Serum concentrations of urea-N and insulin-like growth factor I

Serum concentrations of urea-N were determined by a method described by Fawcett and Scott (1960) using sodium phenate and sodium hypochlorite. The determination of SUN is measured based on the reaction of ammonia with sodium phenate and hypochlorite to yield a blue color to be measured in a spectrophotometer. Absorbance for reactions of standards and samples were read at 625 nm. Samples were considered for re-runs if the coefficient of variation (CV) was greater than 10% among triplicate determinations. Intra- and inter-assay CV were 6.3% and 10.9%, respectively.

Serum concentrations of insulin-like growth factor I (IGF-I) were determined in duplicate via radioimmunoassay (RIA) procedure (Echternkamp et al., 1990; Funston et al., 1995). Insulin-like growth factor binding proteins (IGFBP) in serum were extracted using a 1:17 ratio of sample to acidified ethanol (12.5% 2 N HCl: 87.5% absolute ethanol) (Daughaday et al., 1980). Extracted samples were centrifuged (12,000 × g at 4°C) to separate IGFBP. A portion of the resulting supernatant was removed and neutralized with 0.855 M Tris base, incubated for an additional 4 h at 4°C, and then centrifuged at 12,000 × g at 4°C to remove any additional IGFBP. When samples of this extract, equivalent to the original serum sample, were subjected to Western ligand blot analysis and subsequent phosphorimagery, no detected binding of I-IGF-I to IGFBP was observed. Inhibition curves of the neutralized extracted serum ranging from 12.5 to 50 µL were parallel to the standard curve. Recombinant human IGF-I (GF-050; Austral

Biological, San Ramon, CA, USA) was used as the standard and radioiodinated antigen. Antiserum AFP 4892898 (National Hormone and Peptide Program, National Institutes of Diabetes, Digestive and Kidney Diseases, Bethesda, MD, USA) was used at a dilution of 1:62,500. Sensitivity of the assay was 14.7 pg/tube. Samples were considered for re-runs if the CV was greater than 10% among duplicate determinations. No samples were considered for re-runs; the RIA was completed in a single assay and the intraassay CV was 7.7%.

Growth Performance Calculations and Carcass Data Collection

Steers were individually weighed and harvested after an average of 124 d on feed. Weight gain was based upon initial un-shrunk BW (average of d −1 and 1 BW) and final BW was calculated from HCW/0.625. All steers that were pulled from their home pen for health evaluation were then monitored in individual hospital pens prior to being returned to their home pens. When a steer was moved to a hospital pen the appropriate amount of feed from the home pen was removed and transferred to the hospital pen. If the steer in the hospital returned to their home pen, this feed remained credited to the home pen. If the steer did not return to their home pen, all feed that was delivered to the hospital pen was deducted from the feed intake record for that particular pen back to the date the steer was hospitalized.

Cattle were on feed for an average of 124 d post-implantation before being marketed and harvested at a commercial abattoir (Tyson Fresh Meats, Dakota City, NE) when the population reached sufficient fat cover to grade USDA Choice. Carcass data including HCW, REA, 12th rib fat (RF), kidney, pelvic, and heart fat percent, and USDA marbling score were collected by the camera grading system at the abattoir. Yield grade

(YG) was calculated by using the USDA regression equation (USDA, 1997). Estimated empty body fat (EBF) from carcass traits was calculated according to (Guiroy et al., 2002b). Retail Yield (RY) as percentage of HCW was calculated according to Murphey et al. (1960).

Statistical Analysis

Growth performance and carcass data were analyzed as a randomized complete block design experiment using the GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC), considering implant treatment and block (pen location) as fixed effects. Pen served as the experimental unit for growth performance and carcass traits. Treatment effects were evaluated by the use of orthogonal polynomials (Steel and Torrie, 1960). All results are reported as least squares means.

Serum concentrations of urea-N and IGF-I data were analyzed according to randomized complete block design appropriate for repeated measures using the MIXED procedure of SAS 9.4 (SAS Inst. Inc.). The model included the fixed effects of implant, day, and their interaction. Day was included as the repeated variable and pen served as the experimental unit. Day 0 values for serum concentrations of urea-N and IGF-I were used as covariate adjustments ($P \leq 0.06$) in the repeated measures model. The covariance structure with the lowest Akaike information criterion was used (Littell et al., 1998). Compound Symmetry was the covariance structure used for serum concentration of urea-N and Huynh-Feldt was the covariance structure used for serum concentration of IGF-I. All results are reported as least squares means. A *P*-value of 0.05 ($\alpha = 5\%$) determined significance and a *P*-value between 6% and 10% was considered a tendency.

RESULTS

Animal Growth Performance

Initial bodyweight at time of implant did not differ ($P = 0.51$) between treatments. A quadratic effect ($P = 0.01$) on carcass adjusted final BW was noted; CH was increased 4.5% and PL was increased 5.6% relative to the NI control. Increasing doses of TBA and EB resulted in a linear increase ($P = 0.01$) in cumulative ADG, the increases compared to the NI control group were 18.4% and 21.6%, respectively, for CH and PL treatments. Increasing doses of TBA and EB also resulted in a linear increase in DMI ($P = 0.02$). Dry matter intake was increased by 2.3% and 7.0% for CH and PL treatments, respectively, relative to NI. A quadratic effect on G:F was observed for implanted treatments, increasing by 21.1% and 19.5% for CH and PL, respectively, compared to NI.

