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Effect of Supplemental Trace Mineral Source (Organic versus Inorganic) on Bull Semen Quality

Matt Rowe
University of Arkansas

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By

Matt P. Rowe
University of Arkansas
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University of Arkansas

ABSTRACT

Studies indicate that organic forms of trace minerals can improve cow reproductive performance, particularly during periods of stress. However, limited information is available on the effects of organic trace mineral supplementation on bull fertility. The objective of this study was to evaluate the effect of organic versus inorganic trace mineral supplementation on bull semen quality before and after freezing, as measured by computer-assisted sperm analysis (CASA). Angus and Balancer bulls were assigned to inorganic (n = 9) and organic (n = 10) trace mineral treatments, based on initial semen quality, breed, body weight, and age. The bulls were maintained in a dry lot pen and fed mixed grass hay. Three times each week bulls were individually fed a ration containing either inorganic or organic Zn, Cu, Co and Mn trace mineral for 123 d (mid May to mid September). Treatments were supplemental Zn (450 mg/d), Cu (150 mg/d), Co (12 mg/d), Mn (300 mg/d), Se (3 mg/d), and I (5 mg/d) as either inorganic or as a portion of the same levels as organic sources. Starting on day 60, semen was collected by electroejaculation weekly for 9 weeks. Semen was evaluated by CASA for percent motile, progressive and rapid sperm within 5 min of each collection. On collection weeks 1, 4, and 8, sperm was extended, slowly cooled to 4° C, loaded into 0.5 mL straws, and frozen in liquid nitrogen. After thawing, semen was washed to remove extender and then re-suspended in TALP media. Semen was then evaluated using CASA at 0 and 2 h post-thaw. Data were analyzed by dietary treatment, collection week and their interaction, using the mixed procedure of SAS for repeated measures. For sperm motility parameters, no interaction occurred between collection week and dietary treatment, nor was collection week significant ($P > 0.05$); therefore, data were analyzed for the effects of treatment on sperm motility parameters, with weekly collections as

repeated measures over time. At collection, motile (69.1 vs. 55.2%) and progressive (50.3 vs. 38.5%) sperm were greater ($P < 0.05$) for bulls receiving the organic trace mineral supplementation compared with bulls receiving the inorganic trace mineral supplementation. Likewise, progressive sperm were improved ($P = 0.004$) for bulls receiving organic (70.0%) compared with inorganic (55.4%) trace mineral supplementation. The percentage of motile sperm with rapid motility (path velocity > 50 μm/sec) was also greater ($P = 0.002$) for bulls supplemented with organic compared with inorganic trace mineral (50.7 and 38.0%, respectively). After thawing, motile (16.3 and 7.9%) and progressive (8.9 and 4.1%) sperm were also greater ($P < 0.05$) for semen from bulls in the organic compared with inorganic trace mineral treatments, respectively. At 2 h post-thaw, motile sperm remained greater (8.5 and 3.7%; $P < 0.05$), but progressive sperm (4.2 and 1.7%) was similar ($P > 0.05$) for the organic and inorganic trace mineral treatments, respectively. Sperm motility is the single most important semen quality parameter influencing bull fertility. These results indicated organic trace mineral supplementation improved bull semen quality both before and after freezing. Additional studies are needed to determine if this improvement in semen quality translates into higher pregnancy rates.

This thesis is approved for recommendation
to the Graduate Council.

Thesis Director:

_____
Dr. Jeremy G. Powell

Thesis Committee:

_____
Dr. Rick W. Rorie

_____
Dr. David L. Kreider

_____
Dr. Elizabeth Kegley

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DEDICATION

I would like to dedicate this thesis to my wife, Johanna who has loved, helped and, supported me throughout this process and my college career. Thank you for always giving me words of encouragement.

I would also like to dedicate this thesis to my family. Thank you for all of your continuous support throughout my college career. Thank you for allowing me to pursue my Master's Degree.

TABLE OF CONTENTS

Chapter 1: Review of Literature

Introduction

Trace mineral supplementation has been shown to be very important to the reproductive performance of bulls; however, diets commonly fed to beef bulls may not meet all currently recommended requirements for trace minerals (NRC, 1996). In order to maximize bull reproductive performance key trace mineral nutritional demands must be met. One example is that zinc has been shown to be important for membrane and chromatin stability and also is important in the structure of sperm tail morphology (Baccetti et al., 1973). Cattle producers and semen companies depend on consistent reproductive performance of bulls in order to maximize efficiency. This is especially true in bull studs, where semen is obtained for commercial sale for artificial insemination of cows, where each collection can be of considerable value.

Minerals supplemented in beef cattle diets are broken down into the two major categories of macrominerals (Ca, P, Mg, K, Na) and microminerals (Fe, Mn, Zn, Cu, Co, I, and Se). Beef cattle requirements for minerals are dependent on the stage and level of production (NRC, 1996). Macrominerals are generally required in larger amounts and microminerals, also referred to as trace minerals, are required in smaller amounts.

Most minerals are commonly supplemented in their inorganic forms. Today there are several organic trace mineral supplements available for cattle diet supplementation. It has been shown in some studies that organic mineral supplementation provides a greater bioavailability than inorganic trace mineral supplementation. This review will focus on trace minerals, different sources of these trace minerals, and how they impact bull reproductive performance. Current methods of semen evaluation will also be briefly reviewed.

Minerals perform several different functions within the body which can be summarized into four main roles. The first role is to serve as structural components of body organs and tissues (Suttle and Underwood, 1999). Secondly, minerals are involved as electrolytes in physiological functions such as maintaining osmotic pressure, acid/base balance, membrane permeability, and the transmission of nerve impulses. Thirdly, minerals serve as catalysts in enzyme reactions and in endocrine systems. Lastly, minerals also serve an important role in regulation of cell replication and in directing cell differentiation.

There are many different forms of mineral supplements. Most common mineral supplements are inorganic molecules (i.e. salts [including sulfates, oxides, or chlorides]). Minerals are also found in organic compounds (i.e. amino acid complexes and chelates). Both inorganic (i.e. salts or ions) and organic forms of minerals can be found naturally in forages. Bioavailability is variable for different inorganic trace minerals as well as for different classes of organic trace minerals. Bioavailability is defined as the proportion of the ingested mineral that is absorbed, transported to its site of action, and converted to a physiologically active form (O'Dell, 1984). In order for a mineral to be absorbed, it must be soluble in the gastrointestinal tract; however, solubility in the rumen may be associated with formation of insoluble complexes that render the trace mineral unavailable in the small intestine (Kellogg and Kegley, 2002). Therefore assessing the bioavailability of trace mineral sources through in vitro assays is problematic.

There are several different categories of organic trace minerals as defined by the Association of American Feed Control Officials, Feed Ingredient Definitions (2009). One form is the metal amino acid complex. This complex results from the combination of a soluble metal salt with one or more amino acids. An example of a metal amino acid complex is zinc methionine with the name indicating the metal and the specific amino acid to which it is bound.

Another form, a metal amino acid chelate is a product resulting from the reaction of the metal ion from a soluble metal salt with amino acids occurring with a mole ratio of one mole of metal to between one and three (preferably two) moles of amino acids to form coordinate covalent bonds. Metal polysaccharide complexes result from the combination of a soluble salt with a polysaccharide solution. A metal proteinate is a product resulting in the chelation of a soluble salt with amino acids and/or a partially hydrolyzed protein. Finally, metal propionate is a product resulting from the reaction of a metal salt with propionic acid. Currently these are the only forms of organic trace minerals that may be marketed as feed additives in the United States.

Zinc

Zinc is an essential component of over 300 enzymes (Dibley, 2001; Valee and Falchuk, 1993). Zinc is required for the structural and functional integrity of over 2,000 transcription factors and almost every signaling and metabolic pathway is dependent on one or more zinc-requiring proteins (Beattie and Kwun, 2004; Cousins et al., 2006).

The requirement for zinc for all classes of beef cattle is 30 mg/kg of DM (NRC, 1996). This level of zinc is not usually met by forage diets (Greene, 2000). Zinc requirements have not been well defined for all classes of livestock and it may be argued that the zinc requirement for optimal reproduction is greater than the dietary zinc requirement for growth or maintenance.

Semen and its constituents generally contain high zinc concentrations, although these may vary among animal species. Furthermore, zinc deficiency has been shown to have a negative impact on bull calf testes development (Pitts et al., 1966) and spermatogenesis (Hidiroglou, 1979). In one study, when lambs were fed a zinc deficient diet of 2.4 mg/kg of DM for 20 weeks spermatogenesis practically ceased, but recovered completely during a repletion period (Martin and White, 1992).

