Broiler Breeder Semen Quality as Affected by Trace Minerals In Vitro[1]

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ABSTRACT Research has shown that trace elements, such as Se, Mn, and Zn, can alter reproductive functions. The aim of the current study was to evaluate the sperm quality index (SQI) and sperm viability as affected by various levels and sources of Se, Mn, and Zn when added in vitro to broiler breeder semen. In vitro treatments consisted of the following sources and levels of minerals: Control, no minerals added to sperm; seleno L-methionine, 4 levels ranging from 8.78 to 7,896 μg/L; sodium selenite, 4 levels ranging from 8.78 to 7,896 μg/L; $MnSO_4$, 8 levels ranging from 6,500 to 65,000 mg/L; Zn 180 (Zinpro Corporation), 4 levels ranging from 0.65 to 650 mg/L; and $ZnSO_4$, 4 levels ranging from 0.65 to 650 mg/L. The addition of 7,896 μg of sodium selenite/L to semen was detrimental to sperm motility. Also, $MnSO_4$ adversely affected SQI and sperm viability at concentrations of 6,500 mg/L and greater. Sperm viability was decreased when 650 mg/L of Zn 180 was added to semen. Sperm motility was depressed by exposure to Zn 180 at 650 mg/L and $ZnSO_4$ at 65 and 650 mg/L. Our results suggest that these trace minerals must act at the reproductive tissue level during spermatogenesis to improve semen quality. Direct in vitro application of these elements to semen appears to be detrimental to spermatozoa.

(*Key words*: broiler breeder, trace minerals, sperm motility, sperm viability)

2005 Poultry Science 84:100–105

INTRODUCTION

Deficiencies of the trace minerals Se, Zn, and Mn have been linked to impaired reproductive performance in male and female farm animals (Smith and Akinbamijo, 2000). Research has shown that trace elements such as Mn, Zn, and Se can alter reproductive functions in vivo. However, very limited research exists regarding the direct effects of these trace elements on avian semen quality in vitro.

A selenoprotein contributes to the stability of spermatozoa; therefore when there is a Se deficiency, the quality of the sperm is decreased (Leonhard, 2000). For many years, reports have shown that Se deficiencies can cause impaired male fertility in cattle, boars, rats, and mice. In particular, Se-deficient cattle exhibit reproductive disorders, including weak or silent periods, delayed conception, poor fertilization, cystic ovaries (Corah and Ives, 1992), reduced sperm motility, mastitis (Olson, 1995), and reduced uterine motility (Smith and Akinbamijo, 2000). Surai (2000) reported that Se-dependent glutathione peroxidase is an essential component of the antioxidant system in avian semen. Research has shown that the main problem to arise in Se-deficient spermatozoa is an imprecise architecture of the sperm midpiece (Flohe et al., 2001).

Although the Se concentration in avian semen is not known, Heimann et al. (1983) reported a Se concentration of 0.461 mg/L in bull semen. After administration of Se to bulls, Se concentrations of semen and various tissues increase; however, supplementation does not influence semen quality (Bartle et al., 1980), Se content, or sperm cell viability (Segerson and Johnson, 1979). Research has shown that in Se-deficient mice, the percentage of abnormal sperm greatly increases (6.8 to 49.6%) compared with that of the control (4.0 to 15.0%; Watanabe and Endo, 1991). Edens and Sefton (2002), found a decrease in normal sperm from 98.0 and 97.7% to 91.9 and 85.4%, when broiler breeders were fed Se-Met compared with sodium selenite, respectively.

Selenium added directly to spermatozoa in vitro also alters sperm function. Incubation of ram spermatozoa with selenite, Se-Cys, or Se-Met ranging from 10^{-6} to 2.5×10^{-5} M, significantly improved sperm motility and oxygen consumption (Alabi et al., 1985). On the other hand, Marin-Guzman et al. (2000) observed a decline in boar sperm motility when 0.3 to 0.9 ppm of Se was added to semen samples.