Carcass Characteristics

No linear ($P \geq 0.14$) or quadratic ($P \geq 0.40$) effects were observed for DP, RF, YG, or USDA marbling scores. However, a quadratic increase ($P = 0.01$) in HCW was noted. Hot carcass weight was increased by 4.6% and 5.5% for CH and PL, respectively, compared to NI. A linear increase ($P = 0.01$) in REA was observed. Ribeye area was increased by 4.1% and 7.7% for CH and PL treatments, respectively compared to NI steers.

Serum concentrations of urea-N and insulin-like growth factor I

A significant implant × day interaction ($P = 0.09$) was not noted for serum concentrations of urea-N (Figure 1). The main effect of implant decreased ($P = 0.01$) serum concentrations of urea-N. Steers from CH tended ($P = 0.07$) to have decreased serum concentrations of urea-N compared to NI by 5.8%, steers from PL had decreased

(P = 0.01) serum concentration of urea-N compared to NI by 9.8%. Serum concentration of urea-N increased (P = 0.01) as days post-implantation increased. No implant × day interaction (P = 0.76) was detected for concentrations of serum IGF-I (Figure 2). However, the main effect of implant increased (P = 0.04) serum concentrations of IGF-I. Steers from CH had increased (P = 0.04) serum concentration of IGF-I by 20.1% compared to NI steers; steers from PL had increased (P = 0.02) serum concentration of IGF-I by 23.2% compared to NI steers. Serum concentration of IGF-I was not influenced by days post-implantation (P = 0.01).

DISCUSSION

Animal Growth Performance

Increasing doses of TBA and EB from 100 mg TBA + 14 mg EB (CH) to 200 mg TBA + 28 mg EB (PL) resulted in a linear increase in cumulative ADG. These results agree well with previously reported findings regarding gain responses for cattle following implantation with a single androgenic + estrogenic combination implant (Duckett et al., 1997; Johnson and Beckett, 2014). In the present study, DMI increased linearly with increasing doses of TBA and EB. Increased DMI due to exposure to a combination androgenic + estrogenic implant also concurred with previous research findings (Duckett et al., 1997; Reinhardt and Wagner, 2014; Smith et al., 2018b). Increases in DMI as a result of anabolic implant exposure is likely linked to concurrent increases in final BW (Guiroy et al., 2002a). However, in the present study there was a quadratic effect on carcass adjusted final BW; CH was increased 4.5% and PL increased 5.6% relative to the NI control group. In the present study, the highest dose of TBA and EB (PL) did not result in increased performance relative to the CH treatment. Therefore, the linear

increase of DMI as a response to increasing levels of TBA and EB may not be so simply explained as a result of increasing final BW due to exposure to a more potent terminal implant. Use of a terminal implant, in the present study, caused a quadratic effect on G:F, increasing by 21.1% and 19.5% for CH and PL treatments respectively, compared to NI steers. This positive response in gain efficiency following administration of a terminal implant is in agreement with reported information from a meta-analysis by Wileman et al. (2009) as well as a number of other analyses (Duckett et al., 1997; Reinhardt, 2007; Johnson and Beckett, 2014) in which single implant protocols were compared against a non-implanted control treatment.

Carcass Characteristics

In the present study, use of a combination TBA + EB implant did not influence DP which is similar with previously reported information using TBA + E_2 (Duckett et al., 1997). It has been well documented that the use of combination TBA + E_2 implants in steers results in a significant increase in HCW relative to a non-implanted steers (Bartle et al., 1992; Duckett et al., 1997; Pritchard, 2000; Smith et al., 2018b). Implants increase the amount of protein deposition and decrease the amount of fat deposition at a given weight, thus causing implanted animals to reach similar body composition to that of a non-implanted animal at a heavier weight, thus the increase in HCW occurs concurrently with increases in live BW. In the present study, increasing doses of TBA + EB from 100 mg TBA + 14 mg EB (CH) to 200 mg TBA + 28 mg EB (PL) did not result in additional HCW between the two implants.

Reduced marbling score, and corresponding lowered quality grades have long been a concern when using combination TBA + E_2 terminal implants. Reduced or

delayed subcutaneous and intramuscular fat deposition often occurs in implanted steers fed for equal days due to a shift in composition of gain (Smith et al., 2018a), and also, as reported by Smith et al. (2017), a decrease in expression of important adipogenic genes in the skeletal muscle of steers due to exposure to combination TBA + E_2 implant. It is then of interest, in the present study, that use of combination TBA + EB terminal implant of differing doses did not result in a significant decrease in marbling score compared to NI controls. This agrees with findings from Johnson et al. (1996a), but runs counter to a considerable volume of previous work which has indicated that use of a combination TBA + E_2 implant results in decreased marbling score (Duckett et al., 1997; Pritchard, 2000; Smith et al., 2018b). Bruns et al. (2005), reported that excessive anabolic exposure at key growth stages can have a detrimental impact marbling deposition in beef steers. The level of anabolic exposure experienced by steers from both CH and PL treatments was likely not excessive as evidenced by the lack of an impact on USDA marbling score following implantation with TBA + EB implant. Use of steroidal implants containing a combination of TBA and EB increased HCW, and REA at equal RF accumulation without detriment to USDA marbling score.

Serum concentrations of urea-N and insulin-like growth factor I

Serum concentration of urea-N did not differ at the time of implantation. Serum concentration of urea-N decreased following implantation and this is consistent with work from (Parr et al., 2014b; Smith et al., 2018b). In the present study, implantation with 100 mg or 200 mg of TBA and 14 mg or 28 mg of EB resulted in an increase in serum concentration of IGF-I which is consistent with other findings (Johnson et al., 1996c; Bryant et al., 2010; Parr et al., 2014b; Smith et al., 2018b; Smith et al., 2019).