Research comparing the relative bioavailabilities of organic zinc sources to zinc sulfate has been inconsistent, but zinc lysine and zinc methionine appear to be at least equally bioavailable to zinc oxide or zinc sulfate, and there is some data that indicates the organic complexes are metabolized differently. Stressed cattle have tended to respond more favorably to supplemental zinc methionine compared to inorganic zinc; whereas zinc source in non-stressed cattle has minimal effect (Kellogg and Kegley, 2002).

Arthington et al. (1995) investigated zinc supplementation and its affect on bull fertility. Zinc was supplemented in the diet via three methods including 1) 40 mg of Zn supplied by zinc sulfate/kg of DM, 2) 40 mg of Zn supplied by 1/3 zinc sulfate combined with 2/3 zinc proteinate/kg of DM, and 3) 60 mg of Zn supplied by zinc sulfate/kg of DM. Following 126 days of zinc supplementation, bulls receiving 60 mg of Zn as zinc sulfate/kg of DM and bulls receiving 40 mg of Zn as zinc sulfate/zinc proteinate/kg of DM exhibited an improved percentage of normal sperm cells compared with bulls receiving 40 mg of Zn as zinc sulfate/kg of DM. Kumar et al. (2006) documented that bulls receiving six months of organic Zn propionate supplementation exhibited improved semen characteristics compared with bulls receiving inorganic Zn supplementation over the same time period.

Copper

Copper is a cofactor in many oxidation reduction enzyme systems. Copper may also protect tissues against oxidant stress from free radicals, including those generated during respiration (Suttle and Underwood, 1999). Copper deficiency can lead to ataxia, abnormal wool and hair growth, and bone disorders. Reproductively a deficiency in copper can cause prenatal mortality (Hidiroglou, 1979). Copper is involved in spermatozoa motility and it may also act at pituitary receptors which control the release of LH (Slivkova et al., 2009). Rams fed

supplemental molybdenum and sulfate, dietary copper antagonists, for a year had ejaculates of lower volume, lower sperm concentration, and decreased sperm motility compared to rams receiving just supplemental molybdenum or supplemental copper (Van Niekerk and Van Niekerk, 1989). The rams fed both molybdenum and sulfate were determined to be severely copper deficient versus marginal or adequate in copper status for the other treatment groups, respectively, When the rams were repleted with copper, these semen characteristics returned to normal values (Van Niekerk and Van Niekerk, 1989).

Presently the NRC recommended Cu dietary concentration is 10 mg/kg of DM for all classes of beef cattle, including bulls (NRC, 1996). However, according to reports (Mullis et al., 2003; Ward et al., 1995), copper requirements may vary by breed and some breeds maintain greater plasma concentrations of copper compared with other breeds. In ruminants, determining the optimal dietary concentration of copper is further complicated by the negative interactions of molybdenum, sulfur, and copper within the rumen where thiomolybdates form and bind copper dramatically reducing copper's bioavailability.

Limited research has investigated the impact of copper on bull fertility, although copper deficiency is associated with delayed or depressed estrus in beef cows (Phillippo et al., 1982). In humans, Wong et al. (2001) reported a weak but significant positive correlation between plasma copper concentrations and sperm motility. In a similar study, plasma copper concentration was significantly correlated with seminal plasma copper concentration and sperm count, sperm motility, and normal sperm morphology (Jockenhovel et al., 1990).

Cobalt

In ruminants, cobalt is required by rumen microorganisms for vitamin B_{12} synthesis. Vitamin B_{12} is required, in microorganisms and mammals, for the formation of succinate from

propionate, and it assists methionine synthase in transferring methyl groups from 5-methyletrahydrofolate to homocysteine, which is important in nucleotide synthesis (Suttle, 2010). According to Greene (2000), a dietary concentration of 0.1 mg cobalt/kg of DM provides adequate cobalt for normal rumen function. Cobalt deficiencies are not widespread in the United States and typically would occur only in tropical climates. Sheep are more sensitive to cobalt deficiency compared with cattle (Kincaid, 2000). Rumen vitamin B_{12} synthesis is vital for optimal reproductive fertility in cows (Suttle, 2010) but no data have been reported on the impact of cobalt deficiency on bull fertility.

Limited information is available on the bioavailability of different sources of supplemental Co for cattle. In sheep, cobalt glucoheptonate was 85% as bioavailable as cobaltous sulfate (Ammerman et al., 1995).

Manganese

Manganese is essential for normal bone growth, onset of puberty, reproductive efficiency of cows, and energy metabolism (Kellogg and Kegley, 2002).

The NRC recommended dietary concentration for bulls is 40 mg/kg of DM (NRC, 1996). A severe deficiency of dietary manganese can cause a loss of libido and testicular degeneration (Boyer, et al., 1942).). Manganese deficiency restricted testicular growth in growing ram lambs, although Mn concentrations in the testes were not reduced (Masters et al., 1988). In rats, Mn deficiency was associated with impaired spermatogenesis (Orent and McCollum, 1931). One hypothesis is that reproductive problems may relate to decreased synthesis of cholesterol which is needed for synthesis of sex hormones (Doisey, 1973); however, cholesterol concentrations are not always reduced with Mn deficiency (Hansen et al., 2006a, b). Other data in male rats suggest that Mn is a stimulator of lutenizing hormone secretion and thus affects spermatogenesis (Lee et

al., 2006). In vitro, addition of manganese to extenders for bovine sperm has been reported to reduce oxidative stress and improve the quality/fertility of semen (Cheema et al., 2009).

Manganese methionine averaged a 125% relative bioavailability value in two trials with sheep as compared to manganese sulfate (Ammerman et al., 1995), but little other research has investigated the bioavailability of Mn sources in ruminants.

Collection methods and semen analysis

Common techniques for bull semen collection include the artificial vagina (AV) and electroejaculation (Baracaldo, 2007). Both techniques commonly yield satisfactory ejaculates that contain spermatozoa and seminal plasma, but the AV will produce a more physiological sample. An AV can typically only be used on trained bulls and is very commonly used by bull collection centers when collecting semen for artificial insemination purposes. Since semen collection by AV imitates natural breeding, utilizing this method usually requires two or more people, one to handle the AV and one or more to handle the bull and/or the mount. The bull mounts a cow in heat, a steer, or a dummy while the collection is being performed.

The electroejaculator (EE) is the most common method utilized for semen collection. The EE stimulates the bull with a mild electrical current leading to ejaculation. Some bulls so not successfully respond to the EE stimulation, and therefore cannot be effectively collected using this method. This electrical stimulation can be delivered either by manual or programmed methods leading to a gradual build-up of stimulation culminating in ejaculation.

According to Linford et al. (1976), standard evaluation methods exist for the evaluation of bovine semen. These allow consistent assessment of bull semen to determine if satisfactory standards are met for fertility evaluation. Sperm motility is subjectively scored by an observer using bright field microscopy at 40 to 400 x magnification. Both gross motility and individual

progressive motility is evaluated. Gross motility can be determined while viewing the vigor of the wave-like motion occurring to a freshly collected sample. An observer would assign a score to describe gross motility from a five point scale which includes: very good (VG) - rapid dark swirls and eddies; good (G) - slower swirls and eddies; fair (F) - no swirls, but prominent individual cell motion; poor (P) - little or no individual cell motion (Baracaldo, 2007) while viewing at 40 x magnification. Individual progressive motility evaluates the progressive movement of the sperm cells and is typically determined by placing a drop of diluent (i.e. saline solution or sodium citrate) onto a warm slide and adding a small drop of semen to the solution. An observer would view the diluted sperm sample at 400 x magnification to assess the number of sperm cells that progressively moving across the field. Sperm morphology and percent dead sperm are commonly evaluated after semen is stained with eosin-nigrosin stain (commonly referred to as a live/dead stain) and observed with bright field microscopy. Assessment of sperm morphology would be conducted as described by Menon et al. (2011).

Computer-Assisted Sperm Analysis (CASA)

Currently, highly discriminative and repeatable imaging equipment exist for performing computer-assisted sperm analysis (CASA; Farrell et al., 1997). This technology can be used to evaluate several parameters including sperm variables such as: motile, progressive, rapid, medium, slow, static, path velocity, progressive velocity, track speed, lateral amplitude, beat frequency, straightness, linearity, elongation, and area (Table 1). Utilizing this equipment allows an objective standardized measurement of semen parameters permitting the laboratory analysis of a large number of samples and yielding valid data sets for scientific comparison. Farrell et al. (1998) evaluated bull semen used for artificial insemination and found various combinations of

sperm motility parameters measured by CASA were highly correlated with non-return rates in dairy cattle.