Received for publication May 19, 2004.

Accepted for publication October 11, 2004.

[1]This is Journal Article Number J10531 from the Mississippi Agricultural and Forestry Experiment Station. Use of trade names in this publication does not imply endorsement by the Mississippi Agricultural and Forestry Experiment Station of the products, nor similar ones not mentioned.

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Abbreviation Key: SQI = sperm quality index.

Conversely, reports have shown that the male reproductive system is quite sensitive to excessive levels of Se. Ingestion of 2 to 4 ppm of Se for 5 wk by the house rat caused a dose-dependent reduction in testicular and cauda epididymis weights as well as body weights. In the same experiment, sperm concentrations, motility, and percentage of live spermatozoa decreased, with an immediate increase in the percentage of atypical sperm (Kaur and Parshad, 1994).

It has been known for decades that Mn is abundant in bones and mitochondria, and that a Mn deficiency may cause symptoms such as impaired or depressed reproductive functions when dietary or tissue concentrations of Mn are low (Rojas et al., 1963; Anke and Groppel, 1970; Anke et al., 1973; Underwood, 1977). Cao et al. (1990) found that lactic dehydrogenase activity in the testes was reduced in birds fed 300 ppm of Mn; and birds fed 0 and 300 ppm of Mn had 35.1 and 31.6% fewer spermatozoa per gram of testis, respectively, as compared with birds fed 100 ppm. A study by Lapointe et al. (1996) demonstrated an increase in sperm motility by 53% when $MnCl_2$ was added directly to bovine semen at 12.5 mg/L.

Impairment of reproductive function in the presence of Zn deficiency has been widely reported (Chesters, 1978; Hidiroglou, 1979; Apagar, 1985). A role for Zn in reproduction may be as a vital component of enzymes involved in steroidogenesis. It has been shown that Zn may act indirectly through the pituitary to influence gonadotropic hormones (Hurley and Doane, 1989). When there is a Zn deficiency, research has shown that the amount of Zn found in the testis, epididymis, and dorsolateral prostate are reduced (Millar et al., 1958). Although semen and its components are high in Zn, the link between semen quality and Zn is not completely apparent. Calvin et al. (1975) found that Zn is concentrated in the tail region of the sperm. Studies show that Zn is crucial in membrane stability and for mechanical properties of accessory fibers, tail morphology, and sperm motility (Swarup and Sekhon, 1976). It has also been shown that Zn is involved in the control of sperm motility through its association with ATP in contraction and its regulation of phospholipid energy reserves (Hidiroglou and Knipfel, 1984). Bakst (1985) demonstrated that adding 110 mg/L of $ZnSO_4$ in vitro to turkey semen suppresses sperm O_2 uptake, but does not affect sperm fertility. Therefore, Zn may function as a metabolic inhibitor in turkey sperm, thereby decreasing sperm motility and prolonging survivability in the sperm storage tubules (Bakst, 1985; Bakst and Richards, 1985).

The current research was conducted to determine the direct in vitro effects of Mn, Zn, and Se on broiler breeder sperm. By understanding these in vitro effects, additional information may be gained about the mechanisms these minerals use to bring about their effects within the rooster's reproductive tract.

MATERIALS AND METHODS

Twenty-eight Cobb broiler breeder males were obtained from a local integrator, housed in individual cages, and maintained at 24°C during this experiment. The birds were 52 to 64 wk of age during the trial. They were fed the Mississippi State University male breeder diet (3,080 kcal of ME/kg, 13.9% CP, and 1% Ca) and were feed restricted according to the primary breeder's recommendations. All males received 16 h of light immediately upon placement.