Serum concentration of IGF-I did not increase as days on feed increased which is inconsistent with what others have demonstrated (Johnson et al., 1996b; Bryant et al., 2010; Parr et al., 2014a; Smith et al., 2019). An anticipated increase in anabolism occurred following administration of a TBA + EB implant and can be identified by a reduction in serum concentration of urea-N following implantation, this coupled with a simultaneous increase in serum concentration of IGF-I aligns well with what has been demonstrated previously in beef steers (Johnson et al., 1996b).

CONCLUSIONS

In yearling crossbred beef steers harvested 124 d post-implantation, the use of steroidal implants containing a combination of 100 mg TBA + 14 EB or 200 mg TBA + 28 EB increases final BW, ADG, DMI, gain efficiency, HCW, and REA at equal RF accumulation without detriment to marbling score compared to non-implanted steers. Use of TBA and EB combination implants, in this study, resulted in increased anabolism as suggested by the observed reduction in serum concentration of urea-N and increased serum concentration of IGF-I compared to NI steers. These results indicate that use of a lower dose implant containing 100 mg TBA + 14 mg EB can result in comparable growth performance to an implant containing 200 mg TBA + 28 mg EB. Additionally, these results provide further evidence that one can capture carcass trait related benefits that TBA + EB implants offer without detriment to marbling score.

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Table 3.1. Composition of finishing diets (DM basis)[a]

Item	Finishing diet
Dry-rolled corn, %	69.70
Dried distillers grains plus solubles, %	17.00
Oatlage[b], %	8.37
Liquid supplement[c], %	4.93
Dry matter, %	77.50
Crude protein, %	14.20
Neutral detergent fiber, %	16.60
Acid detergent fiber, %	6.84
Ash, %	5.25
Ether extract, %	5.13
NEm[d], Mcal/kg	2.10
NEg[e], Mcal/kg	1.40

[a]All values except dry matter or a DM basis.
[b]Due to insufficient oatlage supply, grass hay was used as roughage source for final 12 days of the experiment.
[c]Liquid supplement: formulated to add 30 g/907-kg of monensin sodium to diet DM and vitamins and minerals to meet NASEM (2016) requirements.
[d]Net energy for maintenance
[e]Net energy for gain

Table 3.2. Effect of implant on cattle performance and carcass characteristics

	Implant[a]				Contrast *P*-value	
Item	NI	CH	PL	SEM	L	Q
Pens	10	10	10	-	-	-
Days on feed	124	124	124	-	-	-
Initial body weight, kg	400	397	397	3.4	0.51	0.79
Final BW, kg [b]	553	578	584	2.5	0.01	0.01
Average daily gain, kg/d	1.25	1.48	1.52	0.022	0.01	0.10
Dry matter intake, kg/d	9.66	9.93	10.34	0.196	0.02	0.77
ADG/DMI, kg/kg	0.123	0.149	0.147	0.0030	0.01	0.01
Dressing percentage, %	62.64	62.82	62.92	0.246	0.44	0.89
Hot carcass weight, kg	346	362	365	1.67	0.01	0.01
Ribeye area, cm^2	79.81	83.10	85.94	0.924	0.01	0.86
Rib fat, cm	1.12	1.17	1.14	0.033	0.66	0.56
Marbling [c]	463	458	447	10.4	0.28	0.83
Estimated empty body fat, %[d]	28.64	28.71	28.52	0.205	0.70	0.61
Calculated yield grade	2.92	2.92	2.79	0.062	0.14	0.40
Retail yield, %[e]	50.62	50.64	50.92	0.142	0.15	0.45

[a]Treatments: 1) negative control given no implant (NI); a steroidal implant containing 100 mg TBA and 14 mg estradiol benzoate administered subcutaneously in the center one-third of the ear on d 1 (Synovex Choice, Zoetis, Parsippany, NJ; CH); a steroidal implant containing 200 mg TBA and 28 mg estradiol benzoate administered subcutaneously in the center one-third of the ear on d 1 (Synovex Plus, Zoetis; PL).
[b]Calculated from HCW/0.625.
[c]400 = $Small^{00}$ (USDA Low Choice).
[d]According to Guiroy et al. (2002)
[e]As a percentage of HCW according to Murphey et al. (1960).

FIGURE CAPTIONS

Figure 3.1. Effect of implant treatment on serum concentration of urea-N (SUN) in finishing steers (n = 10 pens/treatment; pooled implant × day; SEM = 0.206). Day 0 SUN values were included as a covariate ($P = 0.01$) in the model. Treatments were: 1) negative control given no implant (NI); 2) a steroidal implant containing 100 mg TBA and 14 mg estradiol benzoate administered subcutaneously in the center one-third of the ear on d 1 (Synovex Choice, Zoetis, Parsippany, NJ; CH); 3) a steroidal implant containing 200 mg TBA and 28 mg estradiol benzoate administered subcutaneously in the center one-third of the ear on d 1 (Synovex Plus, Zoetis; PL).