Semen extender methods

The function of semen extenders is to stabilize the spermatozoa while maintaining their fertile properties for cryopreservation. Semen extenders should buffer pH, and provide proper osmolarity, and viscosity to maintain the spermatozoa without significant changes. Seminal pH is used as an indicator of semen quality; when it rises to 7.6 or more, sperm cells become hyperactive and die in short period of time (De Pauw et al., 2003). Semen extenders have a buffer to maintain pH around 6.6 to inhibit motility (De Pauw et al., 2003; Vishwanath and Shannon, 2000). Sperm membranes function at an osmolarity of around 320 mOsm (Chaveiro et al., 2006; De Pauw et al., 2003). It has been reported that ~60% of bull sperm do not survive cryopreservation procedures; possibly due to their osmotic tolerance (Guthrie et al., 2002). Another important property of semen extenders is viscosity. Semen extenders with elevated viscosity negatively affect spermatozoa parameters such as motility, linearity, and swimming velocity (Hirai et al., 1995). Hence, optimal pH, osmolarity, and viscosity are necessary in a good semen extender.

Different extender components are reported to protect spermatozoa from processing and storage damage. The most preferred components in semen extenders for bull semen are egg yolk and whole milk (Thun et al., 2002). Egg yolk has specific components such as lecithin and some phospholipids that protect spermatozoa from cold shock and freeze-thaw damage (Vishwanath and Shannon, 2000). A low density lipoprotein fraction (LDL) in egg yolk also protects sperm during the cooling process (Bergeron et al., 2004). These LDL fractions in egg yolk bind detrimental components (proteins) in seminal plasma that induce lipid loss and destabilize sperm

membranes (Manjunath et al., 2002, De Pauw et al., 2003). Likewise, caseins in milk decrease the binding of these proteins to sperm, reducing sperm lipid loss, while maintaining sperm motility and viability during storage (Bergeron et al, 2007). Other compounds usually found in semen extenders with reported protective activity are highly metabolizable sugars. Most fresh semen extenders use fructose or glucose as the source of energy for spermatozoa (Dalvit et al., 1995).

Objectives

Two experiments were conducted to determine the impact of trace mineral supplementation on the effects of bull semen quality as determined by computer-assisted sperm analysis (CASA). The first experiment was conducted on freshly collected semen to determine the effects of treatment on various sperm parameters.

The objective of the second experiment was to determine the impact of different sources of trace mineral supplements on the effect of bull semen quality of frozen bull semen as determined by CASA.

References

Ammerman, C. B., D. H. Baker, and A. J. Lewis. 1995. Bioavailability of Nutrients for Animals. London, Academic Press.

Arthington, J. D., K. R. Johnson, L. R. Corah, C. L. Willms, and D. A. Hill. 1995. The effect of dietary zinc level and source on yearling bull growth and fertility. KSU Cattlemen's Day Report. 727:109-110.

Baccetti, B., V. Pallini, and A. G. Burrini. 1973. The accessory fibers of the sperm tail. I. Structure and chemical composition of the bull "coarse fibers." J. Submicr. Cytol. 5:237-256.

Baracaldo, M. I., A. D. Barth and W. Bertrand. 2007. Steps for Freezing Bovine Semen: From Semen Collection to the Liquid Nitrogen Tank. Reviews in Veterinary Medicine. International Veterinary Information Service, Ithaca NY.

Beattie, J. H., and I. Kwun. 2004. Is zinc deficiency a risk factor for atherosclerosis? Brit. J. Nutr. 91:177-181.

Bergeron, A., Y. Brindle, P. Blondin, and P.Manjunath. 2007. Milk Caseins Decrease the binding of the major Bovine seminal plasma proteins to sperm and prevent lipid loss from the sperm membrane during sperm storage. Biol. Reprod. 77:120-126.

Boyer, P. D., J. H. Shaw, and P. H. Phillips. 1942. Studies on manganese deficiency in the rat. J. Biol. Chem. 143:417-425.

Chaveiro, A., Machado, L., Frijters, A., Engel, B., and Woelders, H. 2006. Improvement of parameters of freezing medium and freezing protocol for bull sperm using two osmotic supports. Therio. 65:1875-1890.

Cheema, R. S., A. K. Bansal, and G. S. Bilaspuri. 2009. Manganese provides antioxidant protection for sperm cryopreservation that may offer new consideration for clinical fertility. Oxidative Med. Cellular Longevity 2:152-159.

Cousins, R. J., J. P. Liuzzi, and L. A. Lichten. 2006. Mammalian zinc transport, trafficking, and signals. J. Biol. Chem. 281(34):24085–24089.

Dalvit, G.C., Miragaya, M.H., Chaves M.G., and Beconi, M.T. 1995. Energy requirement of bovine spermatozoa for in vitro capacitation. Therio. 44:1051-1058.

De Pauw, I.M.C., Van Soom, A., Mintiens, K., Verberckmoes, S., and de Kruif, A. 2003. In Vitro survival of bovine spermatozoa stored at room temperature under epididymal conditions. Therio. 59:1093-1107.

Dibley, M. J. 2001. Zinc. In Present Knowledge in Nutrition. (8th Ed.). B. A. Bowman and R. M. Russell (Eds.). p 329. Int. Life Sci. Inst. Press, Washington, DC.

Doisey, E. A., Jr. 1973. Micronutrient controls on biosynthesis of clotting proteins and cholesterol. In: Hemphill, D. D. (ed.) Trace Substances in Environmental Health – 6. University of Missouri, Columbia, MO, p. 193.

Farrell, P. B., G. A. Presicce, C. C. Brockett, and R. H. Foote. 1998. Quantification of bull sperm characteristics measured by computer-assisted sperm analysis (CASA) and the relationship to fertility. Therio. 49:671-679.

Feed Ingredient Definitions. 2009. Official publication of the Association of American Feed Control Officials. West Lafayette: Association of American Feed Control Officials.

Greene, L. W. 2000. Designing mineral supplementation of forage programs for beef cattle. Proc. ASAS. 1999. Available at: http://www.fass.org/dasees/nutrition/0913.pdf. Accessed Nov. 17, 2011.

Guthrie, H.D., Liu, J., and Crister, J.K. 2002. Osmotic tolerance limits and effects of cryoprotectants on motility of bovine spermatozoa. Biol. Reprod. 67:1811-1816.

Hansen, S. L., J. W. Spears, K. E. Lloyd, and C. S. Whisnant. 2006a. Growth, reproductive performance and manganese status of heifers fed varying concentrations of manganese J. Anim. Sci. 84:3375-3380.

Hansen, S. L., J. W. Spears, K. E. Lloyd, and C. S. Whisnant. 2006b. Feeding a low manganese diet to heifers during gestation impairs foetal growth and development. J. Dairy Sci. 89:4303-4311.

Hidiroglou, M. 1979. Trace element deficiencies and fertility in ruminants: A Review. J. Dairy Sci. 62(8):1195-1206.

Jockenhovel, F., M. Bals-Pratsch, H. P. Bertram, and E. Nieschlag. 1990. Seminal lead and copper in fertile and infertile men. Andrologia. 22(6):503-511.

Kellogg, D. W. and E. B. Kegley. 2002. Organic-Chelated Minerals. Nutrition – Feed Supplements.

Kincaid, R. L. 2000. Assessment of trace mineral status of ruminants: A review. Proc. ASAS. 1999. Available at: http://jas.fass.org/content/77/E-Suppl/1.24.full.pdf. Accessed Nov. 17, 2011.

Kumar, N., R. P. Verma, L. P. Singh, V. P. Parshney, and R. S. Dass. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bos indicus* x *Bos taurus*) bulls. Reprod. Nutr. Dev. 46:663-675.

Lee, B., M. Pine, L. Johnson, V. Rettori, J. K. Hiney, and W. Les Dees. 2006. Manganese acts centrally to activate reproductive hormone secretion and pubertal development in male rats. Reprod. Toxicol. 22:580-585.

Linford, E., F. A. Glover, C. Bishop and D. L. Stewart. 1976. The relationship between semen evaluation methods and fertility in the bull. J. Repro. Fert. 41:283-291.

Martin, G. B., and C. L. White. 1992. Effects of dietary zinc deficiency on gonadotrophin secretion and testicular growth in young male sheep. J. Reprod. Fert. 96:497-507.

Masters, D. G., D. I. Paynter, J. Briegel, S. K. Baker, and D. B. Purser. 1988. Influence of manganese intake on body, wool and testicular growth of young rams and on the concentration of manganese and the activity of manganese enzymes in tissues. Austral. J. Agric. Res. 39:517-524.

Menon, A. G., J. C. Thundathil, R. Wilde, J. P. Kastelic, and H. W. Barkema. 2011. Validating the assessment of bull sperm morphology by veterinary practitioners Can Vet J. 52:407.

