EFFECTS OF FEEDING 60% DRIED CORN DISTILLERS GRAINS PLUS SOLUBLES ON YEARLING BULL REPRODUCTION

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Effects of feeding 60% dried corn distillers grains plus solubles on yearling bull reproduction

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Thirty-six half-sibling Angus bulls were assigned one of three diets: 1) 60% corn-based (CON; S = 0.18%; n = 12); 2) 60% DDGS replacing corn (60DDGS; S = 0.55% DM; n = 12); 3) CON diet + equivalent sulfur of 60DDGS added as calcium sulfate (SULF; S = 0.54%; n = 12) to evaluate the effects of feeding diets containing DDGS or calcium sulfate on performance and semen characteristics. Bulls began the study at 9 months of age and gained 1.6 kg/day for 112 days. Treatment by day interactions ($P < 0.05$) were observed for glutathione peroxidase and trace mineral concentrations in seminal plasma. Effects of treatment ($P < 0.05$) were observed for semen kinematics and triiodothyronine in serum. Alterations observed when feeding 60% DDGS to developing bulls occurred in a manner that is not dependent on dietary sulfur; therefore, observed changes could be related to other components within DDGS.

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LIST OF ABBREVIATIONS

AA.....amino acid

ACT.....activator of CREM protein

ADG.....average daily gain

ADF.....acid detergent fiber

ALH .....amplitude of lateral head displacement

BCF .....beat cross frequency

BSE .....breeding soundness examination

BW .....body weight

$CaSO_4$.....calcium sulfate

CASA.....Computer Assisted Semen Analyzer

CON .....the control treatment

CP.....crude protein

CREM .....cAMP responsive element modulator

CV .....coefficient of variance

DDGS.....dried corn distillers grains plus solubles

60DDGS.....the treatment containing 60% DDGS

DM .....dry matter

DMR .....distal midpiece reflex

FSH .....follicle stimulating hormone

GnRH .....gonadotropin releasing hormone

GP_x .....glutathione peroxidase

H_2S .....hydrogen sulfide

LH .....luteinizing hormone

NaF.....sodium fluoride

NDF.....neutral detergent fiber

N.....nitrogen

PEM .....polioencephalomalacia

PUFA .....polyunsaturated fatty acids

ROS.....reactive oxygen species

SULF.....the treatment containing $CaSO_4$

SC.....scrotal circumference

SO_2 .....sulfur dioxide

TM.....trace mineral

T3 .....triiodothyronine

UPLC .....Ultra Performance Liquid Chromatography

VAP.....velocity on an average path

VCL.....curvilinear velocity

VSL .....velocity on a straight line

CHAPTER 1: LITERATURE REVIEW

Bull Development

Overview of Spermatogenesis

The ultimate purpose of spermatogenesis is to produce healthy spermatozoa that are capable of fertilization. The testicular parenchyma contains seminiferous tubules and interstitial tissue that are both important for sperm function and sperm maturation. Seminiferous tubules contain Sertoli cells that aid in sperm development and form the blood testes barrier. Leydig cells, within the interstitial compartment, secrete testosterone and estrogen that are crucial for the function of spermatogenesis.

Germ cells are located on the outer layer of the seminiferous tubule, then the germ cells develop into mature spermatids that are released into the lumen. The mature spermatids pass through the rete testis that leads to the caput of the epididymis. As the spermatids move through the epididymis there are many biochemical changes that improve the motility of the spermatozoa (O'Donnell et al., 2001). The duration of one cycle of spermatogenesis is 61 days in the bull. The 61-day cycle consists of eight stages that take 13.5 days to complete, then those stages are repeated 4.5 times to reach spermiation (Senger, 2012; Fig. 1.1).

Stages of the bull seminiferous epithelium	I	II	III	IV	V	VI	VII	VIII
Duration (days)	3.3	2.1	1.9	0.8	0.5	0.9	1.3	2.7
Cell divisions								
According to Ortavant (1959)	B_2	A_1[a]		A_2, M_1, M_2		In		B_1
According to Amann (1961)	A, B^d		A, A^b	A^b, M_1, M_2			In[c]	In[c], B^d
According to Hochereau (1967)	A_1, B_2	A_2[e]	A_3	In, M_1, M_2	A_0[f]			B_1

M_1 = first meiotic division; M_2 = second meiotic division.
[a]Asymetric divisions where A_1 spermatogonia produce A_1 and A_2 spermatogonia.
[b]Asynchronous divisions, some A spermatogonia (A) divide into intermediate spermatogonia, others (A^b) divide into A spermatogonia. The latter undergo a division into intermediate spermatogonia in late stage IV.
[c]Asynchronous divisions, some intermediate spermatogonia divide in stage VII, others in stage VIII.
[d]Asynchronous divisions, some B spermatogonia divide in stage VIII, others in stage I.
[e]Asymetric divisions where A_2 spermatogonia produce A_1 and A_3 spermatogonia.
[f]Asymetric divisions where A_0 spermatogonia produce A_0 and A_1 spermatogonia.

Figure 1.1. Stages of spermatogenesis in the bull (Staub et al., 2018).

The regulation of spermatogenesis is complex and dependent on the communication between germ cells and Sertoli cells. The two cell populations communicate through ligand/ receptor interactions or paracrine interactions. Along with these receptor interactions, hormones are crucial regulators of spermatogenesis. Androgens, primarily testosterone, are produced by the binding of luteinizing hormone (**LH**) to Leydig cells which convert cholesterol to testosterone. Whereas, follicle stimulating hormone (**FSH**) bind to Sertoli cells which allows for the synthesis of progesterone to estrogen, then estrogen to testosterone (O'Donnell et al., 2001). Testosterone is responsible for the continuation of germ cell maturation after meiosis and is required for release of mature sperm into the lumen of the seminiferous tubules (Walker, 2011). Estrogen initiates a negative feedback mechanism with testosterone to decrease LH and FSH secretion. At low concentrations of estrogen testicular descent, epididymal maturity, and tubule development is impacted (O'Donnell et al., 2001).

Spermatogenesis is a complex process that involves proliferation, differentiation, and maturation of germ cells to become mature spermatozoa. Throughout the eight stages of spermatogenesis the mitotic and meiotic divisions are essential to produce healthy and viable spermatozoa. During the differentiation stage of spermatogenesis, transformation of spermatids to sperm occurs by 1) formation of the acrosome, 2) condensation of nuclear chromatin, and 3) growth of the tail. Not only do spermatozoa mature in the seminiferous tubules, but they also mature while traveling through the epididymis.

Additionally, development of the accessory sex glands is crucial for the maturation and survival of spermatozoa in the female tract. The accessary sex glands provide the sperm with seminal plasma, which supplies the sperm with specific nutrients to enhance the motility and quality of sperm. Seminal plasma is primarily secreted by the seminal vesicles during ejaculation

and is mainly composed of ions (Na^+, K^+, etc.), energy substrates (fructose, sorbital), and organic compounds (citric acid, amino acids, and hormones; Juyena and Stelleta, 2012). Additionally, components within seminal plasma can vary depending on species, stage of puberty, and diet composition (Killian and Amann, 1973; Singh et al., 2018).

Puberty Attainment

Sexual maturation and puberty attainment are primarily controlled by hormones through the hypophyseal- pituitary- gonadal axis. Early rise in LH secretion is crucial for early onset of puberty (Rawlings and Evans, 1995). From the hypothalamus, secretion of gonadotropin releasing hormone (**GnRH**) begins at approximately 2 weeks of age; however, LH secretion is not produced until approximately 8 weeks of age. A rise of FSH has been observed between 2 to 12 weeks of age and concentrations remain elevated until 20 weeks of age (Kenny and Byrne, 2018). The role of FSH for sexual maturation is to enhance Sertoli cell differentiation early in life, meanwhile, the role of LH is to enhance Leydig cell differentiation for the production of testosterone (Brito, 2014). The presence of testosterone is vital for testicular growth in bull calves and the production of motile sperm in pubertal and post pubertal bulls (Kenny and Byrne, 2018).

Lunstra et al. (1978) conducted a study on young bulls (7 to 13 months of age) to determine the age at which they become pubertal based on hormone concentrations, growth, testicular development, sperm production, and sexual aggressiveness. It was observed that LH receptors on Leydig cells became more responsive to LH as the bull approached puberty, resulting in the increased production of testosterone (Lunstra et al., 1978; Weinbauer et al., 2010). The increase of circulating LH and testosterone concentrations will result in the animal achieving pubertal status. Therefore, minimizing the age at puberty in beef bulls is necessary to

maximize the growth and reproductive performance in the future. It is well documented that body weight is essential for the establishment of puberty in the male which is heavily influenced by growth rates and plane of nutrition (Brito et al., 2011; Lunstra et al., 1978).

Growth Rates in Bulls

Brito et al. (2011) conducted a study evaluating different growth rates and the effect growth rates have on reproductive development of beef bulls from 6 to 16 months of age. Puberty was determined when an ejaculate contained ≥ 50×10^6 sperm, ≥ 10% motility, and the bull had a scrotal circumference ≥ 26 cm. It was concluded that a bull could gain between 1 to 1.6 kg per day without observing any deleterious effects to reproductive performance.

However, two factors influencing growth rates in bulls is the amount of energy and protein in the diet. High energy diets (≥ 60% concentrate) can accelerate weight gain, increase testes weight, and reduce age at puberty; however, disadvantages are associated with feeding a high energy diet. Some disadvantages associated with high energy diets include excess fat accumulation in the scrotum, decreased motility, and an increase in abnormal sperm (Brito et al, 2011; Crane et. al., 2018; Klopfenstien et al., 2008). Alternatively, bulls fed low energy diets may result in a decrease in scrotal circumference, a decrease in the number of motile and morphologically normal sperm, and a decrease in sperm velocity parameters (Singh et al., 2018).

Bulls fed a high protein diet (14.5% DM) have been observed to have a larger scrotal circumference, an increase in body weight, and improved semen concentration and motility compared with bulls fed a low protein (8% DM) diet (Rekwot et al., 1988). However, low protein diets have been observed to reduce amino acid and glucose uptake in the liver which can affect the hypothalamic- pituitary- gonadal axis by reducing the secretion of GnRH, thereby,

suppressing the release of LH and affecting sexual development (Brown et al., 1994; Singh et al., 2018).

Reproductive Technology

A technique utilized in the cattle industry to determine the breeding potential of a bull without waiting until the next calving season is a breeding soundness examination (**BSE**). A BSE includes a physical assessment, scrotal circumference measurement, and sperm evaluation. A bull that is less than 15 months of age must have a scrotal circumference > 32 cm, > 30% progressively motile sperm, and > 70% morphologically normal sperm to be considered a satisfactory breeder (Koziol et al., 2018). Greater scrotal circumference measurements have been correlated with increased daily sperm production and increased testosterone (Palasz et al., 1994). Additionally, sperm motility and morphology has been positively correlated with increased potential to fertilize the oocyte (Kathirivan et al., 2011).

The process to evaluate sperm has traditionally been conducted using a microscope and hemocytometer. Evaluation of sperm by hemocytometer is dependent on the experience of the technician and can be time consuming, but the benefit is that it is inexpensive. However, during the past 50 years, the Computer Assisted Semen Analyzer (**CASA**; IVOS II) has been utilized to enhance the sperm evaluation process. The CASA microscopically examines the sperm and images are obtained using a camera. The images are then used to analyze total concentration, percent motile, percent progressive, percent slow, and percent static. Subpopulations of motile, progressive, and slow sperm are then further evaluated for kinematic parameters including, and not limited to, velocity on an average path (**VAP**), curvilinear velocity (**VCL**), velocity on a straight line (**VSL**), linear, straight, wobble, elongation, amplitude of lateral head displacement (**ALH**), beat cross frequency (**BCF**), distal mid-piece reflex (**DMR**) and area (Table 1.1).

Table 1.1. Descriptions of sperm motion parameters adopted from HT CASA II Software Manual, 2017.

Category	Abbreviation	Units	Description/ Calculation
Motile	-	%	A sperm that moves more than its head length from its original position during the evaluation period.
Progressive	-	%	sperm that are moving in a mostly straight line with increasing velocity on an average path (VAP).
Slow	-	%	Sperm with a velocity on a straight line (VSL) that is less than the maximum set for that population.
Static	-	%	Sperm with a velocity on a straight line (VSL) less than the maximum value set for that population.
Velocity Parameters			
Velocity on an average path	VAP	μm/s	Average velocity/microns per s.
Curvilinear velocity	VCL	μm/s	Distance between sperm head positions in each frame divided by elapsed time.
Velocity on a straight line	VSL	μm/s	Distance from first and last point on sperm track divided by elapsed time.
Straight	-	%	Ration of VSL/VAP multiplied by 100.
Linear	-	%	Measures sperm direction. Equation is the ratio of VSL/VCL multiplied by 100.
Wobble	-	%	Ratio of VAP/VCL.
Elongation	-	%	Ratio of head width to head length.
Amplitude of lateral head displacement	ALH	μm/s	The maximum value of the motion of the sperm head from the sperm track. Calculated using the maximum distance between each sperm position and the average sperm position across all points.
Beat cross frequency	BCF	Hz	The frequency from which the sperm head crosses the average path line.
Morphology			
Distal mid-piece reflex	DMR	%	Counts the amount of sperm with a bent tail in the distal region of the mid-piece.
Bent tail	-	%	If the tail bending rate exceeds the tail bending rate (degrees/μm).
Coiled tail	-	%	If the tail bend exceeds 180°.
Distal droplet	-	%	If the droplet is located at a distance greater than the distal droplet minimum. These parameters are set before samples are analyzed.

The CASA is beneficial because it is more accurate, highly repeatable, and more information is obtained compared to semen evaluation by hemocytometer; however, it is expensive (Farrell et al., 1998). Additionally, it is not certain which velocity parameters correlate with semen quality. For years, sperm motility has been considered to be important for selection of superior bulls for natural breeding or artificial insemination (Kathirivan et al., 2011). Research is continually being conducted to correlate kinematic parameters such as VAP, VCL, and VSL to fertilization potential of a bull. Nagy et al. (2015) observed that VAP is the best indicator to determine the fertilization potential of a bull. Some researchers have observed that more than one trait should be used to evaluate semen quality. It has been observed that using ALH and BCF with VAP or VCL is highly correlated with fertility (Kasimanickam et al., 2006). However, data correlating CASA parameters to bull fertility is contradictory and more research is necessary to further elucidate which CASA parameters are useful to predicting bull fertility.

Dried Corn Distillers Grains Plus Solubles

Processing of DDGS

With the continued increase of the ethanol industry in the United States, coproducts such as corn oil, wet/dried distillers grains, modified distillers grains, and condensed distillers solubles are produced (U.S. Grains Council Handbook). Dried corn distillers grains plus solubles (**DDGS**) is commonly incorporated into cattle diets to provide the animal with an excellent source of rumen undegradable protein and increased energy compared to corn (Crane et al., 2017). Furthermore, DDGS has greater protein, fat, fiber, sulfur, and phosphorus concentrations because of the distillation process (Klopfenstein et. al., 2008). Sulfur concentrations are greater because of the addition of sulfuric acid during processing to maintain pH for fermentation (Klopfenstein et. al., 2008). The sulfur within DDGS consists of organic (S- containing amino acids) and

inorganic (sulfuric acid) sources of sulfur. However, most of the sulfuric acid will be in the form of sulfates after the fermentation process.

The NASEM recommends to not exceed 0.4% dietary sulfur, but this percentage does vary depending on the amount of roughage that is in the diet (NASEM, 2016). One of the main concerns with using DDGS as a feed source for cattle is the variability of sulfur between batches or across ethanol plants that can range from 0.87% to 1.2% (Buckner et al., 2008).