Figure 3.2. Effect of implant treatment on serum concentration of insulin-like growth factor I (IGF-I) concentrations in finishing steers (n = 10 pens/treatment; pooled implant × day; SEM = 26.376). Day 0 IGF-I values were included as a covariate ($P = 0.06$) in the model. Treatments were: 1) negative control given no implant (NI); 2) a steroidal implant containing 100 mg TBA and 14 mg estradiol benzoate administered subcutaneously in the center one-third of the ear on d 1 (Synovex Choice, Zoetis, Parsippany, NJ; CH); 3) a steroidal implant containing 200 mg TBA and 28 mg estradiol benzoate administered subcutaneously in the center one-third of the ear on d 1 (Synovex Plus, Zoetis; PL).

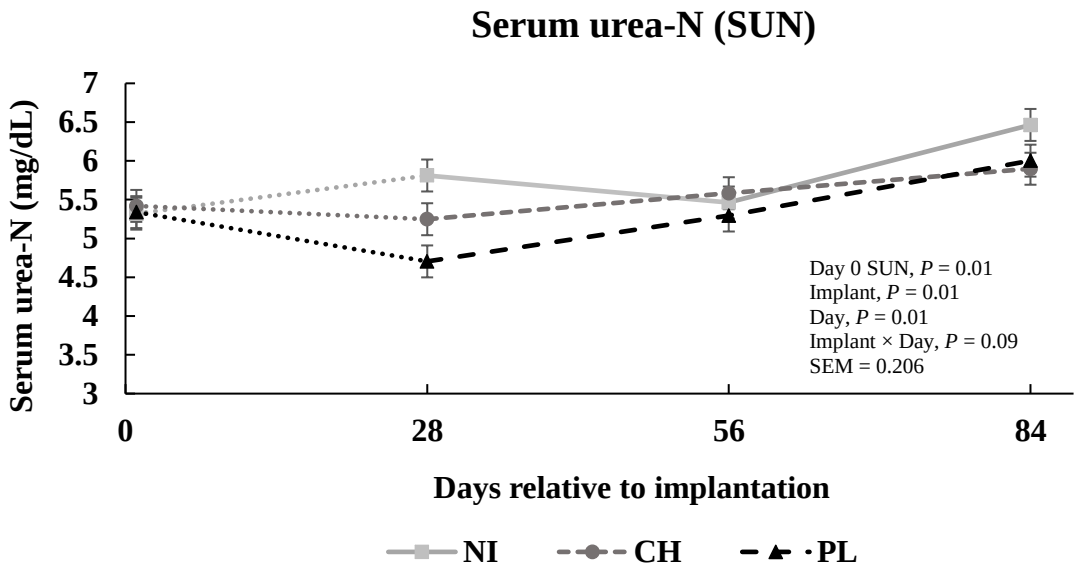

Figure 3.1. Effect of implant treatment on serum concentration of urea-N (SUN) in finishing steers.

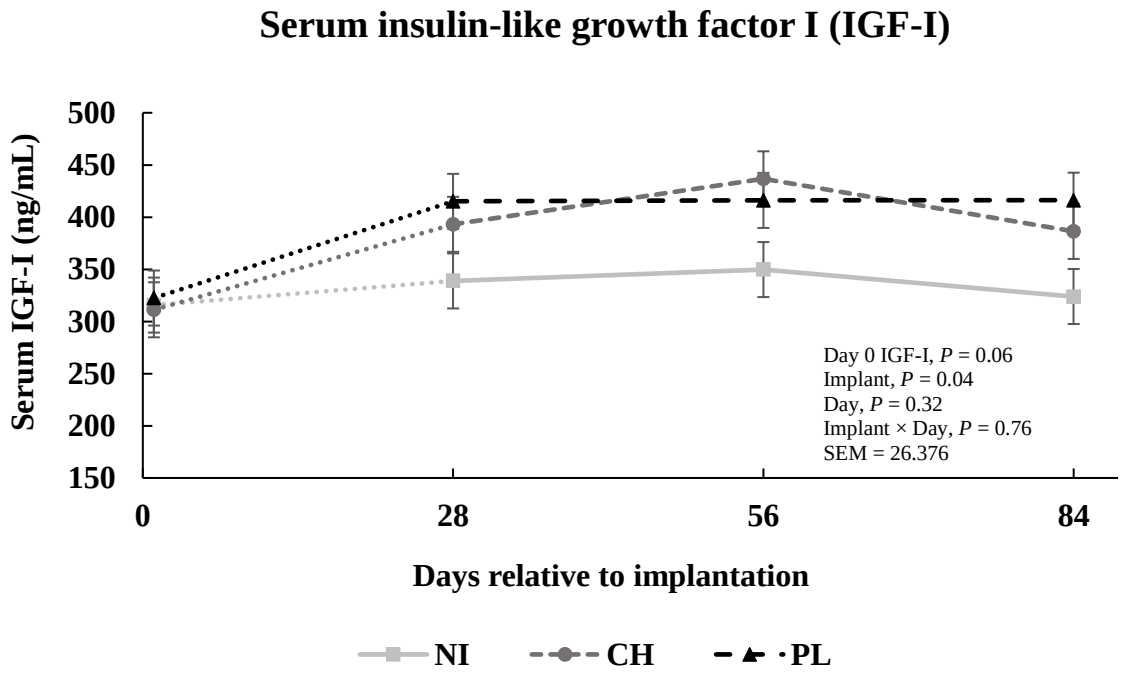

Figure 3.2. Effect of implant treatment on serum concentration of insulin-like growth factor I (IGF-I) concentrations in finishing steers.