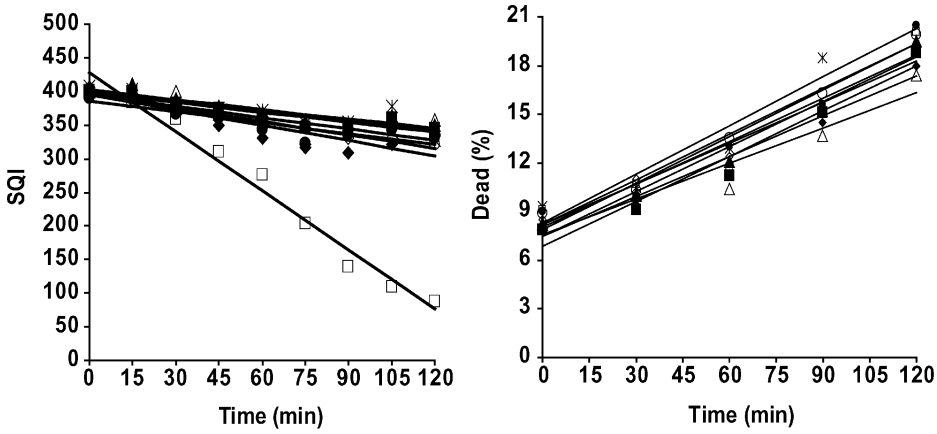
Three experiments, one each for evaluating Se, Mn, and Zn, were conducted. For each experiment, the semen samples from 28 males were pooled to give a composite semen sample. The common semen pool was then divided up into 9 smaller pools for 9 treatments so that any pretreatment variation in sperm concentration, viability, or motility across treatments would be reduced. The average sperm concentration was 6.7 billion sperm per mL for the original sample. Immediately following collection, the semen samples were incubated at 37°C.

In experiment 1, Se was tested using Se-Met and sodium selenite sources at the following levels for both (μg/L): 8.78, 78.96, 878, and 7,896. In experiment 2, Mn was tested using a $MnSO_4$ source with the following levels (mg/L): 6,500, 15,000, 23,000, 32,000, 40,000, 48,000, 57,000, and 65,000. Zinc was tested in experiment 3 using Zinpro Zn 180[3] and $ZnSO_4$ at the following levels for both sources (mg/L): 0.650, 6.5, 65, and 650. Controls containing no added trace minerals were also represented in each experiment. Because of trace mineral additions, semen samples were ultimately diluted 2-fold compared with the original sample. Following additions of trace elements, semen samples were kept in open vials at 37°C throughout each experiment.

Overall semen quality was assessed by the Sperm Quality Analyzer,[4] which produces the sperm quality index (SQI). The SQI is generated from disruptions in a light path created by sperm movement within a capillary tube (McDaniel et al., 1998). The SQI was obtained for each treatment every 15 min for 2 h. Three semen pools, from each treatment at each incubation time, were evaluated for SQI simultaneously on 3 Sperm Quality Analyzers using the method of McDaniel et al. (1998). The CV across these 3 machines was 3.6%. Immediately before SQI analysis, semen was further diluted 5-fold with physiological saline, resulting in a 10-fold dilution of the original semen sample. For each analyzer, treatments were randomly tested. Sperm viability was obtained in duplicate every 30 min of incubation for each treatment using the method of Bilgili and Renden (1984).

To provide experimental replication, the entire study was repeated with 32-wk-old Ross males. Therefore, the 2 times of replication served as blocks in a randomized complete block design with a split plot over incubation time. To further analyze data over incubation time, lin-

[3]Zinpro Corporation, Eden Prairie, MN.
[4]Sperm Quality Analyzer, Medical Electronic Systems Ltd., Migdal Haemek, Israel.