Mullis, L. A., J. W. Spears, and R. L. McCraw. 2003. Effects of breed (Angus vs Simmental) and copper and zinc source on mineral status of steers fed high dietary iron. J. Anim. Sci. 81:318-322.

National Research Council. 1996. Nutrient Requirements of Beef Cattle. 7th Ed. Washington, DC: National Academy of Sciences.

O'Dell, B. L. 1984. Bioavailability of Trace Elements. Nutrition Reviews 42:9 301-308.

Orent, E. R., and E. V. McCollum. 1931. Effects of deprivation of manganese in the rat. J. Biol. Chem. 92:651-678.

Phillippo, M., W. R. Humphries, C. B. Lawrence, and J. Price. 1982. Investigation of the effect of copper therapy on fertility in beef suckler herds. J. Agric. Sci., Cambridge 109:315-320.

Pitts, W. J., W. J. Miller, O. T. Fosgate, J. D. Morton, and C. M. Clifton. 1966. Effect of zinc deficiency and restricted feeding from two to five months of age on reproduction in Holstein bulls. J. Dairy Sci. 49:995-1000.

Slivkova, J., M. Popelkova, P. Massanyi, S. Toporcerova, R. Stawarz, G. Formicki, N. Lukac, A. Putala and M. Guzik. 2009. Concentration of trace elements in human semen and relation to spermatozoa quality. J. Envir. Sci. Health. 44:370-375.

Suttle, N. F. 2010. Mineral Nutrition of Livestock, 4th Ed. CABI, Oxfordshire, UK.

Suttle, N. F. and E. J. Underwood. 1999. The Mineral Nutrition of Livestock. 3rd Ed. CABI, Oxon

Thun, R., Hurtado, M., and Janett, F. 2002. Comparison of Biociphos-Plus® and TRIS-egg yolk extender for cryopreservation of bull semen. Therio. 57:1087-1094.

Vishwanath, R., and Shanon, P. 2000. Storage of bovine semen in liquid and frozen state. Anim. Reprod. Sci. 62:23-53.

Vallee, B. L., and K. H. Falchuk. 1993. The biological basis of zinc physiology. Physiol. Rev. 73:79-118.

Ward, J. D., J. W. Spears, and G. P. Gengelbach. 1995. Differences in copper status and copper metabolism among Angus, Simmental and Charolais cattle. J. Anim. Sci. 73:571-577.

Van Niekerk, F. E., and C. H. Van Niekerk. 1989. The influence of experimentally induced copper deficiency on the fertility of rams. I. Semen parameters and peripheral plasma androgen concentration. J. S. Afr. Vet. Assoc. 60:28-31.

Table 1: Description of Sperm Variables Measured by the Hamilton-Thorne Sperm Analyzer

Variable	Description
Motile	Path velocity ≥ 30 μm/sec and progressive velocity ≥ 15 μm/sec
Progressive	Path velocity ≥ 50 μm/sec and straightness ≥ 70%
Rapid	Progressive % with path velocity > 50 μm/sec
Medium	Progressive % with path velocity < 50 μm/sec but > 30 μm/sec
Slow	Path velocity < 30 μm/sec and progressive velocity < 15 μm/sec
Static	Sperm not moving at all.
Path velocity (VAP)	Average velocity of the smoothed cell path in μm/sec
Progressive velocity (VSL)	Average velocity measured in a straight line over entire track
Track speed (VCL)	Average velocity measured over the actual point-to-point track
Lateral amplitude (ALH)	Mean width of the head oscillation as the sperm swims
Beat frequency (BCF),	Frequency of sperm head crossing the sperm average path
Straightness (STR)	Departure of average sperm path from straight line (VSL/VAP)
Linearity (LIN)	Departure of actual sperm track from straight line (VSL/VCL)
Elongation	Ratio (%) of head width to head length
Area	Average size in square microns of all sperm heads

Chapter 2: Evaluation of organic versus inorganic trace mineral supplementation on blood plasma mineral concentration, seminal plasma mineral concentration, and bull semen quality

Abstract

Studies indicate that organic forms of trace minerals can improve cow reproductive performance, particularly dairy cows during periods of stress. However, limited information is available on the effects of organic trace mineral supplementation on bull fertility. The objective of this study was to evaluate the effect of trace mineral supplementation on bull semen quality, as measured by computer-assisted sperm analysis (CASA). Angus and Balancer bulls were assigned to inorganic (n = 9) and organic (n = 10) trace mineral treatments, based on initial semen quality, breed, body weight, and age. Bulls were maintained in dry lot pens and fed mixed grass hay. Three times each week bulls were individually fed a ration containing either inorganic or organic Zn, Cu, Co, and Mn trace mineral sources for 123 d (mid May to mid September). Starting on d 60, semen was collected by electroejaculation weekly for a 9 week period from mid-July until early-September. Semen was evaluated by CASA for percentages of motile, progressive and rapid sperm within 5 minutes of collection. Data were analyzed by treatment, week and their interaction, using SAS PROC Mixed for repeated measures. Bulls supplemented with organic trace minerals had more ($P = 0.019$) motile sperm than those supplemented with inorganic trace minerals (67.3 versus 56.3%, respectively). Likewise, percentage of progressive sperm was improved ($P = 0.004$) for bulls receiving organic (70.0%) versus inorganic (55.4%) trace mineral supplementation. Percentage of motile sperm with rapid motility (path velocity > 50 μm/sec) was also greater ($P = 0.002$) for bulls supplemented with organic compared to bulls receiving inorganic trace mineral (50.7 versus 38.0%, respectively).

Sperm motility is the single most important semen quality parameter influencing bull fertility. These results suggest organic trace mineral supplementation may improve bull semen quality. Additional studies are needed to determine if this improvement in semen quality translates into higher pregnancy rates.

Introduction

Mineral supplements commonly fed to livestock are mainly in inorganic forms (i.e., a molecule that does not contain carbon). Organic minerals consist of a mineral ion bound to an organic molecule (usually an amino acid or carbohydrate). Trace minerals are present in the body almost entirely as organic complexes or chelates and not as free inorganic ions. Thus, it has been suggested that dietary organic trace minerals could be more efficiently utilized.

It has been well documented that trace mineral supplementation has an impact on reproductive performance. Zinc deficiency has been shown to have a negative impact on spermatogenesis (Hidiroglou, 1979). Supplemental zinc has also been shown to improve the percentage of normal sperm cells in beef bulls (Arthington et al., 1995). Studies indicate that organic forms of trace minerals can improve dairy cow reproductive performance, particularly during times of stress (Kellogg et al., 2003). Limited information has been available on the effects of organic trace mineral supplementation on bull fertility. The objective of this study was to evaluate the effect of source of supplemental trace minerals on bull semen quality as measured by computer-assisted sperm analysis (CASA).

Materials and Methods

This study utilized 19 Angus (n = 5) and Balancer (Gelbvieh × Angus, n = 14) bulls housed at the University of Arkansas Savoy Cow-Calf unit. The bulls averaged 827 kg body weight and 6 yr of age at the start of the study, and were maintained and cared for in accordance

to procedures approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol #11001). Semen was collected by electroejaculation and evaluated a minimum of 3 times prior to the start of the study. Bulls were assigned to inorganic (n = 9) or organic (Availa®4, ZinPro Corp., Eden Prairie, MN; n = 10) trace mineral treatments based on initial semen quality, breed, body weight, and age. Treatments consisted of supplemental Zn (450 mg/d), Cu (150 mg/d), Co (12 mg/d), Mn (300 mg/d), Se (3 mg/d), and I (5 mg/d) supplied as either inorganic or for Zn, Cu, Co, and Mn a mixture of inorganic and organic sources (Table 1). Assuming an intake of 2% of body weight, these supplemental trace mineral levels would be approximately 27 mg of Zn, 9 mg of Cu, 0.7 mg of Co, 18 mg of Mn, 0.18 mg of Se, and 0.3 mg of I/kg of DM.

Bulls were maintained in two dry lot pens throughout the study, each pen housed approximately the same number of bulls on each treatment. Bulls had ad libitum access to water and shade was provided. Bulls were fed mixed grass hay from large round bales to maintain body condition (88.9% DM; 11.6% CP; 75.1% NDF; 43.8% ADF; % ash, 0.32% Ca; 0.24% P; 0.14% Mg; 24.8 mg of Zn/kg; 5.9 mg of Cu/kg; 73.6 mg of Mn/kg; and 0.1 mg of Co/kg; DM basis). Three times each week bulls were individually fed 1.2 kg (DM basis) of a grain supplement (Table 2) that served as the carrier for treatments containing either inorganic or organic trace mineral treatments for 123 d starting in mid-May and extending to early-September. Starting on d 60 of the study (July 15), semen was collected by electro ejaculation weekly for 9 consecutive weeks.