Inclusion of DDGS in Ruminant Diets

There has been a significant amount of research evaluating the optimal inclusion level of DDGS to increase profitability, while maintaining herd health. Schauer et al. (2008), evaluated the effects of increasing levels of DDGS on performance and carcass characteristics in ram lamb diets. They fed DDGS at 20, 40 and 60% of the diet and observed no effects of inclusion level on performance or health of lambs. Feeding DDGS in ram lamb diets at 15 (19.9% CP; 4.2% crude fat; 0.3% S DM), 30 (23.3% CP; 4.8% crude fat; 0.3% S DM), and 60% (26.7% CP; 5.4% crude fat; 0.4% S DM) of the diet resulted in a decrease in testosterone concentrations, scrotal circumference, sperm morphology, and sperm concentration as the concentration of DDGS increased (Crane et al., 2015). Another study was conducted on growing lambs to evaluate the effects of feeding 0, 15 (16% CP; 3.7% crude fat; 0.37% S DM), and 30% (19.4% CP; 4.6% crude fat; 0.5% S DM) DDGS on performance and reproductive traits (Van Emon et al., 2013). As the percentage of DDGS increased in the diet, ADG increased, sperm concentration decreased linearly, but there was no difference observed for scrotal circumference.

In cattle diets, ADG is observed to be greatest when DDGS is fed at 20 to 30% of the diet and maximal G:F was achieved at 10 to 20% of the diet (Klopfenstein et al., 2008). Additionally, a study by Depenbusch et al. (2009) fed DDGS up to 75% of the diet and observed that

performance decreases when DDGS is fed above 15% of the diet. Inclusion levels in cattle have been recommended at 15-30% of the diet without any deleterious effects observed for carcass quality, semen characteristics, and performance (Buckner et al., 2008; Depenbusch et al., 2009; Klopfenstein et al., 2008).

Sulfur

Sulfur Toxicity

Feeding DDGS at greater concentrations can influence DMI, ADG, and feed efficiency possibly because of the greater percentage of dietary sulfur. Along with the decrease in performance characteristics, sulfur toxicity may occur causing a disorder called polioencephalomalacia (**PEM**). Although the mechanism of how sulfur causes PEM is not fully elucidated, increased ruminal hydrogen sulfide (**H_2S**) has been highly correlated with PEM (Gould et al., 1998). Animal performance, ruminal H_2S, and carcass characteristic data were collected to evaluate the effects of feeding 20, 40, or 60% DDGS on sixty steers (Neville et. al., 2012). It was observed that ruminal H_2S increased as the concentration of DDGS increased; however, ADG, DMI, and feed efficiency decreased as DDGS increased in the diet. Other reports have also observed a negative relationship between ruminal H_2S and animal performance (Richter et al., 2012; Uwituze et al., 2011). These studies observed that as ruminal H_2S increased, DMI and ADG decreased when animas were fed sulfur at 0.6-0.65% of the diet.

Effects of Sulfur on Reproductive Traits in Mice

Research by Zhang et al. (2006) and Meng et al. (2004) hypothesized that the decrease in male fertility may be a consequence of the sulfur dioxide (**SO_2**) in pollution. Young male mice were used to research the effect of inhaling SO_2 on reproduction and the possible mechanisms of action that lead to the decrease in reproductive performance. One study evaluated ninety- six

mature male rats that were assigned to one of four treatments: 1) SO_2, 2) sodium fluoride (**NaF**), 3) NaF + SO_2, and 4) control (untreated). Testosterone from blood serum and sperm motility were decreased in the SO_2 and SO_2 + NaF treated mice. They concluded that the excess sulfur dioxide may influence spermatogenesis, more specifically, sperm motility. A similar study was conducted to evaluate the effects of SO_2 or NaF on the blood testes barrier in 8- week old mice. It was concluded that there was a decrease in body weight and a decrease in sperm production and morphology. There was an increase in gap junctions between seminiferous tubules within the blood testes barrier for the SO_2 + NaF treated mice which may have caused the decrease in sperm production and morphology (Zhang, et al., 2016).

The mechanism for how SO_2 is influencing semen quality and testicular morphology was investigated by Meng et al. (2004). They evaluated the effects of exposure of three different SO_2 concentrations on groups of mice to assess the oxidative stress within the testicles. With the increase in SO_2, there was an increase in lipid peroxidation within the testes that could influence testicular function. Another study observed that SO_2 augmented the expression of CREM and ACT proteins in rat testes which resulted in decreased sperm motility and irregular testicular histology (Zhang et al., 2016).

Antioxidants

Antioxidants are crucial for the protection of sperm from oxidative stress by reactive oxygen species (**ROS**). Lipid peroxidation is the process in which electrons are taken from the lipid membrane of a cell resulting in cell damage and further decreases semen motility and quality (de Lamirande et al., 1997). Excess sulfur can influence sperm by lipid peroxidation from reactive oxygen species which include hydrogen peroxide, superoxide anion, nitric oxide, and hydroxyl radicals.

In bull semen, the production of reactive oxygen species primarily comes from dead sperm; however, there are many processes in sperm that need reactive oxygen species to function properly (Juyenna and Stelleta, 2012). Reactive oxygen species in sperm are necessary for capacitation, acrosome reaction, and stabilization of the mitochondria in the mid-piece (O'Flaherty et al., 1999). However, when concentrations of ROS become too high it can cause damage to sperm DNA through a process called oxidative stress. To maintain the balance of reactive oxygen species the presence of antioxidants are important to cell function. Catalase, superoxide dismutase, and glutathione peroxidase are the major antioxidants within seminal plasma. Catalase has been observed to be secreted into the lumen of the epididymis where superoxide dismutase and glutathione peroxidase has been observed to be secreted by the seminal vesicles to protect the sperm during ejaculation (Zubkova and Robaire, 2004).

The process to synthesize glutathione peroxidase begins with inactive thyroxine converted to active triiodothyronine by type 1 and type 2 deiodinases. Glutathione peroxidase, an enzyme containing selenium, aids in the catalysis of peroxidase degradation while type 1 deiodinase aids in the formation of triiodothyronine by undergoing a double replacement reaction. With the reduction in peroxidases and availability of selenium, glutathione peroxidase forms a selenenyl residue and is then regenerated by thiols. Concurrently, the deiodination of thyroxine produces an intermediate also used to form glutathione peroxidase (Köhrle et al., 2005).

DNA in Sperm

Changes in DNA during early stages of spermatogenesis could impact the viability of sperm. At the end of the mitotic stage (~44 d into spermatogenesis), secondary spermatocytes (haploid cells) undergo meiosis to become primary spermatocytes (diploid cells). When

chromatin remodeling occurs at this stage of spermatogenesis it could result in DNA strand breaks. Other potential processes that may cause DNA damage include: apoptosis and ROS. Meng et al. (2004) researched the effects of sulfur dioxide inhalation on various organs, including brain, lung, liver, spleen, kidney, intestine and testicles of mice. They found an increase in single- strand DNA damage in all organs which suggests that sulfur could affect the disulfide bonds that help DNA to maintain its shape and function.

Conclusion

Feeding DDGS to developing bulls may influence their reproductive performance; however, literature is lacking in this area. Studies in mice have demonstrated that sulfur can have negative effects on male reproduction by influencing seminiferous tubule function. Additionally, studies in rams have observed decreases in sperm motility and morphology when the animal is fed greater concentrations of dietary sulfur. Therefore, the current study was designed to evaluate the effects of feeding 60% DDGS or the equivalent sulfur as $CaSO_4$ on 1) performance and semen characteristics and 2) nutrient and metabolite concentrations in serum and seminal plasma.

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CHAPTER 2: EFFECTS OF FEEDING 60% DRIED CORN DISTILLERS GRAINS PLUS SOLUBLES OR THE EQUIVALENT SULFUR AS $CASO_4$ ON PERFORMANCE AND REPRODUCTIVE TRAITS OF YEARLING ANGUS BULLS[1]

Abstract

The objectives of this study were to investigate the effects feeding 60% DDGS or the equivalent S as $CaSO_4$ on semen quality and performance characteristics in yearling bulls. Thirty-six half-sibling Angus bulls [291 ± 8 d; initial body weight (BW) = 320 ± 2 kg] were assigned one of three diets: 1) 60% corn-based concentrate diet (CON; S = 0.18%; n = 12); 2) diet containing 60% DDGS as a replacement for corn (60DDGS; S = 0.55% DM; n = 12); 3) CON diet + equivalent S of the 60DDGS diet added as $CaSO_4$ (SULF; S = 0.54%; n = 12). Bulls were fed for 112 d to target an ADG of 1.6 kg/d. Blood samples were collected on d 0, 56, and 112, and evaluated for testosterone, thyroxine, triiodothyronine (**T3**) and glutathione peroxidase (**GP_x**) activity. Ruminal H_2S was measured on d 0, 14, and 42. Scrotal circumference and semen were collected on d 0, 28, 56, 84, and 112 to evaluate sperm characteristics and GP_x activity in seminal plasma. A computer assisted semen analysis instrument was used to evaluate kinematic profiles in motile and progressive sperm. Data were analyzed as repeated measures using the MIXED procedures of SAS. No differences ($P \geq 0.14$) were observed for final BW, ADG, or scrotal circumference; however, SULF tended ($P = 0.07$) to have reduced gain:feed compared with CON, with 60DDGS being intermediate. Concentrations of ruminal H_2S on d 42 were

[1] The material in this chapter was co-authored by Joel S. Caton, James D. Kirsch, Sheri T. Dorsam, Kacie L. McCarthy, Matthew S. Crouse, Kevin K. Sedivec, Bryan W. Neville, and Carl R. Dahlen. Cierrah Kassetas had the primary responsibility of collecting samples in the field, drafting, and revising all versions of this document. James Kirsch, Sheri Dorsam, Kacie McCarthy, Carl Dahlen, Joel Caton, and Matthew Crouse had the primary responsibility of collecting samples and proofreading this document. Kevin Sedivec and Bryan Neville had the responsibility of proofreading this document and preparing the grant for the funding of this project. This chapter was submitted as a manuscript to the journal of Theriogenology.

greatest ($P < 0.01$) for SULF. Increased ejaculate volume was observed for 60DDGS ($P < 0.01$) compared with SULF, with CON intermediate. For motile populations of sperm, velocity on an average path (VAP) and curvilinear velocity (VCL) were reduced ($P \leq 0.02$) for SULF compared with CON, with 60DDGS being intermediate. In progressively motile sperm, VAP and VSL were reduced ($P \leq 0.05$) in 60DDGS and SULF compared to CON. For VCL, SULF was reduced ($P \leq 0.02$) compared with CON, with 60DDGS being intermediate. In serum, concentrations of T3 were reduced ($P = 0.009$) in 60DDGS compared with CON or SULF. A treatment by day interaction ($P = 0.03$) was observed for seminal plasma GP_x. At d 56, GP_x activity was greater ($P = 0.03$) for 60DDGS compared with CON, with SULF intermediate; and at d 112, 60DDGS had the greatest ($P \leq 0.02$) GP_x activity. Therefore, feeding 60% DDGS to developing bulls altered semen kinematics, T3 concentrations, and GP_x activity leading to the conclusion that these differences may not be solely dependent on concentrations of dietary sulfur.

Key words: beef bulls, dried corn distillers grains plus solubles, semen characteristics, sulfur

Introduction

Healthy growth and development from birth until puberty is essential to obtain optimal performance in bulls. High energy diets fed post weaning have been positively correlated with increased testes weight, whereas animals on low energy diets had decreased testes weights and delayed onset of puberty (Barth et al., 2008). Not only does overall nutrition influence puberty attainment, but specific nutrients may influence animal performance and productivity. Dried corn distillers grains plus solubles is one ingredient that serves as an excellent source of rumen undegradable protein and energy for livestock (Klopfenstein et al., 2008).

Dried corn distillers grains plus solubles is a co-product from the ethanol industry that has been increasingly utilized because it is affordable and readily available. During ethanol

processing, sulfuric acid is added to maintain pH levels which causes an increase in the percent sulfur of DDGS which can range from 0.33 to 0.74% (Buckner et al., 2011). Consequently, diets including DDGS can exceed the maximum tolerable concentration of sulfur (0.4%; NASEM, 2016) and reduce animal performance (Drewnoski et al., 2014a).

Potential also exists for sulfur and DDGS to negatively impact male reproductive parameters. Studies in mice reported a decrease in sperm motility and morphology when sulfur dioxide, a component of pollution, was released into ambient air (Meng et al. 2004; Zhang et al.; 2016a; Zhang et al., 2016b). Feeding increasing levels of DDGS to ram lambs has also been observed to decrease testosterone levels and alter sperm characteristics (Crane et al. 2018; Van Emon et al. 2013). However, there is a lack of data regarding impacts of feeding elevated concentrations of DDGS to developing beef bulls on reproductive parameters. Therefore, the objectives of this study were to determine the effects of feeding 60% DDGS or the equivalent sulfur as $CaSO_4$ to developing beef bulls on performance and semen characteristics.

Materials and Methods

All procedures were approved by the North Dakota State Institution for Animal Care and Use Committee (#A19035).

Animals and Treatments

Thirty-six half-sibling Angus bulls [291 ± 8 d of age; mean initial body weight (**BW**) = 320 ± 2 kg] originating from the Central Grasslands Research Extension Center near Streeter, ND, were used in this experiment. Bulls were ranked by initial BW and sperm concentration then randomly assigned to one of three treatments (Table 2.1): 1) corn-based diet containing 60% concentrate [**CON**; S = 0.18% dry matter (**DM**); n = 12]; 2) diet containing 60% DDGS as a replacement for corn (**60DDGS**; S = 0.55 % DM; n = 12); 3) CON diet + equivalent sulfur of the

60DDGS diet added as $CaSO_4$ (**SULF**; S = 0.54 % DM; n = 12). By design, diets were formulated with differing concentrations of nitrogen, starch, and total fat to accomplish the objectives of this study which was to investigate the effects of sulfur specifically from DDGS. With this objective, 60DDGS and SULF diets were formulated to contain similar concentrations of sulfur. All bulls were housed indoors in the Animal Nutrition and Physiology Center in Fargo, ND. Bulls were individually fed in a Calan gate system and individual intakes adjusted to target a 1.6 kg/d average daily gain (**ADG**).

Diets were prepared by adding the vitamin premix, soybean meal, and $CaSO_4$ (for the SULF diet only) to the mixer then the cracked corn or DDGS, hay, and corn silage were added after. It is important to note that the additional $CaSO_4$ for the SULF diet was mixed in with the entire ration. After each diet was made and distributed to its respective bunk, the mixer and feed cart were emptied and cleaned. Diets were formulated to be equal in forage, concentrate, and total fat on a dry matter basis. Furthermore, the SULF diet was formulated to be equal in sulfur in the form of $CaSO_4$ compared with sulfuric acid and sulfur containing amino acids in the 60DDGS treatment.

Table 2.1. Diet composition, nutrient composition, and % dry matter (DM) inclusion for control (CON), dried corn distillers grains plus solubles (60DDGS), and equivalent sulfur diet (SULF).