Se Source and Level	SQI-Regression equation over incubation time	SQI Mean	% Dead-Regression equation over incubation time	% Dead Mean
✳ Control	y = –0.5[a] x + 401	371[a]	y = 0.098 x + 8.4	14.1
◆ Seleno-Met - 8.78 μg/L	y = –0.8[a] x + 395	350[a]	y = 0.082 x + 7.5	12.4
● Seleno-Met - 78.96 μg/L	y = –0.5[a] x + 385	353[a]	y = 0.096 x + 8.9	13.6
▲ Seleno-Met - 878 μg/L	y = –0.5[a] x + 404	373[a]	y = 0.092 x + 7.5	13.0
■ Seleno-Met - 7,896 μg/L	y = –0.5[a] x + 398	369[a]	y = 0.093 x + 6.8	12.4
◇ Sodium Selenite - 8.78 μg/L	y = –0.7[a] x + 395	355[a]	y = 0.084 x + 8.2	13.2
○ Sodium Selenite - 78.96 μg/L	y = –0.6[a] x + 395	361[a]	y = 0.094 x + 8.2	13.8
△ Sodium Selenite - 878 μg/L	y = –0.5[a] x + 400	373[a]	y = 0.074 x + 7.5	11.9
□ Sodium Selenite - 7,896 μg/L	y = –2.9[b] x + 428	252[b]	y = 0.089 x + 8.0	13.3

FIGURE 1. Effect of Se source and level on sperm quality index (SQI) and percentage of dead sperm over incubation time. [a–b]Slopes or means in a column with different superscripts are significantly different at $P < 0.05$. The SQI means in the table represent 2 replicates per treatment averaged over 9 incubation times (n = 18, SEM = 17.8). The percentage dead sperm means in the table represent 2 replicates per treatment averaged over 5 incubation times (n = 10, SEM = 0.61).

ear and curvilinear regression was used. Linear and quadratic slopes, as well as intercepts were partitioned using Fisher's Protected LSD (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Experiment 1—Selenium

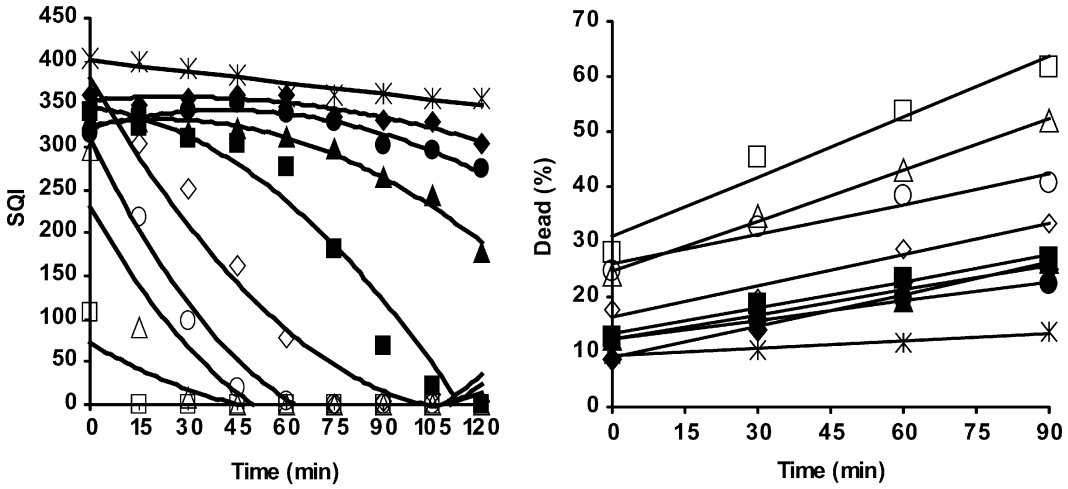
As expected, the SQI of the control sample in every experiment deteriorated with increasing length of incubation due to declining sperm motility and sperm viability (Figure 1, 2, and 3). The SQI was unaffected by addition of Se except at the highest level of sodium selenite (Figure 1). The highest level of sodium selenite (7,896 μg/L) was detrimental to the SQI after 45 min of testing as shown by a rapid linear decline in SQI with incubation time. When averaged over the entire incubation period, the SQI was depressed 32% by the highest level of sodium selenite compared with the control. By 120 min of incubation, very little sperm movement (SQI = 87) was detected in the semen sample containing the highest level of sodium selenite. However, for the control sample, the SQI was 327 units at 120 min of incubation.