APPENDIX A: INCREASING HAY INCLUSION IN SILAGE BASED RECEIVING DIETS AND ITS EFFECTS ONPERFORMANCE AND ENERGY UTILIZATION IN NEWLY WEANED BEEF STEERS[1]

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ABSTRACT

The influence of grass hay (GH) inclusion in replacement of corn silage in receiving diets on growth performance and dietary net energy (NE) utilization was evaluated in newly weaned beef steers (n = 162 Charolais-Red Angus cross steers; initial BW = 278 ± 13.4 kg). Treatments were (DM basis): 1) 0% GH, 2) 10% GH, or 3) 20% GH inclusion in replacement of corn silage in receiving diets fed to newly weaned beef steers for 56-d. The study was conducted from October to December of 2019. Data were analyzed as randomized complete block design with pen serving as the experimental unit for all analyses. Increasing dietary inclusion of hay had no influence ($P \geq 0.11$) on final BW, ADG, gain:feed or observed/expected dietary NE_m and NE_g, observed/expected DMI, or observed/expected ADG. Grass hay inclusion increased (linear effect, $P = 0.01$) DMI. Observed DMI for all treatments was approximately 15 to 17% less than anticipated based upon steer growth performance and tabular NE values. Evaluation of observed/expected ADG was 31 to 37% greater than expected for the steers in the present study. Particles less than 4 mm increased (linear effect, $P = 0.01$) and greater than 4 mm

decreased (linear effect, $P = 0.01$) as grass hay replaced corn silage in the receiving diet. As the proportion of particles greater than 4 mm increased cumulative ADG was decreased. These data indicate that GH should be considered in corn silage based receiving diets to improve DMI. In high-risk calves, improved DMI could result in a lesser incidence of morbidity, although no morbidity was observed in any steers from the present study.

Key words: corn silage, grass hay, naïve calves, net energy

INTRODUCTION

The period that new cattle are received following weaning and transportation to the feedlot is a critical time in beef cattle production. A primary challenge during this receiving phase is the stress of: weaning, transportation, lack of feed and water, and introduction to unfamiliar feed resources (Loerch and Fluharty, 1999; Blom, 2019). Feed intake of newly received feedlot cattle can range from 1% of body weight (BW) in morbid calves to 1.6% of BW in healthy calves (Hutcheson and Cole, 1986). Thus, dry matter intake (DMI) of newly received cattle is often managed in accordance with set protocols developed by the consulting nutritionist or veterinarian and feed yard managers. This is to ensure cattle are consuming feed above maintenance as quickly as possible post-arrival to the feed yard in order to minimize morbidity and reduced animal growth performance. Preston (2007) indicated that in lighter weight calves, the addition of roughage to receiving calve diets might not be beneficial since the calves are at an inadequate DMI level. Preston (2007) postulated that offering newly weaned calves a more energy dense diet with a lower roughage content may help in achieving energy demands of the beef calve at a lower DMI. In the most recent feedlot nutritionist survey only 4.2% of respondents indicated that they use corn silage as a primary roughage source in receiving calf diets (Samuelson et al., 2016). However, corn silage is a primary feed ingredient for beef production in the Midwest. It is a readily digestible energy and NDF source and is an option for marketing home-raised feedstuffs through cattle. The sources of dietary roughage in receiving diets fed to feedlot cattle are important in facilitating adaptation to the new diet in naïve, newly weaned feeder calves. Dry forage feedstuffs are more familiar to cattle transitioning into the feedlot from pasture, however,

many feedlots in the upper Midwest region of the United States use ensiled forages. A primary deterrent to the use of ensiled feed for naïve calves is that it is an unfamiliar feedstuff to calves coming off of pasture (Blom, 2019). The objective of the present study was to evaluate the influence of increasing levels of dietary grass hay inclusion to corn silage based receiving diets on animal growth performance and efficiency of dietary net energy (NE) utilization in newly weaned beef steers.

MATERIALS AND METHODS

Animal care and handling procedures used in this study were approved by the South Dakota State University Animal Care and Use Committee (Approval Number: 19-054E).

Animal Management and Dietary Treatments

One hundred and sixty-two, newly weaned, Charolais × Red Angus beef steers (278 ± 13.4 kg) were transported 513 km from a sale barn in western South Dakota to the Ruminant Nutrition Center (RNC) in Brookings, SD in October of 2019. Upon arrival to the RNC, steers were housed in 7.62 m × 7.62 m concrete surface pens with 7.62 m of linear bunk-space and provided *ad libitum* access to long-stem grass hay (6.18% crude protein, 39.50% NDF, 30.22% ADF, and 4.58% ash) and water. The following day (d -1), all steers were individually weighed (readability 0.454 kg), applied a unique identification ear tag, vaccinated for viral respiratory pathogens: IBR, BVD 1 and 2, PI_3, and BRSV (Bovi-Shield Gold 5, Zoetis, Parsippany, NJ) and clostridials (Ultrabac 7/Somubac, Zoetis). The afternoon following initial processing, all steers were allotted to their study pens (n = 9 steers/pen and 6 pens/treatment). The following morning (d 1) all steers were again individually weighed as well as administered pour-on moxidectin