	Treatment[1]		
Item	CON	60DDGS	SULF
Dietary Composition, %			
Cracked corn	50	-	49.9
Soybean meal	10	-	10
Corn silage	25	28	25
DDGS	-	60	-
Corn oil	2	-	2
Triticale hay	10	10	10
$CaSO_4$ [2]	-	-	0.10
Corn premix	3	-	3
DDGS premix	-	2	-
Nutrient Composition [2]			
Ash, %	6.1	8.3	6.9
Crude Protein, %	13.4	22.0	13.9
Nitrogen, %	2.1	3.5	2.2
ADF, %	14.3	19.2	12.5
NDF, %	29.6	49.2	26.6
Fat, %	4.2	4.7	4.3
Starch, %	36.5	11.9	36.7
GE, cal/g	4474.0	4690.9	4467.4
ME	1.27	1.25	1.32
NE_m, Mcal/kg	0.38	0.38	0.40
NE_g, Mcal/kg	0.79	0.79	0.27
Ca, %	0.96	1.05	1.12
P, %	0.3	0.7	0.3
S, %	0.18	0.53	0.51

[1] CON = corn-based diet containing 60% concentrate (n = 12)**;** 60DDGS = diet containing 60% DDGS as a replacement for corn (n = 12)**;** SULF = CON diet + equivalent sulfur of 60DDGS in diet added as $CaSO_4$ (n = 12).

[2] ADF = acid detergent fiber; NDF = neutral detergent fiber; GE = gross energy; ME = metabolizable energy; NE_m = net energy for maintenance; NE_g = net energy for gain; Ca = calcium; P = phosphorus; S = sulfur.

Sample Collection

Body weights were recorded every 14 d during the 112-d study with a 2-day weight at the beginning and end of the study. Blood samples were collected in tubes containing heparin and spray coated silica for plasma and serum; respectively, before the morning feeding on d 0, 56, and 112 via jugular venipuncture. All blood samples were centrifuged at 1,500 × g for 20

minutes at 4°C (Sorvall ST 16R; Thermo Scientific Inc.; Waltham, MA). The supernatant from the serum and plasma blood tubes were pipetted into 2-mL screw cap tubes and stored at -20°C.

Ruminal H_2S samples were taken via rumen puncture for determination of H_2S concentrations at 4 to 6 h post feeding on d 0, 14, and 42 (Neville et al. 2012). Briefly, landmarks to determine the site for rumen puncture were the midpoint between the last rib and spine on the left side of the bull. The site was clipped and prepared by alternating isopropyl alcohol and Betadine scrubs three times. A 14-gauge needle was used to puncture the rumen and concentrations of H_2S were determined by drawing rumen gas through H_2S detector tubes connected to a volumetric gas pump (Gastec; Kanawaga, Japan).

Scrotal circumference measurements (**SC**) were recorded on d 0 and every 28 d thereafter by placing a scrotal tape around the widest portion of the scrotum while holding the neck of the scrotum (Koziol and Armstrong, 2018). This measurement was conducted by the same technician for the duration of the study. Semen was collected on d 0, 28, 56, 84, and 112 via electroejaculation (Pulsator IV; Lane Manufacturing Inc; Denver, CO) into disposable plastic semen collection bags.

Analysis of Fresh Semen

A sub-sample of semen was diluted with a buffer (Easy Buffer B; IMV Technologies U.S.A.; Maple Grove, MN) to target 60 to 80 cells per field, and 3 µL of semen were pipetted onto a 20µm capillary chamber slide (Leja; IMV Technologies U.S.A; Maple Grove, MN) for analysis via computer assisted semen analysis (**CASA**; IVOS II; Hamilton Thorne; Beverly, MA). Each of 10 fields were analyzed for determination of total concentration, percent motile, percent progressive, percent slow, and percent static. Subpopulations of motile, progressive, and slow sperm were each further evaluated for kinematic parameters including velocity on an

average path (**VAP**), curvilinear velocity (**VCL**), velocity on a straight line (**VSL**), linear, straight, wobble, elongation, amplitude of lateral head displacement (**ALH**), beat cross frequency (**BCF**), distal mid-piece reflex (**DMR**) and area (Table 2). Puberty was defined as bulls that had a SC ≥ 30 cm, ≥ 30% progressively motile sperm, and ≥ 70% morphologically normal sperm (Koziol and Armstrong, 2018).

After semen was collected, it was centrifuged at 1,500 × g for 10 minutes at 4°C (Sorvall ST 16R; Thermo Scientific Inc.; Waltham, MA) to separate sperm from seminal plasma. Seminal plasma and sperm pellets were then pipetted into a 2-mL screw cap tube and stored at -20°C until further analysis.

Laboratory Analysis

Corn silage and individual diets were sampled three times per week to obtain a percent DM for the diet. Samples of corn silage, DDGS, and diets were analyzed for chemical composition which included: nitrogen with a Kjeltec Auto 1030 Analyzer (Foss Tecator AB; Höganäs, Sweden), neutral detergent fiber (**NDF**; assayed with a heat stable amylase and expressed inclusive of residual ash) and acid detergent fiber (**ADF**; expressed inclusive of residual ash; Van Soest et al., 1991). Crude protein (**CP**) was calculated by multiplying nitrogen concentration × 6.25. Standard procedures 934.01, 988.05, 942.05, 920.39 were used to analyze for DM, nitrogen, NDF, ADF, ash and ether extract, respectively (AOAC, 1990; Table 1).

Serum samples were evaluated using commercial kits for the Immulite 1000 immunoassay analyzer (Siemens Healthcare; Malvern, PA) for determination of testosterone, triiodothyronine, and thyroxine concentrations (Kit # LKTW1, LKT41, LKT31, respectively). The intra-assay CV for the testosterone analysis was 5.9% and the controls were 8.5%, 4.5%, and 0.1% for low, medium, high; respectively. The intra-assay CV for triiodothyronine was 4.4% and

the controls were 12.2%, 4.4%, and 3.5% for low, medium, and high; respectively. For thyroxine, the intra-assay CV was 4.4% and the controls were 2.5%, 4.2%, and 1.3% for low, medium, and high; respectively.

The precipitate from blood collected in heparinized tubes was used to obtain red blood cells by removing the buffy coat layer and the supernatant. Two hundred µL of erythrocyte was aliquoted into two separate microtubes with 800 µL of ice-cold 18Ω water. Microtubes were centrifuged at 22,000 x g for 15 minutes at 4°C (Allegra X-22R; Beckman Coulter; Brea, CA). After centrifugation, the supernatant was collected and stored at -80°C.

Glutathione peroxidase activity was evaluated in erythrocyte supernatant and seminal plasma using a commercial kit (Item # 703102; Cayman Chemical Company Inc; Ann Arbor, MI). The positive control consisted of diluted glutathione peroxidase (1:50; intra-assay CV = 7.8%, 3.1%, 10.5%, 3.1%; inter-assay CV = 6.1%) supplied by Cayman Chemical. All blood samples were diluted 1:80 and absorbance measured on a microplate spectrophotometer (BioTek Instruments, Inc.; Winooski, VT) at 39°C (intra-assay CV = 6.61%, 5.98%, 5.5%, 4.9%; inter-assay CV = 6.1%). Seminal plasma was diluted 1:40 and analyzed on a microplate spectrophotometer (BioTek Instruments, Inc.; Winooski, VT) at 25°C (intra-assay CV = 4.3%, 16.1%, 6.7%, 5.0%, 10.3%; inter-assay CV = 4.3%). For the control samples, the intra-assay CV equaled 16.0%, 12.2%, 4.6%, 0.9%, 2.8% and the inter-assay CV was 7.32%. One unit of enzyme activity was defined as a micromole of substrate converted per minute and expressed as U/mL. Enzyme data were converted from nmol to µmol of activity by dividing by 1000.

Statistical Analysis

For all analyses bull was the experimental unit. Bull growth and feed efficiency variables were analyzed for the entire study and only d 112 using the MIXED procedure of SAS (SAS 9.4;

Cary, NC, USA) with a model including the effects of treatment. The analysis was conducted at d 112 to observe if any differences existed among treatments at the time all bulls were considered pubertal. Ruminal H_2S, scrotal circumference, hormone concentrations, and glutathione peroxidase activity were analyzed as repeated measures using MIXED procedure of SAS with a model including day, treatment, and a treatment × day interaction. Sperm morphology, motility, and kinematic data were analyzed as repeated measures using MIXED procedure of SAS with a model including day, treatment, and a treatment × day interaction with d0 as a covariate. Percent of bulls that were pubertal on respective days of evaluation was analyzed using the GLIMMIX procedures of SAS. For each variable tested, appropriate covariate structures were evaluated and the structure with the smallest AIC was used (Wang and Goonewardene, 2004). Results were considered significant when *P*-values were ≤ 0.05 and tendencies were evaluated when *P*-values were $0.05 \geq x \leq 0.10$.

Results

Bulls in all treatments advanced in sexual maturity as the study progressed. Bulls began the project at 291 d ± 8 d (9 mo) where only 22% were considered pubertal. The proportion of bulls that were pubertal increased ($P < 0.0001$; data not shown) over the evaluation period but was not affected by treatment ($P = 0.81$; data not shown). All bulls were considered pubertal by 403 ± 8 d (13 mo). Similarly, motile and progressive populations of sperm increased ($P < 0.0001$) while the percent of static sperm decreased ($P < 0.0001$) over the duration of the study (data not shown).

By design, there were no differences ($P \geq 0.14$) among treatments for ADG, initial BW or final BW over the 112-d period (Table 2.2). In addition, no differences were present among

treatments for dry matter intake ($P = 0.23$), but bulls consuming SULF tended ($P = 0.07$) to have reduced gain to feed compared with CON, with 60DDGS being intermediate.

Table 2.2. Least square means for the effect of dietary sulfur from DDGS or $CaSO_4$ on beef bull performance characteristics.

	Treatment[1]				*P*- value
Item	CON	60DDGS	SULF	SEM	Trt
Initial BW, kg	320	320	320	5	0.90
Final BW, kg	495	481	477	8	0.20
DMI, kg/d[2]	7.60	7.67	7.85	0.10	0.23
ADG, kg/d[3]	1.57	1.43	1.40	0.06	0.14
G:F[4]	0.20	0.18	0.17	0.01	0.07

[1] CON = corn-based diet containing 60% concentrate (n = 12)**;** 60DDGS = diet containing 60% DDGS as a replacement for corn (n = 12)**;** SULF = CON diet + equivalent sulfur of 60DDGS in diet added as $CaSO_4$ (n = 12).
[2] DMI = dry matter intake.
[3] ADG = average daily gain.
[4] G:F = gain to feed ratio.

Concentrations of ruminal H_2S were influenced by a treatment × day interaction ($P = 0.005$; Fig. 2.1). While no effects of treatment ($P = 0.97$) were observed at d 0, on d 14 bulls fed 60DDGS and SULF had increased ($P \leq 0.004$) concentrations of H_2S compared with CON, and SULF tended (P = 0.06) to be greater than 60DDGS. Further, on d 42 concentrations of H_2S were greater ($P < 0.0001$) in SULF than 60 DDGS, which were greater ($P < 0.0001$) than CON.

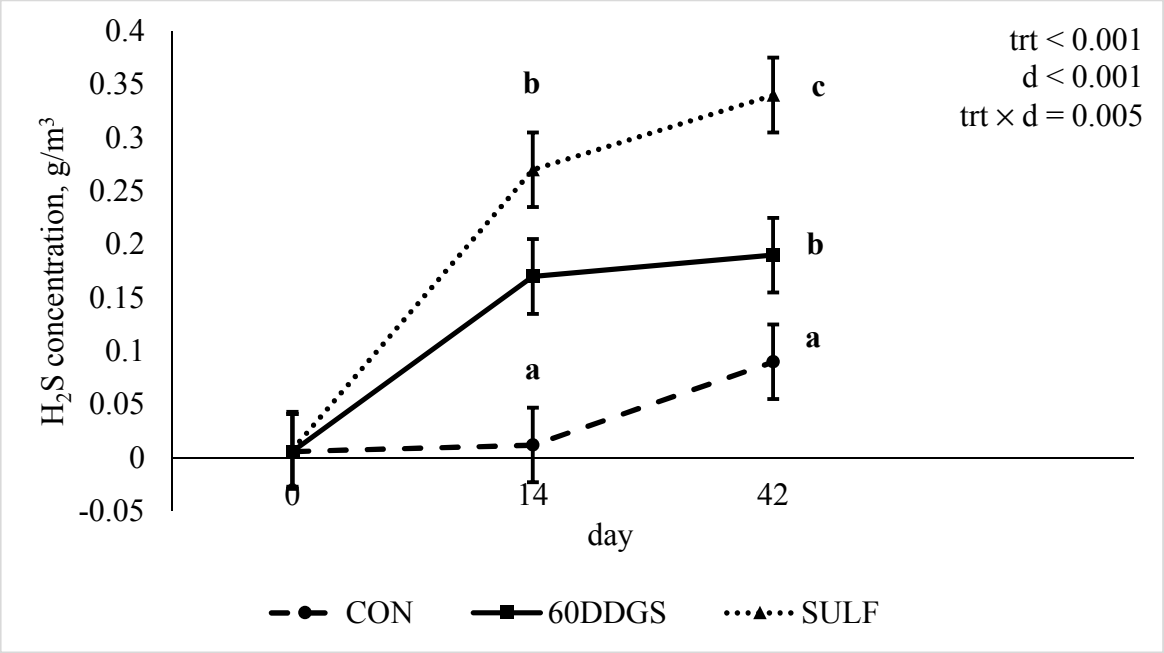

Figure 2.1. Effect of feeding 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$ on ruminal H_2S.
Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated within day when the *P*– values were ≤ 0.05.

No differences ($P \geq 0.12$) were observed among treatments for SC, concentrations of sperm in ejaculate, total sperm; proportion of sperm classified as motile, progressively motile, slow, or static; or proportion of sperm with defects including proximal droplets, bent tails, or DMR (Table 2.3). A treatment difference was observed for ejaculate volume where CON and 60DDGS had greater ($P < 0.01$) ejaculate volume compared with SULF. Percent of sperm with coiled tails was influenced by a treatment × day interaction ($P = 0.02$). At d 28, bulls fed SULF had greater ($P < 0.01$) percentages of coiled tails compared with 60DDGS and CON. At d 56, percent of coiled tails were greater ($P < 0.04$) for 60DDGS compared with CON and SULF (Fig. 2.2). No differences ($P \geq 0.45$) were observed among treatments at d 84 or 112. Additionally,

bulls fed SULF had lower ($P = 0.01$) percent distal droplets compared with CON where 60DDGS was intermediate and equal to both SULF and CON, respectively (Table 2.3).

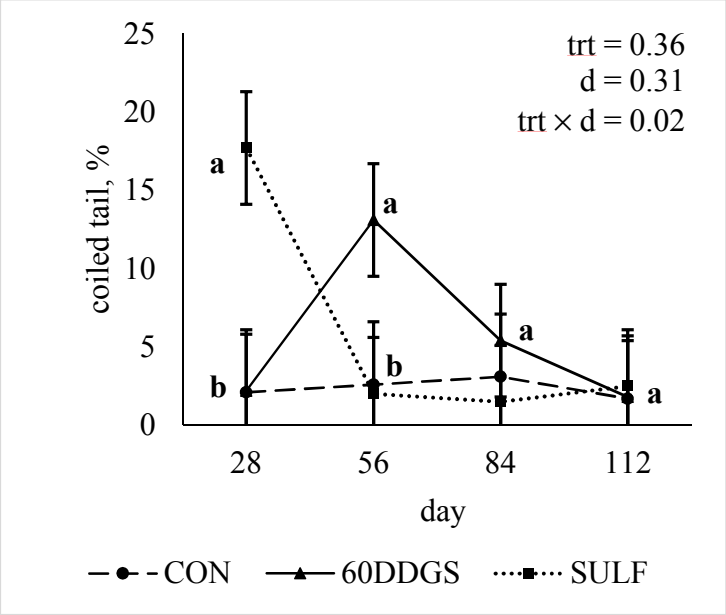

Figure 2.2. Interaction between treatments for coiled tail percent in sperm.
Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated within day when the *P*– values were ≤ 0.05.