Within five minutes of each semen collection, a sample from the collection was placed on a glass slide and stained with eosin-nigrosin stain. A smear was created and viewed at 1000 X magnification under oil immersion. Sperm morphology parameters were measured and a

determination was made for percentage of live and dead sperm (Bishop et al., 1954). Each semen sample was also evaluated by computer-assisted sperm analysis (CASA) for percentage motile, progressive, and rapid sperm within five minutes of each collection (Farrell et al., 1997). Motile sperm were those with a path velocity greater than 30 μm/sec and progressive velocity greater than 15 μm/sec. Progressive sperm had a path velocity greater than 50 μm/sec and straightness of path over 70%. Rapid sperm were those with a progressive path velocity over 50 μm/sec.

Blood samples were collected into heparinized vacuum tubes designed specifically for trace mineral analysis (Becton Dickinson Inc., Franklin Lakes, NJ) from each bull's median caudal vein during the first and ninth week of semen collections for the evaluation of plasma Zn, Cu, and Co levels. After collection, plasma was separated by centrifugation at 2,100 x *g* for 20 min. Plasma was removed and stored at –20° C for subsequent evaluation. Plasma was processed in a similar manner according to the procedure described by Richeson and Kegley (2011); with the exception that 1 mL of plasma was mixed with 9.5 mL of 1 *N* of HNO_3 for plasma deproteinating. Then, the resulting supernatant was analyzed for Zn and Cu by inductively coupled plasma-atomic emission spectroscopy (ICP; Ciros, Spectro Analytical Instruments Inc., Mahwah, NJ) at the Agriculture Diagnostic Laboratory, University of Arkansas, Fayetteville, AR.

During the first and last week of semen collection (mid-July and early-September, respectively), after semen analysis and evaluation were completed, the remaining sample was centrifuged (1,500 *g* x for 7 min; Liu et al., 2009) to separate spermatozoa from seminal plasma. Seminal plasma was then stored frozen at – 20° C for later Zn analysis. Zinc analysis was performed on the seminal plasma using a similar technique as the blood plasma utilizing ICP.

Before analysis hay samples were dried at 50 ºC in a forced air oven. Samples were then ground in a Willey Mill (Arthur H Thompson, Phil., PA) through a 1 mm screen. Samples were analyzed for DM, ash, nitrogen (Rapid combustion method, Elementar Americas, Inc., Mt. Laurel, NJ), sequentially for neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) (Van Soast method, ANKOM Technology Corp., Fairport, NY). Mineral concentrations were determined using ICP after samples were wet ashed using a 1 g sample in a 50 mL centrifuge tube, adding 15 mL of trace mineral grade nitric acid (9598-34 , J.T. Baker, Phillipsburg, NJ) than placing tubes into a heating block and digesting. Samples were brought to a final volume of 40 mL with deionized water after digestion. Triplicate samples from each of three batches of feed were analyzed for DM, ash, nitrogen, and mineral concentrations (0.5 g sample was wet ashed).

Sperm motility data and blood and seminal plasma mineral concentrations were analyzed by treatment, collection week and their interaction, using the mixed procedure of SAS (Version 9.1, SAS Inst. Inc., Cary, NC) for repeated measures. All reported means are least square means ± SE. Differences at $P < 0.05$ were considered to be statistically significant.

Results and Discussion

Sperm production (spermatogenesis) requires about 60 d in bulls. Therefore, bulls were fed trace mineral supplements for 60 d (mid-May to mid-July) prior to starting the weekly semen collections to ensure adequate time for treatments to influence sperm production or quality. The trial consisted of 9 weekly semen collections during which time the mineral supplementation continued. Nine weeks was chosen to cover the entire length of spermatogenesis. Since the effects of high temperature has been shown to negatively affect bull semen quality (Casady et al.,

1953; Skinner and Louw, 1966) the 9 week semen collection period extended from mid-July to early-September when fertility is more likely to be adversely affected by heat stress.

Over the 17 week study, bulls supplemented with the inorganic and organic trace mineral supplements lost an average of 8.4 and 5.9% of their body weight, respectively (Table 3). Weight loss during the study was similar ($P = 0.43$) among supplement trace mineral treatments. Furthermore, a change in scrotal circumference was noted in the bulls during the 17 week study. At the beginning of the study, scrotal circumference was similar ($P = 0.65$) among treatments. At the end of the study, a decrease in scrotal circumference was noted for both treatments; however, the decrease tended ($P = 0.08$) to be greater for the inorganic trace mineral treatment compared with the organic trace mineral treatment (Table 3).

During the collection period, wet conditions developed around the water trough and in the shaded area of the lots. Two bulls in the inorganic trace mineral treatment were removed from the study after the 4th and 6th week of the semen collection period due to lameness. None of the bulls fed the organic trace mineral supplement developed lameness problems. Previous research indicates that supplementation with trace mineral amino acid complexes improves claw integrity in dairy cattle (Siciliano-Jones et al., 2007).

No interaction occurred between collection week and dietary treatment, nor was collection week significant ($P > 0.05$) for sperm motility parameters; therefore, data were analyzed for the effects of treatment on sperm motility parameters, with weekly collections as repeated measures over time. At the start of the study before bulls were assigned to dietary treatments, all sperm parameters were similar across treatments ($P \geq 0.158$; Table 4). Over the 9 week collection period, bulls supplemented with the organic trace mineral supplement had a greater ($P = 0.02$) percentage of motile sperm of 65.5% compared with bulls supplemented with

the inorganic trace mineral treatment of 56.1%. Bulls fed the organic trace mineral supplement also maintained a greater ($P < 0.01$) percentage of progressive motile sperm of 47.0% compared with 38.4% for the inorganic trace mineral supplemented bulls. The percentage of motile sperm with rapid motility was also greater ($P = 0.02$) for bulls supplemented with organic trace minerals compared with bulls receiving the inorganic trace mineral supplement (62.3% versus 52.8%, respectively). Bulls receiving the organic trace mineral supplement had fewer ($P < 0.05$) motile sperm with slow motility, and also tended ($P = 0.06$) to have fewer static sperm compared with sperm collected from bulls receiving the inorganic diet (Table 4).

Comparison of the mean percentage of motile, progressive, and rapid sperm for bulls in each treatment before and during the trial indicates that the organic trace mineral helped to maintain these sperm parameters during the mid-July to early-September study period.

In a similar study, Kumar et al. (2006) documented that bulls receiving six months of organic Zn supplementation, as Zn propionate, exhibited improved semen characteristics compared with bulls receiving inorganic Zn supplementation (Zn sulfate) over the same time period. In that study and the current study, enhanced semen quality in bulls that receive organic trace mineral supplementation may be due to organic minerals possessing more bioavailability than inorganic forms. Furthermore, as a result, there may be more absorption, distribution and uptake of the supplemented minerals, which may account for the improved semen quality demonstrated.

Blood plasma Zn concentration throughout the study was not affected ($P \geq 0.26$) by dietary treatment, collection week, or their interaction (Table 5). However, a collection week by dietary treatment interaction ($P = 0.049$) was noted with plasma Cu concentration. Bulls receiving the inorganic trace mineral supplementation exhibited a declining plasma Cu

concentration over the 9 week semen collection period while bulls receiving the organic trace mineral supplementation exhibited a slight increase in plasma Cu concentrations. Because dietary concentrations of zinc and copper were above the current NRC requirements, plasma zinc and copper concentrations were not expected to be affected by the different sources of trace minerals. Furthermore, although not affected by supplemental trace mineral treatment, all mean plasma Zn concentrations were unexpectedly within the range classified as marginal by Kincaid (1999). Bulls were receiving approximately 53 mg of Zn/kg of DM, 1.8 times the NRC requirement of 30 mg of Zn/kg of DM. Plasma trace mineral concentrations are imperfect measures of trace mineral status because they are affected by other factors, Wegner et al. (1972) reported that serum Zn of dairy cattle was decreased following hyperthermal stress; this could possibly explain the marginal plasma Zn concentrations observed in the present study. Likewise plasma Cu is affected by factors other than nutrition, stress increases plasma Cu concentrations. Concentrations in the present study fell within the adequate (0.7 to 0.9 mg/L) to marginal (0.5 to 0.7 mg/L) classifications of Kincaid (1999). Bulls fed the inorganic trace mineral supplement had increased plasma Cu concentrations on Week 1 of the collection which could indicate that they were under more stress.

The only trace mineral above detection limits identified in seminal plasma was zinc. Seminal plasma Zn concentrations throughout the collection period were not affected ($P = 0.25$) by dietary treatment (Table 6). However, week of semen collection had a tendency ($P = 0.07$) to affect seminal plasma Zn concentrations with a decline being noted in both treatment groups from the first week of semen collection until the ninth week of semen collection.