(Cydectin, Bayer, Shawnee Mission, KS) according to label directions, and test diets were initiated. On study d 14 all steers were implanted with 200 mg progesterone and 20 mg estradiol benzoate (Synovex-S, Zoetis), and an implant retention check occurred on d 42. The initial on test BW was the average of processing BW (d -1 BW) and d 1 BW. Steers were used to evaluate the effect of grass hay (GH) inclusion in corn silage based diets on feedlot receiving phase growth performance and efficiency of dietary NE utilization. Test diets were offered on top of long-stem grass hay for the first 2 d of the receiving period. Treatments consisted of corn silage based growing diets that included (DM basis): 1) 0% GH, 2) 10% GH, or 3) 20% GH inclusion in replacement of corn silage (Table 1). Diets were fortified to provide vitamins and minerals to meet or exceed nutrient requirements and provided monensin sodium (DM basis) at 27.6 g/T (NASEM, 2016). There was no morbidity or mortality noted in the present study. Fresh feed was manufactured twice daily in a stationary mixer (2.35 m^3; readability 0.454 kg). Orts were collected, weighed and dried in a forced air oven at 100°C for 24 h in order to determine DM content if carryover feed spoiled, or was present on weigh days. If carryover feed was present on weigh days, the residual feed was removed prior to the collection of BW measurements. 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. Actual diet formulation and nutrient composition based upon weekly feed analyses [CP, AOAC (1984); NDF and ADF, (Goering and Soest, 1970); ash and DM, (AOAC, 1990)] and corresponding feed batching records were generated. Diets presented in Table 1 are actual DM diet composition, actual nutrient concentrations, and tabular energy values (Preston, 2016).

Growth Performance Calculations

Steers were individually weighed on d -1, 1, 14, 28, 42, and 56. Weight gain was based upon initial un-shrunk on test BW (average of d -1 and d 1 BW) and d 56 BW that was pencil shrunk 4% to account for gastrointestinal tract fill. Daily energy gain (EG, Mcal/d) was calculated according to the large frame steer calf equation: $EG = 0.0493W^{0.75} \times ADG^{1.097}$ (NRC, 1984). Energy gain was the daily deposited energy and W was the average BW from the 56 d receiving period using initial un-shrunk BW and d 56 BW shrunk 4 % (NRC, 1984, 1996). Maintenance energy (EM, Mcal/d) was calculated as: $EM = 0.077W^{0.75}$ (Lofgreen and Garrett, 1968; NASEM, 2016). Using the estimates required for maintenance and gain the performance adjusted (pa) NE_M and NE_G values, Owens and Hicks (2019), of the diet were generated using the quadratic formula: $x = \frac{-b \pm \sqrt{b^2-4ac}}{2c}$, where x = diet NE_M, Mcal/kg, a = -0.41EM, b = 0.877EM + 0.41DMI + EG, c = -0.877DMI, and NE_G was determined from: $0.877NE_M$ -0.41 (Zinn and Shen, 1998; Zinn et al., 2008). Expected DMI (kg/d) was estimated according to the following equation: expected $DMI = (0.0493W^{0.75} \times ADG^{1.097}/tNE_G) + (0.077W^{0.75}/tNE_M)$, where tNE_G and tNE_M are the tabular NE values of the diet based upon formulation [(Preston, 2016), Table 1]. Expected ADG (kg/d) was determined from feed available for maintenance (FFM), feed available for gain (FFG), retained energy (RE; Mcal/d), and W, where $FFM = EM/tNE_M$, FFG = DMI - FFM, and $RE = FFG \times tNE_G$ according to the following equation: expected $ADG = (15.54 \times RE^{0.9116} \times W^{-0.6837})$.

Total Mixed Ration Particle Size Distribution

Total mixed ration (TMR) samples were collected once a week (n = 7 weeks) from each pen in the present study (n = 6 pens/treatment) for a total of 42 replications per

treatment. The TMR samples were separated using the Penn State Particle Separator (PSPS) using the methods described by (Kononoff et al., 2003).

Statistical Analysis

All data were analyzed as a randomized complete block design experiment using the GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC), considering dietary treatment as a fixed effect, pen location for block, and pen served as the experimental unit for all analyses. Treatment effects were evaluated by the use of orthogonal polynomials (Steel and Torrie, 1960). A *P*-value of 0.05 ($\alpha = 5\%$) determined significance and a *P*-value between 5% and 10% was considered a tendency.

RESULTS AND DISCUSSION

Animal growth performance

Limited work in regards to dry roughage inclusion in receiving diets for healthy beef steers has been conducted (Preston, 2007). Much of the work has been in relation to dietary roughage inclusion as a potential ingredient to dilute energy density of the receiving diet (Galyean and Hubbert, 2014) and has been conducted in high risk receiving cattle (Rivera et al., 2005). Dietary treatment effects on steer growth performance are presented in Table A.2. There was no morbidity or mortality recorded during the course of the 56-d receiving period. Increasing dietary inclusion of hay in corn silage based receiving diets had no appreciable influence ($P \geq 0.11$) on final BW, ADG, gain:feed or observed/expected dietary NE_M and NE_G, observed/expected DMI, or observed/expected ADG. Grass hay inclusion in replacement of corn silage in receiving diets increased (linear effect, $P = 0.01$) DMI by nearly 9% for 20% GH compared to 0% GH. Tomczak et al. (2019), noted a 10% increase in DMI for steers offered a roughage based receiving

diet compared to a concentrate diet offered over top of grass hay fed at 0.5% of BW (DM basis) during a 56-d receiving period and a nearly 10% improvement in ADG. It was also noted that steers offered a roughage based receiving diet compared to a finishing diet offered on top of grass hay exhibited greater rumination time for each kg of DMI on d 4, 7, and 12 of the feedlot receiving phase (Tomczak et al., 2019). Although rumination time was not measured in the present study, greater rumination time could potentially offer a myriad of benefits, namely improved ruminal health and greater digestibility of dietary DM.