Table 2.3. Least square means for gross ejaculate characteristics over the development period for yearling Angus bulls fed 60% DDGS or the equivalent sulfur as calcium sulfate.

	Treatment[1]				*P*- value	
Item	CON	60DDGS	SULF	SEM	Trt	Trt × day[2]
Scrotal circumference, cm	33.9	33.4	34.0	0.29	0.14	0.90
Ejaculate volume, mL	6.1[a]	6.6[a]	4.8[b]	0.4	0.002	0.77
Concentration, million/mL	78.3	75.7	86.9	11.1	0.76	0.43
Total sperm, million	575	647	497	131	0.7	0.54
Motile, %	56.3	51.9	53.2	2.7	0.49	0.93
Progressive, %	45.4	41.0	42.4	3.4	0.42	0.83
Slow, %	4.3	6.1	3.6	1.5	0.45	0.63
Static, %	38.5	41.6	40.9	2.9	0.74	0.48
Morphology						
Proximal droplet, %	6.0	6.0	6.6	1.25	0.91	0.19
Bent tail, %	5.2	6.6	4.7	1.2	0.53	0.50
Coiled tail, %	4.6	6.4	5.5	1.66	0.78	0.009
Distal droplet, %	9.4	7.8	6.2	1.0	0.08	0.16
DMR, %[3]	1.8	1.3	1.8	0.4	0.48	0.98

[1] CON = corn-based diet containing 60% concentrate (n = 12); 60DDGS = diet containing 60% DDGS as a replacement for corn (n = 12); SULF = CON diet + equivalent sulfur of 60DDGS in diet added as $CaSO_4$ (n = 12).

[2] Means separation for trt × day interactions can be found in Fig. 2.

[3] DMR = distal mid-piece reflex.

[ab] Means lacking common superscripts within a row indicate differences among treatments ($P \leq 0.05$).

For velocity parameters in motile populations of sperm, a treatment by day interaction ($P = 0.05$; Fig. 2.3) was observed for VSL. At d 28, 60DDGS had reduced ($P \leq 0.04$) motile VSL when compared with CON or SULF, then, at d 84 VSL was lower ($P \leq 0.05$) for SULF compared with CON and 60DDGS. No differences ($P \geq 0.33$) were observed among treatments at d 56 or 112. Additionally, VAP and VCL were reduced ($P \leq 0.04$) for SULF compared with CON, where 60DDGS was intermediate and equal to both CON and SULF (Table 2.4). No differences ($P > 0.10$) were observed for percent elongation, straight, linear, ALH, BCF, area, and percent wobble in the motile population of sperm.

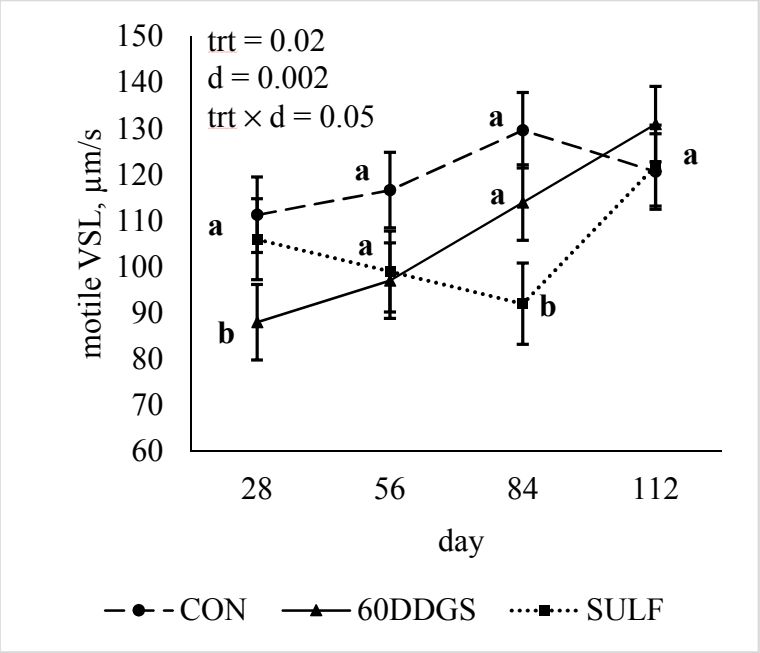

Figure 2.3. Interactions between treatments for velocity on a straight line (VSL). Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated within day when the *P*– values were ≤ 0.05.

Within the progressively motile population of sperm, VAP and VSL were reduced ($P \leq 0.05$) for 60DDGS and SULF when compared with CON. For VCL, SULF was reduced ($P = 0.01$) compared with CON and 60DDGS was intermediate and equal to both CON and SULF (Table 2.4).

Within the slow population of sperm, percent elongation and ALH were reduced ($P \leq 0.03$) for 60DDGS and SULF compared with CON. Percent linear was greater ($P \leq 0.05$) for 60DDGS and SULF compared with CON. No differences were observed among treatments ($P \geq 0.06$) for VAP, VCL, VSL, straight, BCF, area, and wobble (Table 2.4).

Table 2.4. Least square means over the development period for CASA motion parameter measurements in sperm of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$.

	Treatment[1]				*P*- value	
Item[2]	CON	60DDGS	SULF	SEM	Trt	Trt × d[3]
Motile						
VAP, μm/s	132.1[a]	120.3[ab]	114.9[b]	4.5	0.02	0.15
VCL, μm/s	202.7[a]	188.2[ab]	174.6[b]	9.0	0.04	0.49
VSL, μm/s	119.6	107.7	105.1	4.0	0.02	0.05
elongation, %	0.39	0.38	0.39	0.005	0.46	0.39
straight, %	90.4	90.1	90.9	0.6	0.66	0.09
linear, %	61.7	63.5	62.4	1.2	0.60	0.21
ALH, μm/s	6.2	5.4	5.5	0.2	0.07	0.97
BCF, Hz	42.1	43.1	41.4	0.7	0.25	0.38
area, μm^2	17.8	18.2	18.3	0.1	0.10	0.42
wobble, %	67.5	69.3	67.7	0.9	0.39	0.37
Progressive						
VAP, μm/s	139.0[a]	128.8[b]	125.4[b]	3.6	0.02	0.09
VCL, μm/s	209.0[a]	191.3[ab]	184.6[b]	7.4	0.05	0.32
VSL, μm/s	131.3[a]	121.8[b]	119.1[b]	3.4	0.02	0.06
elongation, %	0.39	0.38	0.38	0.005	0.17	0.22
straight, %	94.8	94.8	95.1	0.3	0.56	0.85
linear, %	66.3	68.2	67.8	1.0	0.47	0.92
ALH, μm/s	5.9	5.2	5.2	0.2	0.10	0.81
BCF, Hz	42.7	43.2	42.7	0.5	0.80	0.37
area, μm^2	17.6[a]	18.2[b]	18.2[b]	0.19	0.03	0.19
wobble, %	69.7	71.7	71.0	0.9	0.38	0.91
Slow						
VAP, μm/s	61.7	43.4	45.1	7.2	0.09	0.92
VCL, μm/s	134.1	87.9	101.2	15.7	0.06	0.91
VSL, μm/s	20.2	18.3	18.6	0.9	0.24	0.80
elongation, %	0.45[a]	0.40[b]	0.41[b]	0.01	0.02	0.88
straight, %	53.2	62.4	65.6	3.0	0.08	0.79
linear, %	24.7[a]	34.6[b]	31.9[b]	3.1	0.05	0.83
ALH, μm/s	7.1[a]	4.7[b]	5.1[b]	0.71	0.02	0.79
BCF, Hz	41.2	36.0	38.1	2.2	0.21	0.43
area, μm^2	19.4	19.8	19.2	0.92	0.89	0.28
wobble, %	46.5	53.7	48.6	2.3	0.07	0.76

[1] CON = corn-based diet containing 60% concentrate (n = 12); 60DDGS = diet containing 60% DDGS as a replacement for corn (n = 12); SULF = CON diet + equivalent sulfur of 60DDGS in diet added as $CaSO_4$ (n = 12).
[2] VAP = average path velocity; VCL = curvilinear velocity; VSL = straight line velocity; ALH = amplitude of lateral head displacement; BCF = beat cross frequency. See Table 2 for descriptions.
[3] Mean separations for treatment × day interactions can be found in Fig. 3.
[ab] Means lacking common superscripts within a row indicate differences among treatments ($P \leq 0.05$).

By d 112, all bulls had attained pubertal status and a separate analysis was conducted to evaluate any differences between treatments at the final collection. There was a treatment difference ($P = 0.0008$) for ejaculate volume, where CON and 60DDGS had greater ($P < 0.01$)

ejaculate volume compared with SULF. In addition, percent distal droplet was greater ($P < 0.01$) in CON and 60DDGS ($P \leq 0.04$) compared with SULF (Table 2.5). No differences ($P > 0.22$) were observed among treatments for all kinematic parameters among the motile, progressive, and slow populations of sperm (data not shown).

Table 2.5. Gross ejaculate characteristics at d 112 of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$.

	Treatment[1]				***P* - value**
Item	CON	60DDGS	SULF	SEM	Trt
Scrotal circumference, cm	37.12	36.5	36.8	0.55	0.77
Ejaculate volume, mL	8.5[a]	9.3[a]	6.1[b]	0.71	0.008
Total sperm, million	1,018.6	1,032.5	756.3	289.5	0.75
Concentration, million/mL	116.1	102.6	128.1	28.1	0.81
Motile, %	76.07	73.16	68.8	3.43	0.33
Progressive, %	62.5	62.2	59.8	3.58	0.85
Slow, %	2.82	2.34	2.66	0.47	0.75
Static, %	23.9	26.8	31.2	3.43	0.33
Morphology					
Proximal droplet, %	2.58	3.65	3.85	0.60	0.30
Bent tail, %	3.0	2.9	2.9	0.54	0.98
Coiled tail, %	1.7	1.9	2.5	0.57	0.53
Distal droplet, %	13.4[a]	11.7[a]	3.5[b]	2.29	0.009
DMR, %	1.9	0.8	1.5	0.47	0.20

[1] CON = corn-based diet containing 60% concentrate (n = 12); 60DDGS = diet containing 60% DDGS as a replacement for corn (n = 12); SULF = CON diet + equivalent sulfur of 60DDGS in diet added as $CaSO_4$ (n = 12).

[ab] Means lacking common superscripts within a row indicate differences among treatments ($P \leq 0.05$).

No differences ($P = 0.13$) were observed among treatments for concentrations of testosterone (mean values for CON, 60DDGS, and SULF were 1710 ± 168 ng/dL, respectively) or thyroxine (mean values for CON, 60DDGS, and SULF were 5.2, 4.8, and 4.8 μg/dL, respectively) in serum; however, concentrations of triiodothyronine were reduced ($P = 0.009$) in bulls fed the 60DDGS diet compared with CON and SULF (Fig. 2.4).

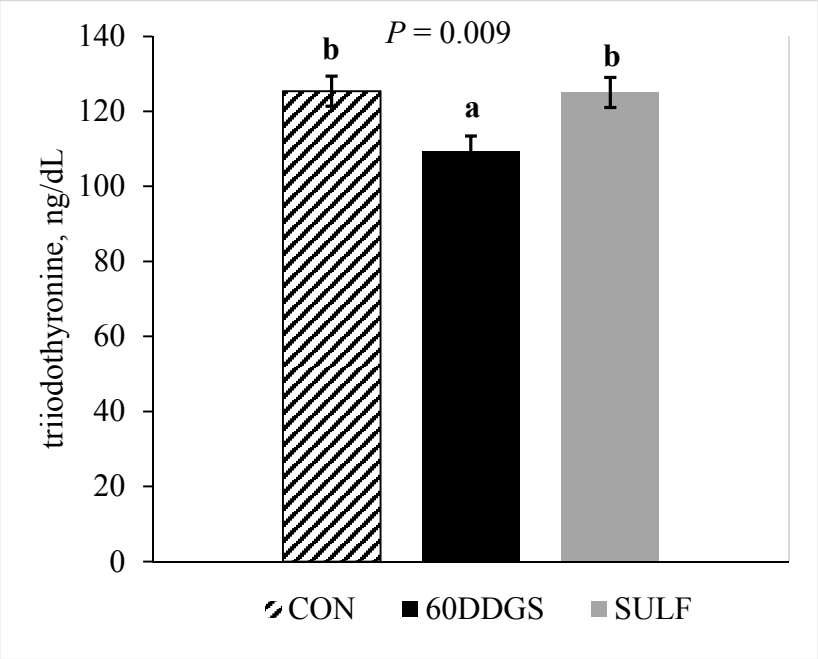

Figure 2.4. Effect of diet on triiodothyronine concentrations in serum of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$.
Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated when *P*–values were ≤ 0.05.

No differences (*P* = 0.66) were observed among treatments for erythrocyte glutathione peroxidase activity (data not shown); however, glutathione peroxidase activity in seminal plasma was influenced by a treatment × day interaction (*P* = 0.03). No differences (*P* > 0.20) were observed at d 0; however, at d 56 bulls fed 60DDGS had greater (*P* = 0.02) activity compared with CON, whereas SULF was intermediate and equal to both CON and 60DDGS. At d 112, glutathione peroxidase remained greater (*P* = 0.008) in bulls fed 60DDGS compared to CON and SULF (Fig. 2.5).

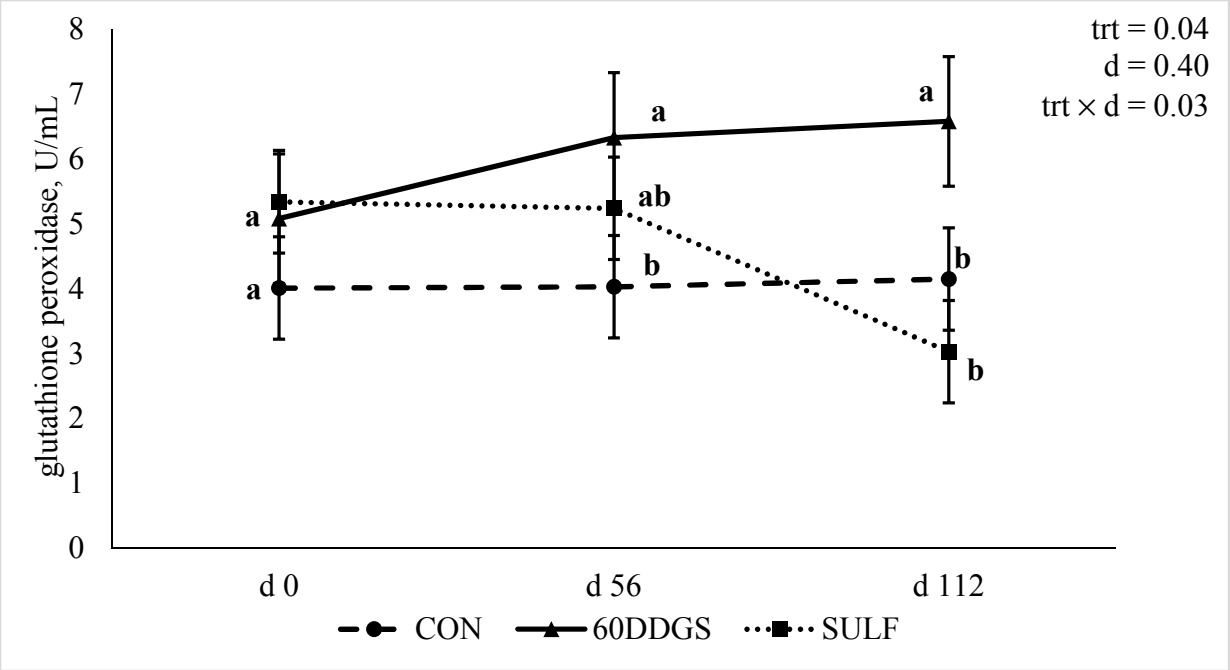

Figure 2.5. Glutathione peroxidase activity in seminal plasma from yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$
Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated within day when the *P*– values were ≤ 0.05.