Conclusion

Overall, the results of this study indicate partial replacement of inorganic Zn, Cu, Co, and Mn with equal levels of organic trace mineral amino acid complexes minimized the deterioration in bull semen motility parameters during the hot summer months compared with bulls receiving inorganic trace mineral supplementation. Additional research studies are indicated to determine if these treatment results yield increased pregnancy rates in cows.

References

Arthington, J. D., K. R. Johnson, L. R. Corah, C. L. Willms , D. A. Hill. 1995. The effect of dietary zinc level and source on yearling bull growth and fertility. KSU Cattlemen's Day Report. 727:109-110.

Bishop, M.W.H., R. C. Campbell, J. L. Hancock, and A. Walton. 1954. Semen characteristics and fertility in the bull. J. Agric. Sci. 44:227.

Casady, R. B., R. M. Myers, and J. E. Legates. 1953. The effect of exposure to high ambient temperature on spermatogenesis in the dairy bull. J. Dairy Sci. 36:14.

Farrell, P. B., G. A. Presicce, C. C. Brockett, R. H. Foote. 1998. Quantification of bull sperm characteristics measured by computer-assisted sperm analysis (CASA) and the relationship to fertility. Therio. 49:671-679.

Hidiroglou, M. 1979. Trace element deficiencies and fertility in ruminants: A Review. J. Dairy Sci. 62(8):1195-1206.

Kellogg, D. W., M. T. Socha, D. J. Tomlinson, and A. B. Johnson. 2003. Review: effects of feeding cobalt glucoheptonate and metal specific amino acid complexes of zinc, manganese, and copper on lactation and reproductive performance of dairy cows. Prof. Anim. Sci. 19:1-9.

Kincaid, R. L. 1999. Assessment of trace mineral status of ruminants: a review. Proc. Of the Amer. Soc. of Anim. Sci. www.asas.org/symposia/1998-1999.htm.

Kumar, N., R. P. Verma, L. P. Singh, V. P. Parshney, R. S. Dass. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bos indicus* x *Bos taurus*) bulls. Reprod. Nutr. Dev. 46:663-675.

Liu, D., B. Sie, M. Liu, F. Agresta, and H. W. G. Baker. 2009. Relationship between seminal plasma zinc concentration and spermatozoa-zona pellucida binding and the ZP-induced acrosome reaction in subfertile men. Asian J Androl. 11:499-507.

Richeson, J. T. and E. B. Kegley. 2011. Effect of supplemental trace minerals from injection on health and performance of highly stressed, newly received beef heifers. Prof. Anim. Sci. 27:461-466.

SAS. 2003. Version 9.1. SAS Institute, Inc., Cary, NC.

Siciliano-Jones, J. L., M. T. Socha, D. J. Tomlinson, and J. M. DeFrain. 2007. Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle. J. Dairy Sci. 91:1985-1995.

Skinner J. D., and G. N. Louw. 1966. Heat stress and spermatogenesis in *Bos indicus* and *Bos taurus* cattle. J. Applied Physio. 21:1784-1790.

Wegner, T. N., D. E. Ray, C. D. Lox, and G. H. Stott. 1972. Effect of stress on serum zinc and plasma corticoids in dairy cattle. J. Dairy Sci. 56:478-752.

Table 1. Supplemental sources and levels of trace minerals fed to bulls

Mineral	Organic diet		Inorganic diet
	Inorganic portion, mg/d	Organic portion[1], mg/d	Inorganic portion, mg/d
Zinc	90 as zinc sulfate	360 as zinc amino acid complex	450 as zinc sulfate
Copper	25 as copper sulfate	125 as copper amino acid complex	150 as copper sulfate
Cobalt	0	12 as cobalt glucoheptonate	12 as cobalt carbonate
Manganese	100 manganese sulfate	200 as manganese amino acid complex	300 as manganese sulfate
Selenium	3 as sodium selenite	0	3 as sodium selenite
Iodine	5 as calcium iodate	0	5 as calcium iodate

[1] Organic minerals provided via Availa®4 (Zinpro Corporation, Eden Prairie, MN)

Table 2. Ingredients and nutrient composition of carrier diet containing either inorganic or organic trace mineral supplement.

Supplement constituents:	Inorganic diet	Organic diet
Ingredient, % as fed		
Ground corn	83.4	83.4
Salt, white	5.8	5.8
Limestone	5.8	5.8
Molasses	5	5
Inorganic mineral premix[1]	+	-
Organic mineral premix[2]	-	+
Nutrient composition, DM basis		
DM, %	85.2	86.9
CP, %	10.1	10.0
Ca, %	2.91	2.63
P, %	0.25	0.26
Mg, %	0.11	0.11
Zn, mg/kg[3]	907 ± 35	954 ± 50
Cu, mg/kg[3]	338 ± 28	353 ± 7.4
Mn, mg/kg[3]	698 ± 125	769 ± 91
Co, mg/kg[3]	21.5 ± 0.45	27.1 ± 0.60

[1]Premix formulated to provide desired daily level of trace minerals (Table 1) in this supplement that was fed 3 times each week, 1.2 kg/feeding.
[2]Premix formulated to provide desired daily level of trace minerals (Table 1) in this supplement that was fed 3 times each week. Organic premix contained trace mineral amino acid complexes provided via Availa®4 (Zinpro Corporation, Eden Prairie, MN) and some inorganic trace minerals as sulfates.
[3]Diets mixed 3 times; values reported as the mean ± standard deviation after analyses of samples from each mix.

Table 3. Mean body weight and scrotal circumference of bulls at initiation of the study, at initiation of collection period and at the end of the study. (LSM)

	Trace mineral treatment		
Item	Inorganic	Organic[1]	*P* value
Body weight, kg			
Initiation of study	812.8 (±24.71)	820.9 (±20.67)	0.81
Initiation of collection[2]	773.5 (±23.80)	817.9 (±19.92)	0.13
End of study	744.7 (±25.59)	770.7 (±21.41)	0.44
Average loss during study	68.0 (±17.27)	50.1 (±14.44)	0.43
Average daily gain	- 0.59 (±0.15)	- 0.43 (±0.12)	0.44
Scrotal Circumference, cm			
Initiation of study	41.78 (±0.73)	41.35 (±0.61)	0.65
Initiation of collection[2]	40.14 (±0.61)	40.70 (±0.51)	0.42
End of study	39.36 (±0.57)	40.75 (±0.48)	0.08

[1]Organic minerals provided via Availa®4 (Zinpro Corporation, Eden Prairie, MN)
[2]Weekly semen collections began after 8 weeks of feeding experimental diets

Table 4. Mean sperm parameters of bulls assigned to inorganic and organic trace mineral treatments at the start and over the nine week study. (LSM)

CASA sperm parameter	Trace mineral treatment		*P* value
	Inorganic	Organic[1]	
At initiation of study			
Motile, %	72.0 ± 2.0	71.3 ± 1.8	0.801
Progressive, %	58.5 ± 2.5	58.1 ± 2.3	0.896
Rapid, %	68.2 ± 2.3	66. 9 ± 2.1	0.678
Medium, %	2.7 ± 0.59	4.0 ± 0.67	0.158
Slow, %	11.2 ± 1.8	11.0 ± 2.0	0.935
Static, %	24.5 ± 5.3	22.4 ± 6.0	0.795
Over the 9 wk collection period[2]			
Motile, %	56.1 ± 2.8	65.5 ± 2.6	0.0195
Progressive, %	38.7 ± 2.2	47.0 ± 2.0	0.0069
Rapid, %	53.2 ± 2.9	62.3 ± 2.7	0.0227
Medium, %	3.22 ± 0.33	3.15 ± 0.30	0.854
Slow, %	6.9 ± 0.70	5.1 ± 0.63	0.0465
Static, %	36.4 ± 2.82	29.33 ± 2.56	0.063

[1]Organic minerals provided via Availa®4 (Zinpro Corporation, Eden Prairie, MN)
[2]Weekly semen collections began after 8 weeks of feeding experimental diets

Table 5. Effects of trace mineral source on blood plasma mineral concentrations in beef bulls

	Inorganic		Organic[1]			Contrasts, *P* =		
	Wk 1[2]	Wk 9[2]	Wk 1[2]	Wk 9[2]	**SEM**	Treatment	Wk	Interaction
Plasma Zn, mg/L	0.74	0.63	0.78	0.79	0.086	0.36	0.36	0.26
Plasma Cu, mg/L	0.84	0.63	0.69	0.7	0.054	0.51	0.06	0.05

[1]Organic minerals provided via Availa®4 (Zinpro Corporation, Eden Prairie, MN)
[2]Week 1 indicates first week of semen collection and evaluation (mid-July), and week 9 indicates last week of semen collection and evaluation (early-September), bulls had been fed experimental treatments for 60 days before Week 1 of collection.