There was a tendency (linear effect, $P \leq 0.10$) for increasing inclusion of grass hay to decrease $paNE_{M \text{ and } G}$. However, this was expected as the grass hay had lower tabular NE_M and NE_G values than the corn silage it replaced in the diet (Preston, 2016). Interestingly, observed DMI for all treatments was approximately 15 to 17 % less than expected based upon steer growth performance and tabular NE values, suggesting that high-growth potential steers that exhibit no obvious signs of clinical morbidity do not match model estimates for expected intake and exhibit improved gain efficiency. Additionally, observed ADG was 31 to 37% greater compared to expected when using the large frame steer equation (NRC, 1984) for live weight gain (LWG). Suggesting that the growth potential of the steers used in the present study was greater than the estimates for gain when using the LWG equation for large framed steer calves (NRC, 1984).

Total mixed ration particle size distribution and effects on cumulative ADG

The effect of grass hay inclusion on TMR particle size distribution is presented in Table A.3. The corn silage was estimated to have a grain content of greater than 50%. Corn particles were observed on the upper sieves (larger than 4 mm) of the particle

separator and would have influenced the proportion of larger particles measured in the present study. It is unknown whether or not the influence of receiving diet on larger particles was an artifact of corn, roughage, or both as the mechanical influence of forage processing is drastically different for corn silage and grass hay. As grass hay increased in the receiving diet, there was an increase (linear effect, $P = 0.01$) in the large particles greater than 19 mm. Conversely, as grass hay increased in the receiving diet, there was a decrease (linear effect, $P = 0.01$) in medium sized particles from 8 to 19 mm. There was a decrease (quadratic effect, $P = 0.01$) in small particles from 4 to 8 mm in size as grass hay increased in the receiving diet, being greatest for the 0% GH level and similar for the 10% and 20% GH inclusion diets. Overall, particles less than 4 mm increased (linear effect, $P = 0.01$) and greater than 4 mm decreased (linear effect, $P = 0.01$) as grass hay replaced corn silage in the receiving diet. Effect of the proportion of particles greater than 4 mm delivered on cumulative ADG (kg/d) was determined (Figure 1). As the proportion of particles greater than 4 mm increased cumulative ADG was decreased, this could be related to differences in DMI as proportion of larger particles delivered decreased, and this is similar to what others have determined (Blom, 2019). This effect of particle size on observed ADG could be due to a variety of factors such as increased ruminal fill that influenced daily DMI in addition to altered rate of passage that resulted in reduced digestibility of diet DM, although neither of these variables were measured in the present study.

CONCLUSIONS

Steers in the present study had exceptional DMI, ADG, and gain efficiency. This is likely a function of healthy steers that exhibited a great deal of lean growth potential

and as such were very efficient on a high roughage diet. Increasing GH inclusion in replacement of corn silage resulted in improved DMI. As the proportion of particles greater than 4 mm increases, cumulative ADG is decreased. Measuring the proportion of particles larger than 4 mm could be a useful tool in determining the ADG during the receiving period, however, the practicality of use might be limited as it does not incorporate differences in dietary NE and DMI. These data indicate that GH should be considered in corn silage based receiving diets to improve DMI. In high-risk calves, improved DMI could result in a reduced incidence of morbidity, although no morbidity was observed in any steers from the present study.

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Table A.1. Composition of experimental receiving diets (DM basis).[a]

	Grass Hay Inclusion, % (DM basis)		
Item	0	10	20
Corn silage[b]	73.64	63.67	53.77
Dried distillers grains plus solubles	20.36	20.33	20.29
Grass hay[c]	0.00	10.00	19.94
Pelleted Supplement[d]	6.00	6.00	6.00
Soybean Meal	*(3.936)*	*(3.778)*	*(3.618)*
Soybean hulls	*(0.582)*	*(0.740)*	*(0.900)*
Trace mineralized salt	*(0.300)*	*(0.300)*	*(0.300)*
Calcium Carbonate	*(1.110)*	*(1.110)*	*(1.110)*
Premix[e]	*(0.072)*	*(0.072)*	*(0.072)*
Nutrient Composition[f]			
Dry Matter, %	38.81	41.77	45.38
NE_M, Mcal/kg	1.78	1.74	1.70
NE_G, Mcal/kg	1.16	1.11	1.08
Crude protein, %	13.11	13.08	13.09
NDF, %	37.09	39.82	43.10
ADF, %	26.21	28.08	30.21
ASH, %	6.07	6.31	6.48

[a]All values except Dry Matter on a DM basis.
[b]Corn silage (n = 9 samples) contained (DM basis): 31.50 % dry matter, 6.18% crude protein, 39.50% NDF, 30.22% ADF, and 4.58% ash.
[c]Grass hay (n = 9 samples) contained (DM basis): 86.33% dry matter, 7.23% crude protein, 65.50% NDF, 49.94% ADF, and 7.27% ash.
[d]Inclusion to total diet DM included in parentheses.
[e]Vitamin premix contained (in each 907-kg of supplement): 7,204 g of SBM, 1,972 g of Rumensin-90 (Elanco, Indianapolis, IN) , 48 g of vitamin A (650,000 IU/g), 750 g of vitamin E (500 IU/g), 721 g of intellibond Zn (Micronutrients, Indianapolis, IN) , and 195 g intellibond Cu (Micronutients) for 0% GH; 7,123 g of SBM, 2,022 g of Rumensin-90 (Elanco) , 49 g of vitamin A (650,000 IU/g), 769 g of vitamin E (500 IU/g), 726 g of intellibond Zn (Micronutrients) , and 201 g intellibond Cu (Micronutients) for 10% GH; 7,226 g of SBM, 1,980 g of Rumensin-90 (Elanco) , 48 g of vitamin A (650,000 IU/g), 753 g of vitamin E (500 IU/g), 699 g of intellibond Zn (Micronutrients) , and 184 g intellibond Cu (Micronutients) for 20% GH.
[f]Tabular NE from (Preston, 2016) and actual nutrient compositions from weekly assay of individual dietary ingredients and feed batching records.