Discussion

All bulls in the current study were managed to have a targeted ADG of 1.6 kg/d to ensure that any differences observed were due to their dietary ingredients and not rate of gain. Bulls that are developed at faster rates of gain have greater scrotal circumference and enhanced semen characteristics compared with slower gaining counterparts (Palasz et al., 1994); however, some limitations for growth rates do exist as bulls managed aggressively and gaining >1.75 kg/d had reduced semen parameters compared with bulls managed to gain ≤ 1.0 kg/d (Skinner et al., 1981). In bulls gaining >1.75 kg/d excess fat accumulation in the scrotum has been associated with decreased fertility (Brito et al., 2012). Furthermore, Brito et al. (2012) found that optimal growth rates for yearling bulls was between 1 and 1.6 kg/d.

The observed tendency for reduced efficiency in bulls fed the SULF diet aligns with other reports where elevated sulfur impacted performance. Depenbusch et al. (2009) fed increasing concentrations of DDGS ad libitum (0 to 75% DDGS) to yearling heifers and reported a linear decrease for final BW, DMI, ADG, and G:F. Alternatively, Ritcher et al. (2012) fed a low sulfur (S = 0.34%) or a high sulfur (S = 0.47%) diet to beef steers grazing pasture, but were supplemented with DDGS or a concentrate-based diet during their finishing phase. In the high sulfur treatment, ADG was reduced, DMI tended to decrease, but no differences were observed for G:F. It is important to note that bulls in the current experiment were fed for targeted gains; therefore, if our bulls had ad libitum access to diets we may have also observed divergence in ADG and DMI reported by others.

A factor that is well documented to cause decreases in animal performance is the presence of increased ruminal H_2S from feeding high sulfur diets (Neville et al., 2012; Drewnoski et al., 2014a). In the current experiment, ruminal H_2S was measured during the first 42 d, where increased ruminal H_2S was observed in animals that were fed greater amounts of sulfur, which was expected. A review on the bioavailability of sulfur concluded that sulfur from $CaSO_4$ and sulfuric acid had similar availability when compared with sodium sulfate (Henry and Ammerman, 1995). Similarly, Drewnoski et al. (2014b) conducted a study to investigate the effects of feeding different sources of sulfur on ruminal pH and H_2S. Their results were that ruminal pH was reduced when beef steers were fed $CaSO_4$ compared to feeding DDGS, but no differences were observed for ruminal H_2S. However, their $CaSO_4$ diet was formulated to include 21% DDGS, which contributed to a portion of the sulfur in the diet. Additionally, Sarturi et al. (2013) reported that sulfur containing amino acids are more likely to be converted to other amino acids and inorganic sources (DDGS and $CaSO_4$) are more likely to be converted to

ruminal H_2S. In the current study, elevated ruminal H_2S in bulls fed the SULF diet compared with 60DDGS diet indicate that ruminal availability of sulfur in $CaSO_4$ was greater than that of the combination of sulfuric acid and S-containing amino acids present in DDGS.

Our observation of similarities across treatments for SC and overall sperm concentration may have been related to their targeted rates of gain. Additionally, treatment × day interactions observed for some of the kinematic properties of motile sperm (VSL, ALH, percent linear, and percent straight) were largely driven by differences present among treatments at d 0 that were not expected. Knowing differences among treatments at d 0 were driving these interactions, a separate analysis was conducted at d 112 to observe if any differences existed. This time point reflects the end of the development phase and a time when bulls would be placed in breeding pastures with females. At d 112 (13 mo of age), all bulls were considered pubertal and there were no differences observed for the motile, progressive, and slow populations of sperm. Although ejaculate volume was greatest in the 60DDGS bulls, no differences were observed for total sperm or overall concentrations of sperm in ejaculates. Based on the response variables evaluated by the CASA, it would seem that bulls across all treatments would have the ability to breed; however, further research, either in vivo or in vitro, would be necessary to fully evaluate the fertilization potential of these bulls.

It is well documented that sperm motility is one of the most valuable traits when predicting fertility of sperm in bulls (Kathiravan et al., 2011). Technology like the CASA has increased the accuracy for selection of bulls, but with the many response variables CASA reports it is important to know which parameters are the best indicators for determining semen quality. Nagy et al. (2015) conducted a study to determine which velocity parameters within the motile population correlates best with bull fertility. They observed that VAP had the greatest correlation

to semen quality and may be the best predictor for determining the ability of sperm to fertilize. Therefore, in the current study, overall motility was not affected, but sperm in the 60DDGS and SULF treatments had reduced VAP, VCL, and VSL. The data suggests that modifications may be occurring at the molecular level which could affect the ability of sperm to fertilize when sulfur intake is increased in the diet.

Oxidative stress, availability of selenium, and thyroid hormone concentrations are factors that may influence kinematic properties of sperm when greater concentrations of sulfur are fed. Oxidative stress in sperm cells occurs in the presence of reactive oxygen species; therefore, enzymes like the glutathione peroxidase and superoxide dismutase are synthesized to aid in the protection of sperm (Bansal and Bilaspuri, 2010). Inactive thyroxine is converted to active triiodothyronine by type 1 and type 2 deiodinases. Glutathione peroxidase, an enzyme containing selenium, aids in the catalysis of peroxidase degradation while type 1 deiodinase aids in the formation of triiodothyronine by undergoing a double replacement reaction. With the reduction in peroxidases and availability of selenium, glutathione peroxidase forms a selenenyl residue and is then regenerated by thiols. Concurrently, the deiodination of thyroxine produces an intermediate also used to form glutathione peroxidase (Köhrle et al., 2005).

Additionally, a lack of antioxidant enzymes may result in lipid peroxidation and decreased semen motility and quality (de Lamirande et al., 1997). Lipid peroxidation is the process where free radicals take electrons from lipids that contain double bonds resulting in cell damage. Dried corn distillers grain plus solubles have increased concentrations of polyunsaturated fatty acids (PUFA) which increases the likelihood of lipid peroxidation in sperm. However, PUFAs are necessary in sperm to provide flexibility to the sperm plasma membrane which is needed during fertilization. These alterations in the plasma membrane cause

the sperm to become more susceptible to reactive oxygen species which begins the process of lipid peroxidation further damaging sperm function (Wathes et al., 2007; Hamilton et al., 2016).

Increasing dietary sulfur may also cause a reduction in glutathione peroxidase by influencing the bioavailability of selenium ultimately influencing glutathione peroxidase synthesis (Ivancic and Weiss, 2001). When protein and energy is greater in the diet, it would be expected that concentrations of triiodothyronine would be greater. However, in this study, lower triiodothyronine concentrations were observed in serum in bulls fed the 60DDGS diet. The decrease observed in triiodothyronine concentrations may be a result of alterations to energy metabolism in the bulls fed the 60DDGS diet (Huszenicza et al., 2002).

In other studies, induction of hypothyroidism has been observed to increase Sertoli cell proliferation resulting in larger testes and greater sperm production in peripubertal bulls (Majdic et al., 1998; Waqas et al., 2019). On the contrary, when hyperthyroidism was induced, oxidative stress increased, while glutathione peroxidase activity decreased, and DNA damage increased (Choudhury et al., 2003; Dobrzynska et al., 2004; Chenoweth, 2007). Although neither hypo- nor hyperthyroidism was induced (basal concentrations of triiodothyronine in bull serum = 131 ng/dL; Anderson et al., 1988) in the current study, the mechanisms observed in the previous papers indicate that oxidative stress may have caused the decrease in the velocity parameters and alterations in sperm DNA may have occurred. Alternatively, the sperm cells in bulls fed 60DDGS may have had the ability to fight off reactive oxygen species and prevent the sperm from DNA damage through the presence of glutathione peroxidase in seminal plasma, in turn, protecting the sperm from DNA damage.

Conclusion

In the current study where bulls were fed to gain similarly, 60DDGS- treated bulls had increased glutathione peroxidase activity in seminal plasma with a decrease in triiodothyronine concentration compared with other treatments. These results indicate that bulls fed 60DDGS may have produced more glutathione peroxidase in response to oxidative stress in semen. Furthermore, differences observed for VAP, VSL, and VCL in the motile and progressively motile populations of sperm suggest that sulfur may not be the only factor contributing to decreased semen quality in yearling bulls. Other factors from DDGS that may be influencing semen quality are nitrogen, starch, and fat which will be considered for future studies. However, more research needs to be conducted to investigate if any molecular modifications were made in sperm DNA or RNA by feeding 60% DDGS in the diet to yearling bulls. Additionally, further research may be necessary to elucidate the effects of feeding DDGS to yearling bulls on Sertoli cell and seminiferous tubule phenotype and function.

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CHAPTER 3: EFFECTS OF FEEDING 60% DRIED CORN DISTILLERS GRAINS PLUS SOLUBLES OR THE EQUIVALENT SULFUR AS $CaSO_4$ ON GLUCOSE, UREA NITROGEN, AND METABOLITES IN SERUM AND SEMINAL PLASMA[2]

Abstract

The objectives of this study were to investigate the effects of feeding 60% dried corn distillers grains plus solubles (DDGS) or the equivalent S as $CaSO_4$ on glucose, urea nitrogen (N), and metabolite concentrations in serum and seminal plasma. Thirty-six half-sibling Angus bulls [256 ± 8 d; initial body weight = 320 ± 2 kg] were assigned one of three treatments: 1) corn-based diet containing 60% concentrate (CON; S = 0.18%; n = 12); 2) diet containing 60% DDGS as a replacement for corn (60DDGS; S = 0.55% DM; n = 12); 3) CON diet + equivalent S of the 60DDGS diet added as $CaSO_4$ (SULF; S = 0.54%; n = 12). Blood and semen samples were collected on d 0, 56, and 112 then evaluated for concentrations of glucose, urea N, amino acid (AA), and trace mineral (TM) concentrations in serum and seminal plasma. Data were analyzed as repeated measures using PROC MIXED in SAS. A treatment × day interaction was observed ($P < 0.01$) for serum urea N. At d 0, no differences were observed; however, at d 56 and 112, 60DDGS was greater ($P < 0.01$) compared with SULF and CON. For seminal plasma urea N, an effect of treatment ($P < 0.01$) was observed where 60DDGS was greater ($P < 0.01$) compared with SULF and CON. Treatment × day interactions were observed ($P < 0.02$) for Cu, Se, Mo in serum. For serum Cu, no differences ($P > 0.15$) were observed at d 0 or 56, but at d 112, 60DDGS was reduced ($P < 0.01$) compared with SULF and CON. For Se in serum, at d 0,

[2] The material in this chapter was co-authored by Joel S. Caton, James D. Kirsch, Sheri T. Dorsam, Kacie L. McCarthy, Matthew S. Crouse, Kevin K. Sedivec, Bryan W. Neville, and Carl R. Dahlen. Cierrah Kassetas had the primary responsibility of collecting samples in the field, drafting, and revising all versions of this document. James Kirsch, Sheri Dorsam, Kacie McCarthy, Carl Dahlen, Joel Caton, and Matthew Crouse had the primary responsibility of collecting samples and proofreading this document. Kevin Sedivec and Bryan Neville had the responsibility of proofreading this document and preparing the grant for the funding of this project.

no differences ($P > 0.09$) were observed; however, at d 56 and 112, Se was greater ($P < 0.01$) in 60DDGS compared with CON and SULF. For serum Mo, at d 0, 60DDGS was greater ($P = 0.03$) than CON, whereas SULF was intermediate. Then, at d 56 and 112, CON was greater ($P < 0.01$) than SULF and 60DDGS. Treatment × day interactions were observed ($P \leq 0.02$) for Cu and Mo in seminal plasma. For Cu, no differences ($P \geq 0.09$) were observed at d 0 or 56, but at d 112, CON and 60DDGS were greater ($P < 0.01$) compared to SULF. For Mo in seminal plasma, at d 0, 60DDGS was greater ($P = 0.03$) compared with SULF, whereas CON was intermediate, but at d 56 and 112, CON was greater ($P < 0.01$) than 60DDGS and SULF. In addition, differences ($P = 0.01$) were observed for Se in seminal plasma where Se was greater ($P = 0.02$) for 60DDGS compared with SULF, whereas CON was intermediate. Increases in dietary S altered TM concentrations which could be influencing sperm DNA integrity. However, components of DDGS other than S may be influencing semen characteristics.

Key words: beef bulls, dried corn distillers grains plus solubles, glucose, urea nitrogen, trace mineral concentrations

Introduction

Alterations to the nutrient and metabolite components of seminal plasma can be detrimental to the survival of sperm after ejaculation (Juyena and Stelletta, 2012). Studies have concluded that plane of nutrition can influence testes size, testicular morphology (i.e. seminiferous tubules, interstitial cells), and seminal vesicle development (Barth et al., 2008; Brown, 1993; Kastelic and Thundathil, 2008). Modifications to the development of seminal vesicles may result in alterations to the composition and overall production of seminal plasma.

Specifically, changes to glucose, trace minerals, and amino acid concentrations in seminal plasma may influence bull performance. Glucose is one fundamental component of

seminal plasma that is important for the synthesis of fructose for energy production in sperm cells. Not only is fructose necessary for energy production, it is also necessary for sperm motility and function (Juyena and Stelletta, 2012). In addition, previous studies have observed that with the inclusion of trace minerals, especially Zn, Cu, and Se, semen quality may improve in prepubertal bulls (Geary et al, 2016; Rowe et al., 2014). Zn, Cu, and Se are trace minerals that are necessary for synthesis of important antioxidant enzymes, for example, glutathione peroxidase and superoxide dismutase, that aid in the prevention of oxidative stress to sperm cells (Bansal and Bilaspuri, 2011; Tvrda et al., 2015). Lastly, amino acid composition within seminal plasma is necessary for synthesis of key seminal proteins (Juyena and Stelletta, 2012). Previous literature has stated that seminal proteins are important to maintain plasma membrane stability, sperm motility, and support fertilization (Desnoyers and Manjunath, 1992; Yanagimachi, 1994).

Therefore, alterations observed in seminal plasma may be a direct consequence of the nutritional status of the bull. Additionally, specific dietary ingredients such as DDGS may be influencing yearling bull reproduction by altering nutrient and metabolite concentrations in serum and seminal plasma. A study was conducted to further investigate the effects of feeding 60% DDGS (diet high in sulfur) or the equivalent sulfur as $CaSO_4$ on glucose, urea nitrogen, and metabolite concentrations in developing beef bulls gaining similarly. We hypothesized that nutrient and metabolite concentrations will be altered in serum and seminal plasma of bulls fed diets containing greater concentrations of sulfur from DDGS or $CaSO_4$.

Materials and Methods

All procedures were approved by the North Dakota State Institution for Animal Care and Use Committee (#A19035). Treatments are briefly described below, but further information regarding diets and animal care can be found in Chapter 2.

Animals and Diets

Thirty-six half-sibling Angus bulls [256 ± 8 d; mean initial BW = 320 ± 2 kg] were assigned to one of three treatments: 1) corn-based diet containing 60% concentrate [CON; S = 0.18% DM; n = 12]; 2) diet containing 60% DDGS as a replacement for corn (60DDGS; S = 0.55% DM; n = 12); 3) CON diet + equivalent sulfur of the 60DDGS diet added as $CaSO_4$ (SULF; S = 0.54% DM; n = 12). All bulls were housed indoors in the Animal Nutrition and Physiology Center in Fargo, North Dakota. Bulls began the study at 291 ± 8.5 d of age and were individually fed in a Calan gate system. Individual intakes were adjusted to target a 1.6 kg/d ADG.