Table 6. Effects of trace mineral source on seminal plasma Zn concentrations in beef bulls

	Inorganic		Organic[1]			Contrasts, *P* =		
Seminal conc.	Wk 1[2]	Wk 9[2]	Wk 1[2]	Wk 9[2]	**SEM**	Treatment	Wk	Interaction
Plasma Zn, mg/L	2.23	1.33	1.59	1.05	0.38	0.25	0.07	0.64

[1]Organic minerals provided via Availa®4 (Zinpro Corporation, Eden Prairie, MN)
[2]Week 1 indicates first week of semen collection and evaluation (mid-July), and week 9 indicates last week of semen collection and evaluation (early-September), bulls had been fed experimental treatments for 60 days before Week 1 of collection.

Chapter 3: Effects of organic versus inorganic trace mineral supplementation on bull semen quality before and after freezing

Abstract

Limited information is available on the effects of organic trace mineral supplementation on bull fertility. The objective of this study was to evaluate the effect of trace mineral supplementation on bull semen quality before and after freezing, as measured by computer-assisted sperm analysis (CASA). Angus and Balancer bulls were assigned to inorganic (n = 9) and organic (n = 10) trace mineral treatments, based on initial semen quality, breed, body weight, and age. The bulls were maintained in dry lot pens and fed mixed grass hay. Three times each week bulls were individually fed a grain supplement that served as the carrier for treatments containing either inorganic or organic trace mineral for 123 days (mid May to mid September). Treatments were supplemental Zn (450 mg/d), Cu (150 mg/d), Co (12 mg/d), Mn (300 mg/d), Se (3 mg/d), and I (5 mg/d) as either inorganic or as a portion of the same levels as organic sources. Starting on day 60, semen was collected by electro-ejaculation on weeks 1, 4, and 8 of the study. Semen was evaluated by CASA for percentages motile and progressive sperm within 5 min of each collection. Sperm was then extended, slowly cooled to 4° C, loaded into 0.5 mL straws, and frozen in liquid nitrogen. After thawing, semen was washed to remove extender and then re-suspended in TALP media. Semen was then evaluated using CASA at 0 and 2 h post-thaw. Data were analyzed by dietary treatment, collection week and their interaction, using PROC GLM. Collection week and dietary treatment x collection week were not significant ($P > 0.05$), so they were dropped from the analysis. At collection, motile (69.1 vs. 55.2%) and progressive (50.3 vs. 38.5%) sperm were greater ($P < 0.05$) for bulls in the organic than the inorganic groups. Immediately after thawing, motile (16.3 vs. 7.9%) and progressive (8.9 vs. 4.1%) sperm were also greater ($P < 0.05$) for semen from bulls in the organic vs. inorganic treatments, respectively.

At 2 h post-thaw, motile sperm remained greater (8.5 vs. 3.7%; $P < 0.05$), but progressive sperm (4.2 vs. 1.7%) was similar ($P > 0.05$) for the organic and inorganic groups, respectively. Although post-thaw motility was low for both treatments, results suggest organic trace mineral supplementation may improve bull semen quality both before and after freezing.

Introduction

Trace mineral supplements have been shown to have an impact on reproductive performance. Zinc, manganese, cobalt, and copper have been shown to be essential nutrients in terms of maximizing reproductive performance. Zinc is found in high concentrations in the male reproductive tract and in semen (Hidiroglou, 1979). Zinc has an important role in prostate, epididymal, and testicular functions (Martin and White, 1992). Zinc also influences sperm motility (Kumar et al., 2006) and functions to stabilize sperm membranes. Manganese has been reported to reduce oxidative stress of sperm. Cobalt is a constituent of vitamin B12, which is necessary for fertility. Copper supplementation appears to improve sperm concentration, motility, and percent of live sperm (Jockenhovel et al., 1990).

Most commonly these minerals have been supplemented to livestock as inorganic molecules. Zinc is commonly fed as zinc sulfate ($ZnSO_4$), an inorganic molecule (Kellogg and Kegley, 2002). Organic minerals are minerals that are bound in some manner to an organic molecule, usually an amino acid or carbohydrate. Trace minerals are present in the body almost entirely as organic complexes or chelates and not as free inorganic ions. It has been suggested that dietary organic trace minerals could be more efficiently utilized. This is thought to be due to an increase in bioavailability (Kincaid, 2000). Bioavailability is the proportion of the ingested mineral that is absorbed, transported to its site of action, and converted to a physiologically active form. Therefore, the objective of this study was to evaluate the effect of organic versus

inorganic trace mineral supplementation on bull semen quality before and after freezing, as measured by computer-assisted sperm analysis (CASA).

Materials and Methods

This study utilized 19 Angus (n = 5) and Balancer (Gelbvieh × Angus, n = 14) bulls housed at the University of Arkansas Savoy Cow-Calf unit. The bulls averaged 827 kg body weight and 6 yr of age at the start of the study, and were maintained and cared for in accordance to procedures approved by the University of Arkansas Institutional Animal Care and Use Committee (protocol #11001). Semen was collected by electroejaculation and evaluated a minimum of 3 times prior to the start of the study. Bulls were assigned to inorganic (n = 9) or organic (Availa®4, ZinPro Corp., Eden Prairie, MN; n = 10) trace mineral treatments based on initial semen quality, breed, body weight, and age. Treatments consisted of supplemental Zn (450 mg/d), Cu (150 mg/d), Co (12 mg/d), Mn (300 mg/d), Se (3 mg/d), and I (5 mg/d) supplied as either inorganic or for Zn, Cu, Co, and Mn a mixture of inorganic and organic sources (Table 1). Assuming an intake of 2% of body weight, these supplemental trace mineral levels would be approximately 27 mg of Zn, 9 mg of Cu, 0.7 mg of Co, 18 mg of Mn, 0.18 mg of Se, and 0.3 mg of I/kg of DM.

Bulls were maintained in two dry lot pens throughout the study, each pen housed approximately the same number of bulls on each treatment. Bulls had ad libitum access to water and shade was provided. Bulls were fed mixed grass hay from large round bales to maintain body condition (88.9% DM; 11.6% CP; 75.1% NDF; 43.8% ADF; % ash, 0.32% Ca; 0.24% P; 0.14% Mg; 24.8 mg of Zn/kg; 5.9 mg of Cu/kg; 73.6 mg of Mn/kg; and 0.1 mg of Co/kg; DM basis). Three times each week bulls were individually fed 1.2 kg (DM basis) of a grain supplement (Table 2) that served as the carrier for treatments containing either inorganic or

organic trace mineral treatments for 123 d starting in mid-May and extending to early-September.

Starting on day 60 of the study semen was collected by electro-ejaculation. Three collections took place at the beginning (d 60), middle (d 88), and end (d 116) of the second half of the study. Semen was evaluated by computer aided sperm analysis (CASA) for percentage of motile, progressive, and rapid sperm within 5 minutes of each collection (Farrell et al., 1997). After analysis semen was diluted in fraction A of the semen extender (egg yolk-citrate) in 15 mL conical tubes. These were placed in 34° C water bath and allowed to cool to 5° C over a 2 to 3 h period in a walk in cooler. After equilibration to 5° C, fraction B (milk/glycerol) was added in steps over a 1 h period. Semen was then allowed to equilibrate with fraction B for an additional hour. Equilibrated semen was loaded into 0.5 mL straws and frozen using an MVE vapor freezing unit. Straws were placed in the freezing unit at -10° C and then cooled at a rate of -15° C/min to -100° C. The temperature was allowed to equilibrate for 10 min before plunging the straws into nitrogen.

At evaluation, the straws were removed from liquid nitrogen and thawed in a 35° C water bath. After thawing, semen straws were unloaded into centrifuge tubes and centrifuged to remove extender, then re-suspended in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffered Tyrode's-albumin-lactate-pyruvate (TALP) media. Sperm were analyzed by CASA at 0 and 2 h post thaw. At least 400 sperm were evaluated for each sample and evaluation period. In between analysis, semen was kept in 38.6° C incubator to maintain temperature.

The percentages of motile, progressive and rapid sperm were analyzed by dietary treatment, collection week, and their interaction using PROC GLM (SAS Inst., Cary, NC). Differences at $P < 0.05$ were considered to be statistically significant.

Results

Spermatogenesis requires approximately 60 d in bulls. Therefore, bulls were fed trace mineral supplements for 60 d (mid-May to mid-July) prior to starting the semen collections to ensure adequate time for treatments to influence sperm production or quality. The collections took place from mid-July to early September when fertility is more likely to be adversely affected by heat stress. At the beginning of the study, all sperm parameters were similar across treatments.