Table A.2. Influence of grass hay inclusion in replacement of corn silage on animal growth performance and dietary energetics of newly weaned beef steers during the feedlot receiving phase.

	Grass Hay Inclusion, % (DM basis)				*P* - value	
Item	0	10	20	SEM	Linear	Quadratic
Days	56	56	56	-	-	-
Pen, n	6	6	6	-	-	-
Steers, n	54	54	54	-	-	-
Growth performance[a]						
Initial BW, kg	278	278	277	0.3	0.12	0.30
Final BW, kg	352	353	357	2.7	0.21	0.62
ADG, kg	1.33	1.35	1.43	0.048	0.16	0.54
DMI, kg/d	6.46	6.74	7.04	0.105	0.01	0.93
gain:feed	0.206	0.200	0.204	0.0045	0.72	0.37
Expected DMI, kg	7.60	7.92	8.51	0.208	0.01	0.62
Expected ADG, kg	1.00	1.03	1.05	0.023	0.21	0.91
pa NE, Mcal/kg[b]						
Maintenance	2.05	1.99	1.99	0.022	0.10	0.30
Gain	1.39	1.33	1.34	0.020	0.10	0.30
Observed/Expected						
NE_M	1.16	1.14	1.17	0.013	0.45	0.23
NE_G	1.19	1.20	1.24	0.017	0.11	0.60
DMI	0.85	0.85	0.83	0.011	0.19	0.42
ADG	1.32	1.31	1.37	0.026	0.26	0.35

[a]Initial BW was the average of d -1 and d 1 BW, final BW was from d 56 and was pencil shrunk 4% to account for gastrointestinal tract fill.

[b] performance adjusted dietary NE (paNE) calculated from observed steer growth performance (Zinn and Shen, 1998; Zinn et al., 2008).

Table A.3. Influence of grass hay inclusion in replacement of corn silage on particle size distribution of total mixed ration (TMR) from newly weaned beef steers during the feedlot receiving phase.[a]

	Grass Hay Inclusion, % (DM basis)				*P* - value	
Item	0	10	20	SEM	Linear	Quadratic
Replicates, n	7	7	7	-	-	-
Pens, n	6	6	6	-	-	-
TMR, % (as-is basis)						
Large (≥ 19 mm)	6.4	11.9	16.3	0.27	0.01	0.15
Medium (8 to 19 mm)	61.6	54.1	47.7	0.36	0.01	0.23
Small (4 to 8 mm)	11.4	10.3	9.8	0.07	0.01	0.01
Less than 4 mm	20.6	23.8	26.2	0.27	0.01	0.30
Greater than 4 mm	79.4	76.2	73.8	0.27	0.01	0.30

[a]Determined according to (Kononoff et al., 2003).

FIGURE CAPTIONS

Figure A.1. Effect of the proportion of particles greater than 4 mm delivered on cumulative ADG (kg/d). Cumulative ADG = -0.0198 (proportion of particles greater than 4 mm) + 2.8852; R^2 = 0.2238.

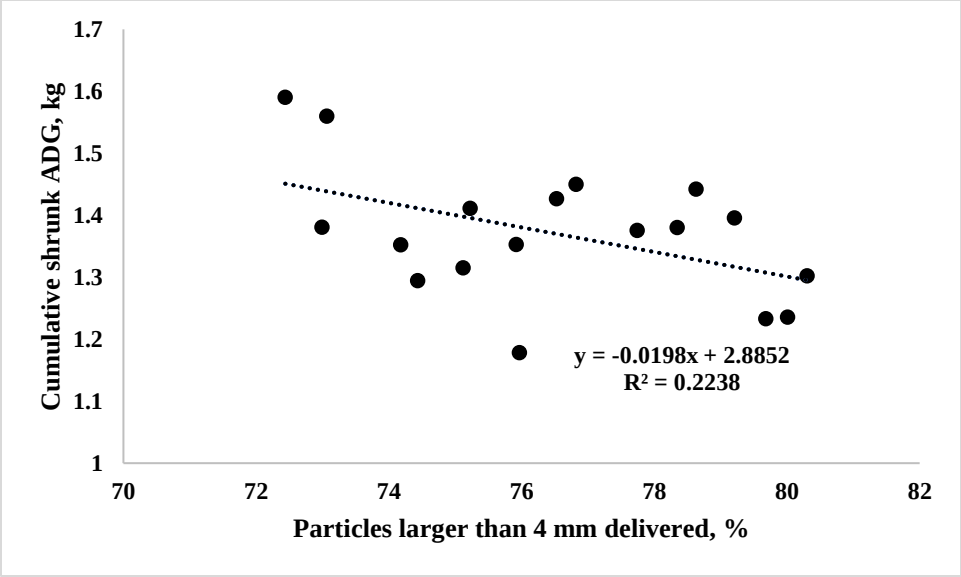

Figure A.1. Effect of the proportion of particles greater than 4 mm delivered on cumulative ADG (kg/d).