Table 3.1. Mineral composition for control (CON), 60% dried corn distillers grains plus solubles (60DDGS), and sulfur (SULF) diets.

	Treatment[1]		
Item	CON	60DDGS	SULF
Mineral Composition, %			
Ca, %	0.96	1.05	1.12
P, %	0.30	0.70	0.30
S, %	0.18	0.55	0.54
Co, %	-	-	-
Cu, %	0.0003	0.0003	0.0003
Fe, %	0.04	0.03	0.04
Mg, %	0.20	0.34	0.19
Mn, %	0.007	0.007	0.006
Mo, %	0.0001	0.0001	0.0001
Zn, %	0.019	0.018	0.016
Se, %	-	-	-

[1] CON = corn-based diet containing 60% concentrate (n = 12)**;** 60DDGS = diet containing 60% DDGS as a replacement for corn (n = 12)**;** SULF = CON diet + equivalent sulfur of 60DDGS in diet added as $CaSO_4$ (n = 12).

Blood and Semen Collection

Body weights were recorded every 14 d during the 112-day study with a 2-day weight at the beginning and end of the study. Before the morning feeding on d 0, 56, and 112 blood samples were collected via jugular venipuncture in serum and trace mineral tubes. Tubes for

serum contained spray coated silica and the trace mineral tubes for plasma lack a lubricant on the stopper to avoid influencing results. Blood collected in serum tubes were used for glucose, urea-N, and amino acid analyses. Semen was collected on d 0, 56, and 112 via electroejaculation (Pulsator IV; Lane Manufacturing Inc; Denver, CO) into disposable plastic semen collection bags. All blood and semen samples were centrifuged at 1,500 × g for 20 minutes at 4°C (Sorvall ST 16R; Thermo Scientific Inc.; Waltham, MA). The supernatant from the serum and plasma blood tubes were pipetted into 2-mL screw cap tubes and stored at -20°C. After centrifugation of semen, seminal plasma was pipetted into 2-mL screw cap tubes and stored at -20°C.

Glucose Analysis in Serum and Seminal Plasma

Glucose was analyzed on a microplate spectrophotometer using the Infinity glucose kit from Thermo Scientific containing the hexokinase/glucose- 6- phosphate dehydrogenase method (Pittsburgh, PA, USA). For control samples, intra-assay CV for serum glucose analysis were 4.8 ± 1.8% and the inter-assay CV was 4.5%. Intra-assay CV's for seminal plasma glucose were 3.3 ± 1.0% and the inter-assay CV was 3.5%.

Urea Nitrogen Analysis in Serum and Seminal Plasma

Serum urea-N was analyzed based on the procedures of Jung et al. (1975). A QuantiChrom Urea Assay Kit (BioAssay Systems; Hayward, CA) containing *o*-phthaldialdehyde and primaquine diphosphate was analyzed on the microplate spectrophotometer. Intra-assay CV for serum urea- N controls was 5.6 ± 3.7%; and the inter-assay CV was 6.6%. For seminal plasma urea-N controls, intra-assay CV was 2.25 ± 0.3%; and the inter-assay CV was 6.8%.

Amino Acid Analysis in Serum and Seminal Plasma

Amino acids were analyzed in serum and seminal plasma by Ultra Performance Liquid Chromatography (**UPLC**). In short, once the samples were prepared they were inserted into the

UPLC and the MassTrac Amino Acid Analysis system was used to profile the amino acids in fluids (Crouse et al., 2016). Derivatization chemistry for physiological samples is a precolumn method and is based on a derivatizing reagent, which converts both primary and secondary amino acids to stable chromophores for UPLC detection (Lemley et al., 2013). Percent of total amino acids were calculated by obtaining a total concentration for all amino acids for one bull. Next, the individual amino acid concentrations were divided from the total and multiplied by 100.

Trace Mineral Analysis in Serum and Seminal Plasma

A trace mineral panel was run on all serum and seminal plasma samples. This panel consisted of Co, Cu, Mn, Mo, Se, Fe, and Zn. All samples were analyzed at the Veterinary Diagnostic Lab at Michigan State University using Inductively Coupled Plasma- Optical Emission Spectrometry (Lansing, MI).

Statistical Analysis

For all analyses, bull was the experimental unit. Serum glucose, urea-N, amino acids, and trace minerals were analyzed using repeated measures in the MIXED procedures of SAS (SAS 9.4; Cary, NC, USA) with a model including treatment, day, and a treatment × d interaction. For each variable tested, appropriate covariate structures were evaluated and the structure with the smallest AIC was used (Wang and Goonewardene, 2004). Results considered significant when *P*-values were ≤ 0.05.

Results

Glucose and Urea Nitrogen

No differences ($P \geq 0.24$) were observed among treatments for concentrations of glucose in serum or seminal plasma; however, effects of day were observed. Bulls had greater ($P < 0.01$;

data not shown) concentrations of glucose at d 56 and 112 compared with d 0 (means = 82.3, 83.8, and 70.0 ± 4.3 mg/dL, respectively) in serum. In seminal plasma, glucose concentrations were greater ($P < 0.02$; data not shown) at d 112 (231.6 mg/dL) compared with d 0 (109.2 mg/dL) and 56 (171.5 mg/dL).

A treatment × day interaction ($P < 0.01$) was observed for urea-N concentrations in serum (Fig. 3.1). At d 0, no differences were observed among treatments for serum urea-N concentrations ($P > 0.77$); however, at d 56 and 112, 60DDGS had greater ($P < 0.01$) concentrations of urea-N compared with SULF and CON (Fig. 3.1). For seminal plasma, differences ($P = 0.002$) were observed among treatments where 60DDGS had a greater ($P < 0.01$) concentrations of urea-N compared with CON and SULF (Fig. 3.2).

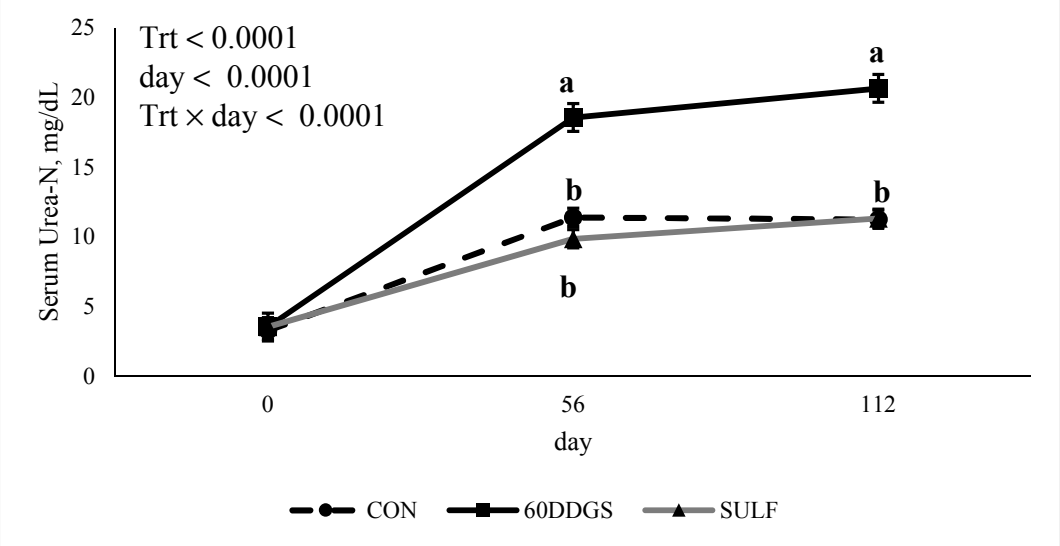

Figure 3.1. Effect of diet on urea nitrogen concentrations in serum of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$.
Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated within day when *P*– values were ≤ 0.05.

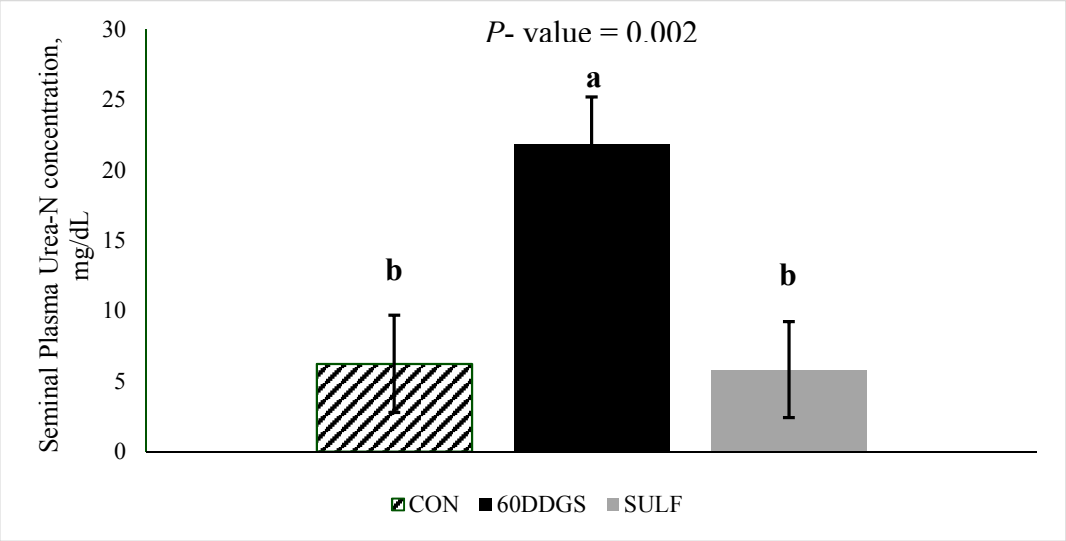

Figure 3.2. Effect of diet on urea nitrogen concentrations in seminal plasma of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$. Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated among treatments when *P*– values were ≤ 0.05.

Serum Amino Acids

In serum, treatment × day interactions were observed for essential AA, Arg, Thr, Lys, Val, Ile, Leu, and Phe (Table 3.2). For essential AA, no differences ($P \geq 0.29$) were observed among treatments on d 0; however, at d 56 and 112 60DDGS was greater ($P \leq 0.01$) compared to SULF and CON.

For Arg, no differences ($P \geq 0.08$) were observed among treatments at d 0, but 60DDGS was greater ($P = 0.005$) at d 56 than all treatments. At d 112, 60DDGS was greater ($P < 0.02$) compared with CON, whereas SULF was intermediate.

For Thr, no differences ($P \geq 0.08$) were observed among treatments at d 0 and 56; however, 60DDGS was greater ($P = 0.002$) than CON and SULF at d 112. For Lys, no differences ($P \geq 0.50$) were observed among treatments at d 0. However, at d 56, 60DDGS was

reduced ($P < 0.01$) compared with CON, whereas SULF was intermediate. At d 112, 60DDGS remained reduced ($P = 0.02$) compared with SULF and CON.

At d 0, no differences ($P > 0.18$) were observed for Val, Ile, and Leu; however, 60DDGS was greater ($P < 0.02$) compared with all other treatments at d 56 and 112. For Phe, no differences ($P \geq 0.16$) were observed at d 0; however, 60DDGS was greater ($P \leq 0.005$) compared with SULF and CON at d 56; however, at d 112 60DDGS was greater ($P < 0.01$) compared with CON, where SULF was intermediate.

Differences ($P < 0.01$) were observed among treatments for Trp where 60DDGS was greater ($P \leq 0.01$) compared with SULF and CON. Effects of day ($P \leq 0.01$) were observed for His and Met. Concentrations of His in serum were greatest ($P = 0.01$) at d 0 compared with d 56, where d 112 was intermediate. For Met, concentrations were greatest ($P < 0.01$) on d 56 compared with d 0 and d 112 (Table 3.2).

For non- essential AA in serum, treatment × day interactions were observed for non-essential AA, Gly, Asp, Glu, Ala, Pro, Tyr, and Tau (Fig. 3.2). For non-essential AA, Gly, Asp, and Glu, there were no differences ($P \geq 0.29$) observed on d 0; however, at d 56 and 112, 60DDGS was reduced ($P < 0.01$) compared to SULF and CON. For Ala, no differences ($P \geq 0.31$) were observed at d 0; however, at d 56, 60DDGS had the lowest ($P \leq 0.04$) percentage of Ala compared CON, with SULF being intermediate. At d 112, Ala was reduced ($P < 0.01$) in 60DDGS compared with SULF and CON.

Pro was slightly greater ($P = 0.03$) in 60DDG at d 0 compared with SULF and CON, but 60DDGS remained greater ($P < 0.01$) at d 56 and 112 compared with SULF and CON. No differences ($P \geq 0.15$) were observed for Tyr at d 0; however, at d 56, 60DDGS was greater ($P =$

0.003) compared with SULF. At d 112, 60DDGS had greater ($P < 0.01$) Tyr compared with SULF and CON (Table 3.2).

For Tau, CON was greater ($P = 0.04$) compared to SULF and 60DDGS at d 0; however, at d 56, no differences ($P \geq 0.10$) were observed among treatments. At d 112, 60DDGS was greater ($P < 0.01$) compared with SULF, with CON intermediate.

Differences ($P \leq 0.03$) were observed among treatments for Ser and Gln in serum. For Ser, 60DDGS and SULF had reduced Ser compared to CON. Gln concentrations were reduced for 60DDGS compared with CON and SULF. An effect of day was observed ($P < 0.01$) for Asn where concentrations were reduced ($P < 0.01$) at d 0 when compared to d 56 and 112 (Table 3.2).

Table 3.2. Least square means for the effect of diet and day on the percent of total amino acids in serum of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$.

		Day					*P*- value		
Item, %	Treatment[1]	0	56	112	Trt Avg[2]	SE	Trt	Day	Trt × d[3]
Essential AA	Day	33.0	34.4	36.9		0.74	<0.01	<0.01	<0.01
	CON	33.0	31.4[b]	33.4[b]	32.6	1.3			
	60DDGS	32.6	40.3[a]	43.8[a]	38.9	1.0			
	SULF	33.4	31.5[b]	33.3[b]	32.7	1.2			
His	Day	3.7[a]	2.7[b]	3.0[c]		0.08	0.69	<0.01	0.11
Arg	Day	5.4	7.2	7.1		0.16	< 0.01	<0.01	0.003
	CON	5.0	7.3[c]	7.3[b]	6.5	0.27			
	60DDGS	5.5	6.2[a]	6.4[a]	6.0	0.27			
	SULF	5.6	7.9[b]	7.5[b]	7.0	0.27			
Thr	Day	4.4	3.2	2.5		0.22	0.01	<0.01	0.004
	CON	4.6	3.0[a]	1.9[b]	3.2	0.38			
	60DDGS	4.1	3.4[a]	3.6[a]	3.7	0.38			
	SULF	4.3	3.0[a]	1.9[b]	3.0	0.38			
Lys	Day	2.8	2.7	3.4		0.12	< 0.01	<0.01	0.002
	CON	2.7	3.2	3.6	3.2	0.21			
	60DDGS	2.8	1.8	3.0	2.5	0.20			
	SULF	2.7	2.8	3.6	3.0	0.20			
Met	Day	1.3[a]	1.5[b]	1.4[a]		0.03	0.19	0.01	0.18
Val	Day	7.5	9.4	10.4		0.25	<0.01	<0.01	<0.01
	CON	7.4	8.4[b]	9.2[b]	8.3	0.45			
	60DDGS	7.4	11.5[a]	12.8[a]	10.6	0.43			
	SULF	7.7	8.2[b]	9.1[b]	8.3	0.43			
Ile	Day	3.3	3.9	4.2		0.09	<0.01	<0.01	0.0007
	CON	3.1	3.7[b]	3.8[b]	3.6	0.15			
	60DDGS	3.2	4.2[a]	4.8[a]	4.1	0.14			
	SULF	3.3	3.5[b]	3.9[b]	3.6	0.14			
Leu	Day	5.5	7.1	7.8		0.29	<0.01	<0.01	<0.01
	CON	3.1	3.7[b]	3.8[b]	3.6	0.15			
	60DDGS	3.2	4.2[a]	4.8[a]	4.1	0.14			
	SULF	3.3	3.5[b]	3.9[b]	3.6	0.14			
Phe	Day	2.7	2.2	2.3		0.05	<0.01	<0.01	0.0002
	CON	2.6	1.8[a]	2.0[a]	2.1	0.09			
	60DDGS	2.6	2.5[b]	2.5[b]	2.5	0.09			
	SULF	2.7	2.2[c]	2.1[a]	2.3	0.09			
Trp	Day	1.9	1.8	1.9		0.04	< 0.01	0.18	0.48
	CON				2.1[a]	0.05			
	60DDGS				2.5[b]				
	SULF				2.3[c]				
Non-essential AA	Day	66.9	65.5	63.1		0.74	<0.01	<0.01	<0.01
	CON	66.9	65.5[a]	63.1[a]	67.3	1.31			
	60DDGS	67.3	59.6[b]	56.1[b]	61.0	1.26			
	SULF	66.5	68.4[a]	66.6[a]	67.2	1.26			
Asn	Day	1.6[a]	1.9[b]	1.8[b]		0.04	0.80	<0.01	0.20
Ser	Day	5.3	4.2	3.9		0.12	0.03	<0.01	0.12
	CON				4.7[a]	0.14			
	60DDGS				4.3[b]				
	SULF				4.2[b]				

Table 3.2. Least square means for the effect of diet and day on the percent of total amino acids in serum of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$ (continued).