Collection week and the dietary treatment by collection week interaction were not significant ($P > 0.05$), so they were dropped from the analysis. Before freezing, semen collected from bulls supplemented with the organic trace mineral had a greater percentage of ($P = 0.047$) motile sperm than semen collected from bulls fed the inorganic trace mineral supplement (Table 3). The percentage of rapid sperm tended ($P \geq 0.06$) to be greater before freezing for bulls supplemented with organic trace minerals compared with bulls supplemented with inorganic trace minerals. Furthermore, the percentage of progressive sperm was greater ($P \leq 0.03$) pre-freezing for bulls receiving the organic compared with inorganic trace mineral supplement. A report by Arthington et al. (1995) concluded that zinc supplementation had a positive effect on bull fertility, and bulls supplemented with 2/3 zinc proteinate with 1/3 zinc sulfate exhibited an improved fertility compared with bulls supplemented at the same level with only zinc sulfate. In a another study comparing zinc supplementation from organic and inorganic sources in bulls, Kumar et al. (2006) determined that bulls receiving organic zinc supplementation exhibited improved semen characteristics compared with bulls receiving inorganic zinc supplementation over the same time period.

Immediately after thawing (0 h), the percent motility remained greater ($P = 0.013$) for semen collected from bulls fed the organic trace mineral (16.2%) treatment compared with semen collected from bulls fed the inorganic trace mineral treatment (7.8%). Percentage of progressive sperm was greater ($P = 0.02$) at 0 h post-thaw in semen from bulls in the organic trace mineral treatment (8.9%) compared with semen from bulls in the inorganic trace mineral group (4.1%). Furthermore, at 0 h post thaw, the bulls on the organic trace mineral supplement treatment still had greater ($P = 0.03$) rapid motility than bulls that received the inorganic trace mineral supplement treatment (10.6% versus 5.0%, respectively).

Following 2 h post thaw, semen collected from the bulls on the organic trace mineral treatment still had greater ($P = 0.025$) motility than semen collected from bulls that received the inorganic trace mineral treatment (8.5% compared with 3.7% respectively). Furthermore, at 2 h post thaw, percentages of progressive and rapid sperm still tended to be greater ($P < 0.07$) for the bulls receiving organic compared with inorganic trace mineral supplementation treatment (4.2% versus 1.7%, and 4.4% versus 2.0%, respectively).

In the current study, bulls that received organic trace mineral supplementation had enhanced measures of semen quality both before and after freezing. However, sperm motility parameters were greatly impacted by the freezing process which may likely be due to semen collections occurring during hot weather. Vogler et al. (1991) compared cryopreserved bull semen collected both before and after scrotal insulation and found there was a significant reduction in sperm motility and morphology in cryopreserved semen collected 3-9 days following scrotal insulation.

Conclusion

Sperm motility is one of the most important semen quality parameters influencing bull fertility and regardless of treatment, sperm motility parameters were greatly impacted by the freezing process. This is likely due to these semen collections occurring during hot weather. Overall, these results suggest replacing a portion of the supplemental Zn, Cu, Co, and Mn with organic trace mineral amino acid complexes may improve measures of bull semen quality both before and after freezing. Additional studies are needed to see if this improvement will have an effect on pregnancy rates.

REFERENCES

Arthington, J. D., K. R. Johnson, L. R. Corah, C. L. Willms , D. A. Hill. 1995. The effect of dietary zinc level and source on yearling bull growth and fertility. KSU Cattlemen's Day Report. 727:109-110.

Farrell, P. B., G. A. Presicce, C. C. Brockett, R. H. Foote. 1998. Quantification of bull sperm characteristics measured by computer-assisted sperm analysis (CASA) and the relationship to fertility. Therio. 49:671-679.

Kellogg, D. W. and E. B. Kegley. 2002. Organic-Chelated Minerals. Nutrition – Feed Supplements.

Hidiroglou, M. 1979. Trace element deficiencies and fertility in ruminants: A Review. J. Dairy Sci. 62(8):1195-1206.

Jockenhovel, F., M. Bals-Pratsch, H. P. Bertram, E. Nieschlag. 1990. Seminal lead and copper in fertile and infertile men. Andrologia. 22(6):503-511.

Kincaid, R. L. 2000. Assessment of trace mineral status of ruminants: A review. Proc. ASAS. 1999. Available at: http://jas.fass.org/content/77/E-Suppl/1.24.full.pdf. Accessed Nov. 17, 2011.

Kumar, N., R. P. Verma, L. P. Singh, V. P. Parshney, R. S. Dass. 2006. Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bos indicus* x *Bos taurus*) bulls. Reprod. Nutr. Dev. 46:663-675.

Martin, G. B., and C. L. White. 1992. Effects of dietary zinc deficiency on gonadotrophin secretion and testicular growth in young male sheep. J. Reprod. Fert. 96:497-507.

National Research Council. 1996. Nutrient Requirements of Beef Cattle. 7th Ed. Washington, DC: National Academy of Sciences.

SAS. 2003. Version 9.1. SAS Institute, Inc., Cary, NC.

Vogler, C. J., R. G. Saacke, J. H. Bame, J. M. DeJarnette, and M. L. McGilliard. 1991. Effects of scrotal insulation on viability characteristics of cryopreserved bovine semen. J Dairy Sci. 74:3827-3835.

Table 1. Supplemental sources and levels of trace minerals fed to bulls

Mineral	Organic diet		Inorganic diet
	Inorganic portion, mg/d	Organic portion[1], mg/d	Inorganic portion, mg/d
Zinc	90 as zinc sulfate	360 as zinc amino acid complex	450 as zinc sulfate
Copper	25 as copper sulfate	125 as copper amino acid complex	150 as copper sulfate
Cobalt	0	12 as cobalt glucoheptonate	12 as cobalt carbonate
Manganese	100 manganese sulfate	200 as manganese amino acid complex	300 as manganese sulfate
Selenium	3 as sodium selenite	0	3 as sodium selenite
Iodine	5 as calcium iodate	0	5 as calcium iodate

[1] Organic minerals provided via Availa®4 (Zinpro Corporation, Eden Prairie, MN)

Table 2. Ingredients and nutrient composition of carrier diet containing either inorganic or organic trace mineral supplement.

Supplement constituents:	Inorganic diet	Organic diet
Ingredient, % as fed		
Ground corn	83.4	83.4
Salt, white	5.8	5.8
Limestone	5.8	5.8
Molasses	5	5
Inorganic mineral premix[1]	+	-
Organic mineral premix[2]	-	+
Nutrient composition, DM basis		
DM, %	85.2	86.9
CP, %	10.1	10.0
Ca, %	2.91	2.63
P, %	0.25	0.26
Mg, %	0.11	0.11
Zn, mg/kg[3]	907 ± 35	954 ± 50
Cu, mg/kg[3]	338 ± 28	353 ± 7.4
Mn, mg/kg[3]	698 ± 125	769 ± 91
Co, mg/kg[3]	21.5 ± 0.45	27.1 ± 0.60

[1]Premix formulated to provide desired daily level of trace minerals (Table 1) in this supplement that was fed 3 times each week, 1.2 kg/feeding.
[2]Premix formulated to provide desired daily level of trace minerals (Table 1) in this supplement that was fed 3 times each week. Organic premix contained trace mineral amino acid complexes provided via Availa®4 (Zinpro Corporation, Eden Prairie, MN) and some inorganic trace minerals as sulfates.
[3]Diets mixed 3 times; values reported as the mean ± standard deviation after analyses of samples from each mix.

Table 3. Mean sperm parameters of bulls assigned to inorganic and organic trace mineral treatments at pre-freeze, 0 h post-thaw and 2 h post-thaw. (LSM)

CASA sperm parameter	Trace mineral treatment		P value
	Inorganic	Organic[1]	
Motile, %			
Pre-freeze	55.2±4.9	69.1±4.5	0.047
0 h post-thaw	7.8±2.4	16.2±2.2	0.013
2 h post-thaw	3.7±1.5	8.5±1.4	0.025
Progressive, %			
Pre-freeze	38.5±3.8	50.3±3.5	0.032
0 h post-thaw	4.1±1.5	8.9±1.4	0.020
2 h post-thaw	1.7±.9	4.2±0.8	0.058
Rapid, %			
Pre-freeze	52.6±5.0	65.9±4.7	0.06
0 h post-thaw	5.0±1.8	10.6±1.7	0.03
2 h post-thaw	2.0±0.97	4.4±0.9	0.07

[1]Organic minerals provided via Availa®4 (Zinpro Corporation, Eden Prairie, MN)