		Day					*P*- value		
Item, %	Treatment[1]	0	56	112	Trt Avg[2]	SE	Trt	Day	Trt × d[3]
Gln	Day	11.1	13.9	14.1		0.39	<0.01	<0.01	0.10
	CON				13.9[a]	0.33			
	60DDGS				11.6[b]				
	SULF				13.4[a]				
Gly	Day	21.8	17.9	16.8		0.57	< 0.01	<0.01	0.004
	CON	21.7	18.1[a]	18.1[a]	19.3	1.0			
	60DDGS	22.2	15.7[b]	13.5[b]	17.1	0.96			
	SULF	21.4	19.6[a]	18.8[a]	19.9	0.96			
Asp	Day	0.46	0.54	0.54		0.016	<0.01	<0.01	<0.01
	CON	0.44	0.59[a]	0.59[a]	0.54	0.02			
	60DDGS	0.48	0.43[b]	0.42[b]	0.44	0.02			
	SULF	0.47	0.60[a]	0.59[a]	0.55	0.02			
Glu	Day	4.2	3.4	2.7		0.13	<0.02	<0.01	0.008
	CON	4.0	3.5[a]	3.0[a]	3.5	0.22			
	60DDGS	4.1	2.6[b]	1.8[b]	2.8	0.21			
	SULF	4.3	3.9[a]	3.1[a]	3.8	0.21			
Ala	Day	7.8	7.9	7.6		0.18	<0.01	0.32	<0.01
	CON	7.7	9.1[a]	8.2[a]	8.3	0.37			
	60DDGS	7.9	6.7[b]	6.1[b]	6.9	0.36			
	SULF	7.6	7.8[c]	8.4[a]	7.9	0.36			
Pro	Day	3.7	3.6[b]	3.3[b]		0.06	<0.01	<0.01	<0.01
	CON	3.5[b]	3.2[a]	3.0[a]	3.3	0.1			
	60DDGS	3.8[a]	4.3[b]	3.7[b]	3.9	0.1			
	SULF	3.5[b]	3.1	2.9	3.2	0.1			
Cys	Day	0.003	4.6[-6]	0.001		0.001	0.29	0.19	0.49
Tyr	Day	2.9	2.8	2.8		0.06	<0.03	0.24	0.003
	CON	2.8	2.4[a]	2.5[b]	2.6	0.11			
	60DDGS	2.8	3.2[b]	3.1[a]	3.0	0.11			
	SULF	3.0	2.7[c]	2.7[b]	2.8	0.11			
Tau	Day	2.7	2.2	2.4		0.12	0.07	0.01	0.05
	CON	3.0[a]	2.0[b]	2.4[ab]	2.4	0.21			
	60DDGS	2.5[b]	2.5[a]	2.7[a]	2.6	0.20			
	SULF	2.5[b]	2.1[b]	2.0[b]	2.2	0.20			

[1] Dietary treatments were: 1) 60% concentrate (CON; n = 12); 2) 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON as $CsSO_4$ (SULF; n = 12).

[2] [ab] Differences are indicated within treatment when *P*– value is ≤ 0.05.

[3] [ab] Differences for treatment × day interactions are indicated within day when *P*– value is ≤ 0.05.

Seminal Plasma Amino Acids

In seminal plasma, a treatment × day interaction was observed for Val ($P = 0.008$; Fig. 3.3). On d 0, CON was greater ($P \leq 0.01$) compared with 60DDGS, where SULF was

intermediate and equal to both CON and 60DDGS. At d 56, 60DDGS had greater Val ($P \leq 0.01$) compared with SULF and CON. At d 112, no differences ($P \leq 0.01$) were observed among treatments.

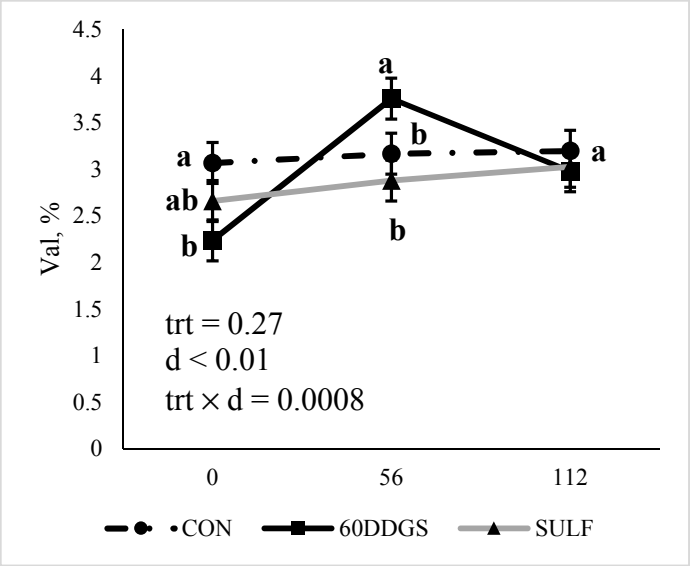

Figure 3.3. Effect of diet on the percentage of the total Val in seminal plasma of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$. Dietary treatments were: 1) 60% concentrate (CON; n = 12); 2) 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON as $CsSO_4$ (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated within day when *P*– values were ≤ 0.05.

Treatment differences ($P = 0.05$) were observed for Glu, where SULF was greater ($P = 0.05$) compared with CON, where 60DDGS was intermediate (Table 3.3). Effects of day were observed ($P \leq 0.05$) for overall essential and non- essential AA, His, Arg, Lys, Leu, Trp, Asn, Ser, Gly, Asp, Ala, and Pro (Table 3.3). For His, concentrations were greater at d 0 ($P \leq 0.01$) compared to d 56 and 112. For essential AA, Arg, Lys, Leu, Asn, and Asp concentrations increased ($P \leq 0.02$) from d 0 to d 112. Non- essential AA and His concentrations decreased ($P \leq 0.01$) from d 0 to d 112. Concentrations for Ser at d 0 were reduced ($P = 0.02$) when compared to d 56, where at d 112 concentrations were intermediate and equal to d 0 and 112. Concentrations of Ala were greatest ($P = 0.003$) at d 0 compared with d 56, where d 112 was intermediate and

equal to d 0 and 56. Concentrations of Gly decreased ($P < 0.01$) from d 0 to 112. Lastly, for Trp, concentrations were greatest ($P < 0.02$) at d 56 compared with d 0 and 112 (Table 3.3).

Table 3.3. Least square means for the effect of diet and day on the percent of total amino acids in seminal plasma of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$.

	Treatment[1]				Day[2]				*P*- value		
Item, %	CON	DDGS	SULF	SE	0	56	112	SE	Trt	Day	Trt × d[3]
Essential AA	24.9	22.7	24.1	1.1	21.0	25.6	25.1	1.1	0.35	< 0.01	0.38
His	2.8	2.7	2.9	0.15	3.4[a]	2.6[b]	2.4[b]	0.15	0.76	< 0.01	0.48
Arg	11.1	11.5	11.2	0.70	8.7[a]	12.8[b]	12.3[b]	0.66	0.88	< 0.01	0.79
Thr	1.4	1.1	1.4	0.19	1.1	1.2	1.5	0.21	0.40	0.39	0.49
Lys	4.6	4.4	4.5	0.27	3.9[a]	4.6[b]	5.1[b]	0.27	0.78	0.006	0.56
Met	0.82	0.68	0.65	0.07	0.60	0.82	0.73	0.09	0.18	0.09	0.38
Val	3.2	3.0	2.9	0.13	2.7	3.3	3.1	0.13	0.27	< 0.01	< 0.01
Ile	1.3	1.1	1.2	0.05	1.1	1.3	1.2	0.05	0.27	0.15	0.27
Leu	7.7	7.0	7.6	0.54	5.7[a]	8.4[b]	8.1[b]	0.58	0.61	< 0.01	0.54
Phe	1.8	1.6	1.6	0.17	1.4	1.8	1.8	0.15	0.62	0.34	0.81
Trp	1.4	1.2	1.5	0.14	1.1[a]	1.7[b]	1.3[a]	0.14	0.41	0.01	0.84
Non- essential AA	75.1	77.3	75.9	1.08	78.9	74.4	74.9	1.1	0.35	< 0.01	0.38
Asn	0.63	0.76	0.67	0.05	0.43[a]	0.67[b]	0.96[c]	0.07	0.22	< 0.01	0.88
Ser	6.3	6.5	6.0	0.43	5.6[a]	6.9[b]	6.2[ab]	0.55	0.71	0.05	0.16
Gln	1.6	2.0	1.9	0.25	0.63	0.88	3.96	0.37	0.50	< 0.01	0.06
Gly	21.6	23.5	19.5	1.9	30.0[a]	20.8[b]	13.8[c]	2.3	0.35	< 0.01	0.74
Asp	2.2	2.1	2.4	0.15	1.9[a]	2.3[b]	2.5[b]	0.14	0.34	0.01	0.72
Glu	10.9[a]	12.3[ab]	14.6[b]	1.03	10.4	11.4	15.9	1.04	0.05	< 0.01	0.80
Ala	13.5	11.8	11.4	0.75	13.7[a]	11.0[b]	12.0[ab]	0.97	0.13	< 0.01	0.52
Pro	1.2	1.2	1.2	0.09	1.4[a]	1.3[a]	0.8[b]	0.08	0.95	< 0.01	0.77
Cys	0.20	0.18	0.22	0.02	0.21	0.19	0.21	0.02	0.40	0.53	0.16
Tyr	1.5	1.4	1.3	0.09	1.0	1.7	1.4	0.09	0.20	< 0.01	0.07
Tau	4.2	4.0	5.4	0.52	4.6	4.3	4.8	0.54	0.14	0.54	0.07

1 Treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12).

2 Bulls were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo).

3 For means separation refer to Fig. 3.3.

[ab] Differences indicated when *P*– values were ≤ 0.05.

Serum Trace Minerals

For trace mineral concentrations in serum, treatment × day interactions were observed for Co, Cu, Zn, Se, and Mo ($P \leq 0.02$; Fig. 3.4). For Cu, no differences ($P \geq 0.15$) were observed on d 0 or 56, but at d 112, DDGS was reduced ($P < 0.01$) compared with SULF and CON (Fig. 3.4A). At d 0, no differences ($P \geq 0.38$) were observed for Co, but on d 56, CON was greater ($P < 0.01$) compared with 60DDGS and SULF; however, no differences ($P \geq 0.09$) were observed among treatments for Co on d 112 (Fig. 3.4B). For Zn, at d 0, SULF was greater ($P < 0.01$) compared with CON and 60DDGS was intermediate and equal to both SULF and CON (Fig. 3.4C). However, no differences ($P > 0.65$) were observed among treatments at d 56 or 112. For Se, at d 0, no differences ($P \geq 0.09$) were observed; however, at d 56 and 112, 60DDGS was greater ($P \leq 0.01$) compared with CON and SULF (Fig. 3.4D). For Mo, at d 0, 60DDGS was greater ($P = 0.03$) than CON, whereas SULF was intermediate. At d 56 and 112, CON was greater ($P < 0.01$) than SULF and 60DDGS for Mo (Fig. 3.4E). An effect of day was observed for Mn where SULF was greater ($P = 0.01$) compared with CON, whereas 60DDGS was intermediate.

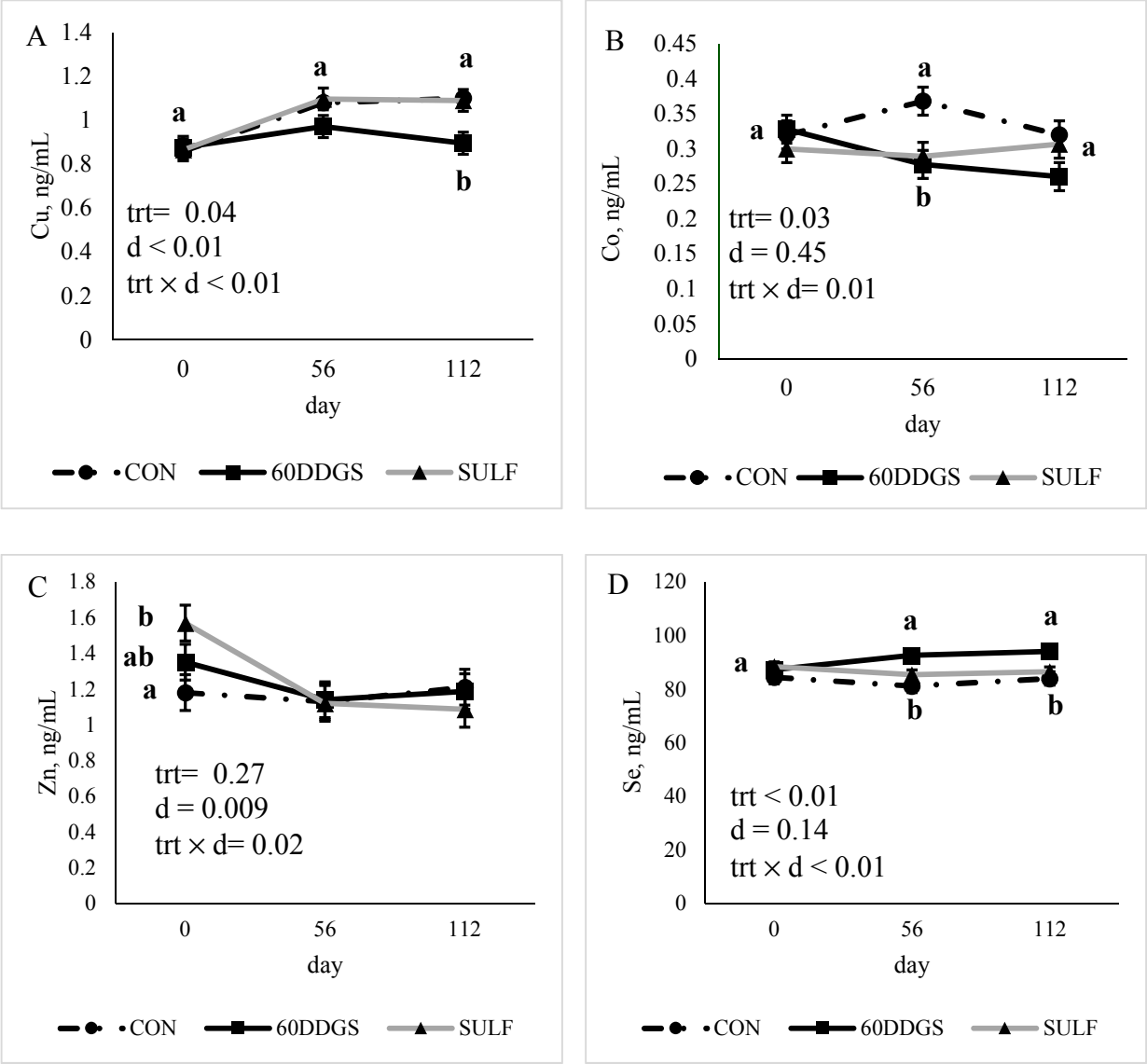

Figure 3.4. Effect of diet on trace mineral concentrations in serum of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$.
Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated within day when *P*– values were ≤ 0.05.

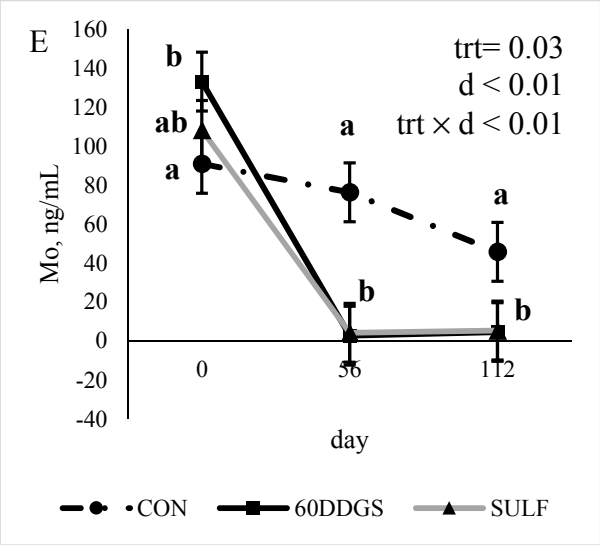

Figure 3.4. Effect of diet on trace mineral concentrations in serum of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$ (continued). Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated within day when *P*– values were ≤ 0.05 (continued).

Seminal Plasma Trace Minerals

For trace mineral concentrations in seminal plasma, treatment × day interactions were observed for Cu and Mo ($P = 0.02$, 0.01, respectively; Fig. 3.5). For Cu, no differences ($P \geq 0.09$) were observed at d 0 or 56, but on d 112, CON and DDGS were greater ($P < 0.01$) compared with SULF. For Mo, at d 0, 60DDGS was greater ($P = 0.03$) compared with SULF, whereas CON was intermediate. At d 56 and 112, CON was greater ($P < 0.01$) compared with 60DDGS and SULF for Mo. Furthermore, a treatment effect was observed for Se where 60DDGS was greater ($P = 0.02$) compared with SULF, whereas CON was intermediate (Fig 3.6). An effect of day was observed for concentrations of Mn. Mn was greatest ($P = 0.01$) at d 56 and least ($P = 0.05$) at d 0, where concentrations of Mn were intermediate at d 112 (data not shown).

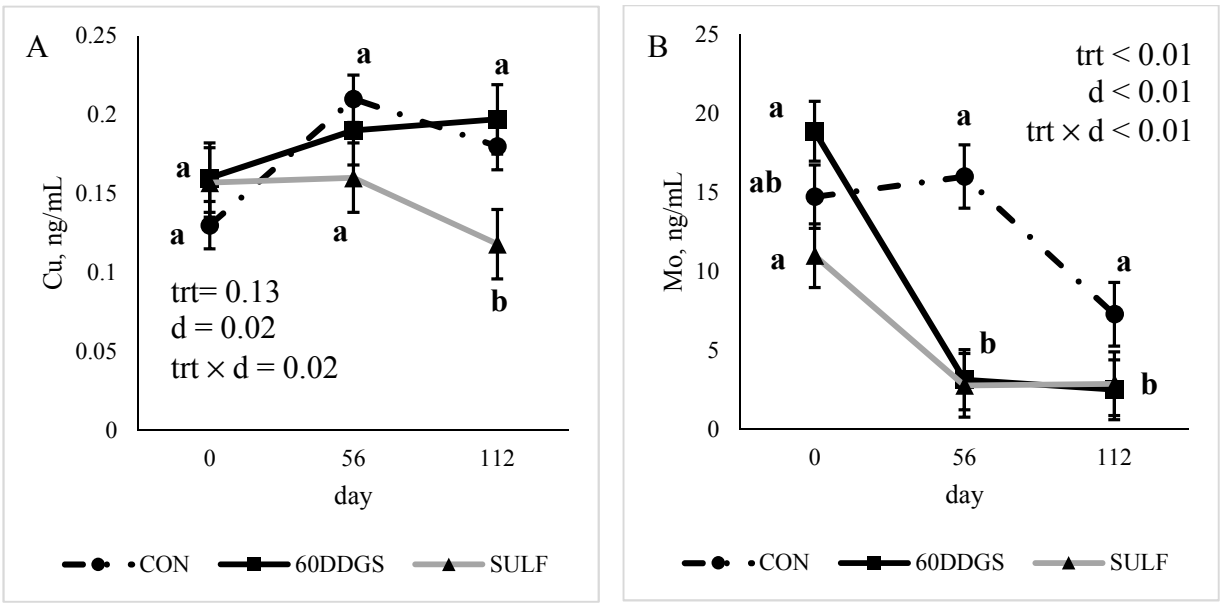

Figure 3.5. Effect of diet on trace mineral concentrations in seminal plasma of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$. Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated within day when P– values were ≤ 0.05.

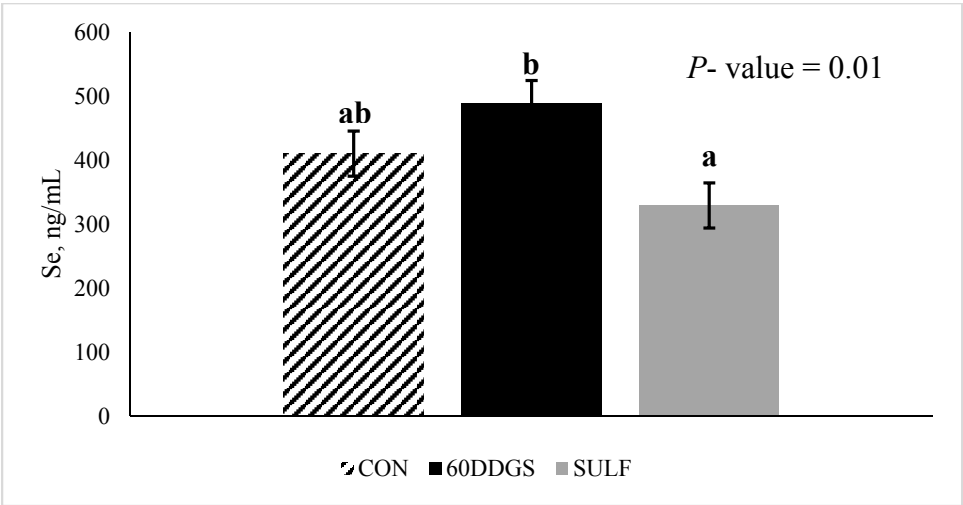

Figure 3.6. Effect of diet on Se concentrations in seminal plasma of yearling Angus bulls fed 60% dried corn distillers grains plus solubles or the equivalent sulfur as $CaSO_4$. Dietary treatments were: 1) corn-based diet containing 60% concentrate (CON; n = 12); 2) diet containing 60%; DDGS as a replacement for corn (60DDGS; n = 12); 3) equivalent sulfur of 60DDGS added to the CON diet as calcium sulfate (SULF; n = 12) and were fed from 291 ± 8.5 d of age (9 mo) to d 112 they were 403 ± 8.5 d of age (13 mo). [ab] Differences indicated among treatments when *P*– values were ≤ 0.05.

Discussion

Glucose and Urea Nitrogen

Although, fructose in seminal plasma is considered the main energy source for sperm and is at greater concentrations (150 to 900 mg/dL); glucose (300 mg/dL) can maintain ATP levels (Juyena and Stelleta, 2012; Martikainen et al., 1980). In the current study, glucose increased for both serum and seminal plasma over the developmental period which was expected. Furthermore, increases in seminal plasma glucose concentrations may be a result of the increase in demand for energy for sperm production.

Concentrations of urea-N were greatest in the 60DDGS treatment for both serum and seminal plasma which may be explained by the percentage of protein in these diets. Previous studies have reported that as protein content in the diet increases, blood urea-N also increases (Bahrami- Yekdangi et al., 2014). However, the presence of urea in seminal plasma is important for protein metabolism and is a product of protein degradation (Vehlo et al., 2018). Additionally, urea can be converted to uric acid which may be beneficial because of the potential for uric acid to act as an antioxidant. If so, greater uric acid concentrations in semen may enhance sperm motility, viability, and morphology (Banihani, 2018). In the current study, uric acid was not measured, but should be considered for future studies. In addition, more research is necessary to evaluate the presence of urea and uric acid in seminal plasma and possible mechanisms for how these nutrients can influence sperm in bulls.

Amino Acids

Many studies have evaluated amino acids concentrations in seminal plasma of bulls to improve semen extenders and cryoprotectants. Additionally, some studies have attempted to correlate specific amino acids to the fertilization potential of a bull. One study evaluated the

effects of amino acid composition on non- return rates in cows bred with high or low fertility bulls. A correlation was observed between non-return rates of the cows and citrate, tryptamine, Tau, and Leu. High fertility bulls had low concentrations of citrate and Ile, and high concentrations of tryptamine, Tau, and Leu in seminal plasma (Kumar et al., 2015). It was concluded that the combination of tryptamine, Tau, and Leu in seminal plasma may improve motility, capacitation, and acrosomal reactions in sperm. Therefore, these metabolites could potentially be used as biomarkers for fertility (Kumar et al., 2015). Additionally, Holden et al. (2017) researched the effects of different amino acid profiles in seminal plasma on pregnancy rates from non- sorted or sex- sorted semen. A positive correlation was observed between Val and Ile for pregnancy rates in non- sorted semen. For sex- sorted semen glutamic acid was positively correlated to pregnancy rates.

In the current study, differences were observed for Tau, Leu, and Ile, but these results were in serum. In seminal plasma, differences were observed for Val at the beginning of the study, but by d 112 no differences were observed among treatments. Interestingly, no differences were observed for the S- containing amino acids Cys and Met in serum or seminal plasma with the increase of dietary sulfur. These amino acids are vital for the regulation of the transsulfuration pathway which synthesize important hormones, enzymes, and proteins (Stipanuk and Ueki, 2011). Therefore, the data in the current study does not suggest that amino acid concentrations were influenced solely by treatments in this study. Differences observed in serum may have been a result of the increase of nitrogen in bulls fed 60DDGS diet. However, the lack of differences in seminal plasma indicate that there are mechanisms within the accessory sex glands that regulate amino acid concentrations in seminal plasma.

Trace Minerals

Trace minerals, especially Cu, Mo, Zn, and Se, are crucial for the development of a bull and maturation of spermatozoa. The primary functions of the trace minerals mentioned previously are to aid in spermatogenesis, enhance motility and morphology of sperm, and synthesize steroid hormones (Preedy et al., 2018). Improvement of sperm motility and morphology was observed when an injectable trace mineral was administered to prepubertal bulls at puberty that contained Cu, Mn, Zn, and Se (Preedy et al., 2018). Additionally, it has been observed that reduced concentrations of Cu and Zn may delay the age at which bulls become pubertal (Geary et al, 2016).

Furthermore, in the presence of increased sulfur and/or Mo, thiomolybdates can be synthesized and absorbed through the rumen wall influencing Cu absorption (Baker et al., 2006; Gould and Kendall, 2011; Spears, 2003). A study by Suttle (1974) reported that Cu concentrations in sheep were reduced when sheep were fed greater concentrations of dietary sulfur as sulfate or S- containing AA. Knowing an interaction between sulfur, Cu, Mo, and sulfur exists; it was expected that Cu and Mo would decrease in serum and seminal plasma in the current study. As expected, serum Cu and Mo in serum and seminal plasma were reduced for bulls fed the SULF and 60DDGS diets. However, a different trend was observed for Cu in seminal plasma resulting in the conclusion that there may have been increased Cu utilization in seminal plasma of bulls fed the SULF diet compared with bulls fed 60DDGS or CON. Moreover, the mechanism of transfer of nutrients from serum to seminal plasma is not fully understood and there is opportunity for research in this area.

Selenium is important for the production of selenoproteins such as iodothyronine deiodinases and glutathione peroxidases. The intermediate iodothyronine deiodinase 1, that is

used to convert inactive triiodothyronine to active thyroxine, is needed for the conversion of glutathione peroxidase (Köhrle et al., 2005). Glutathione peroxidase is an important antioxidant used to protect cells from oxidative stress (Bansal and Bilaspuri, 2011). Diets containing increased amounts of sulfur have been observed to influence glutathione peroxidase concentrations in cattle (Pogge et al., 2014). In the current study, Se concentrations were greatest in the 60DDGS treatment in serum and seminal plasma. In serum, circulating Se concentrations may have been a result of increased Se in the diet; however, Se is essential for the synthesis of glutathione peroxidase to prevent oxidative stress. Increases in seminal plasma Se and glutathione peroxidase may have been in response to increased oxidative stress for the sperm cells.

Conclusion

Glucose concentrations were greater at d 112 in serum and seminal plasma for bulls fed the 60DDGS diet. In addition, urea-N was greater in 60DDGS treatment in serum and seminal plasma which may have been a result of the amount of nitrogen in the 60DDGS diet. Differences were observed for trace minerals in serum and seminal plasma suggesting that sulfur may not be the only factor contributing to decreased semen quality in yearling bulls. Other factors within DDGS may be contributing to differences observed in yearling bull reproductive performance.

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CHAPTER 4: CONCLUSION

In the current study where bulls were fed to gain similarly, bulls fed the 60DDGS diet had increased glutathione peroxidase activity in seminal plasma with a decrease in triiodothyronine concentrations and an increase in Se compared with other treatments. These results indicate that bulls fed 60DDGS may have produced more glutathione peroxidase in response to oxidative stress in semen. Glucose concentrations were greater at d 112 in serum and seminal plasma for the 60DDGS treatment. In addition, urea-N was greater in 60DDGS treatment in serum and seminal plasma which may have been a result of the percent CP in DDGS. Differences observed for trace minerals in serum and seminal plasma indicate that Cu and Mo were altered in bulls fed greater dietary sulfur.

Furthermore, alterations observed for VAP, VSL, and VCL in the motile and progressively motile populations of sperm, hormone concentrations, glutathione peroxidase activity, and trace mineral concentrations suggest that sulfur may not be the only factor contributing to decreased semen quality in yearling bulls. Other factors such as protein, starch, or fat may be contributing to the differences observed for reproductive performance in yearling Angus bulls. However, more research in necessary to investigate if any molecular modifications were made to the acrosome, DNA, or RNA in these sperm populations by feeding 60% DDGS to yearling bulls. Additionally, further research may be necessary to elucidate the effects of feeding DDGS to yearling bulls on Sertoli cell and seminiferous tubule phenotype and function.