Secondary to the SQI data, the percentage of dead sperm increased with incubation length in every treatment (Figure 1). However, exposure to Se-Met or sodium selenite did not alter the percentage of dead sperm.

It is apparent that Se at high levels is very detrimental to avian sperm motility. Seleno L-methionine and sodium selenite were evaluated at the same concentration. However, the amount of Se per gram of Se-Met was 13% less than that in sodium selenite due to the greater molecular weight of methionine. The difference in actual Se content in these 2 treatments may explain the disparity in results for the 2 treatments.

Experiment 2—Manganese

When averaged over all incubation times, the SQI for $MnSO_4$ at every level was significantly less than that of the control (Figure 2). Also, all $MnSO_4$ treatments yielded a quadratic decline in SQI over incubation time, whereas the SQI for the control sample declined linearly and slowly with incubation time. Once the Mn level exceeded 32,000 mg/L there was a drastic decline in SQI with incubation time, with the slopes being considerably



$MnSO_4$ Level	SQI-Regression equation over incubation time	SQI Mean	% Dead-Regression equation over incubation time	% Dead Mean
Control	$y = -0.4\,x + 400$	374^{a}	$y = 0.04^{c}\,x + 9^{c}$	11^{e}
$MnSO_4$ 6,500 mg/L	$y = -0.006^{d}\,x^2 + 0.3^{ab}x + 355^{ab}$	343^{b}	$y = 0.19^{bc}\,x + 9^{c}$	18^{d}
$MnSO_4$ 15,000 mg/L	$y = -0.012^{de}\,x^2 + 1.0^{a}x + 322^{bc}$	321^{b}	$y = 0.11^{c}\,x + 12^{bc}$	18^{d}
$MnSO_4$ 23,000 mg/L	$y = -0.015^{de}\,x^2 + 0.7^{ab}x + 325^{bc}$	289^{c}	$y = 0.15^{c}\,x + 12^{bc}$	19^{cd}
$MnSO_4$ 32,000 mg/L	$y = -0.023^{e}\,x^2 - 0.4^{b}x + 346^{abc}$	203^{d}	$y = 0.16^{bc}\,x + 13^{bc}$	20^{cd}
$MnSO_4$ 40,000 mg/L	$y = 0.027^{b}\,x^2 - 6.5^{d}x + 380^{a}$	129^{e}	$y = 0.19^{bc}\,x + 16^{b}$	25^{c}
$MnSO_4$ 48,000 mg/L	$y = 0.044^{a}\,x^2 - 7.7^{e}x + 312^{c}$	73^{f}	$y = 0.18^{bc}\,x + 26^{a}$	34^{b}
$MnSO_4$ 57,000 mg/L	$y = 0.043^{a}\,x^2 - 6.7^{de}x + 232^{d}$	44^{g}	$y = 0.31^{ab}\,x + 25^{a}$	38^{b}
$MnSO_4$ 65,000 mg/L	$y = 0.015^{c}\,x^2 - 2.2^{c}x + 72^{e}$	12^{h}	$y = 0.36^{a}\,x + 31^{a}$	47^{a}

FIGURE 2. Effect of Mn level on sperm quality index (SQI) and percentage dead sperm over incubation time. [a–h]Linear slopes, quadratic slopes, y-intercepts, or means in a column with different superscripts are significantly different at $P < 0.05$. The SQI means in the table represent 2 replicates per treatment averaged over 9 incubation times (n = 18, SEM = 7.4). The percentage dead sperm means in the table represent 2 replicates per treatment averaged over 4 incubation times (n = 8, SEM = 1.9).

higher than those of the lower levels. Even at the very beginning of incubation, the SQI for the 65,000-mg/L level was only 108 units compared with 403 units for the control. By 15 min, the 65,000-mg/L treatment exhibited no sperm motility, as evidenced by an SQI of zero.

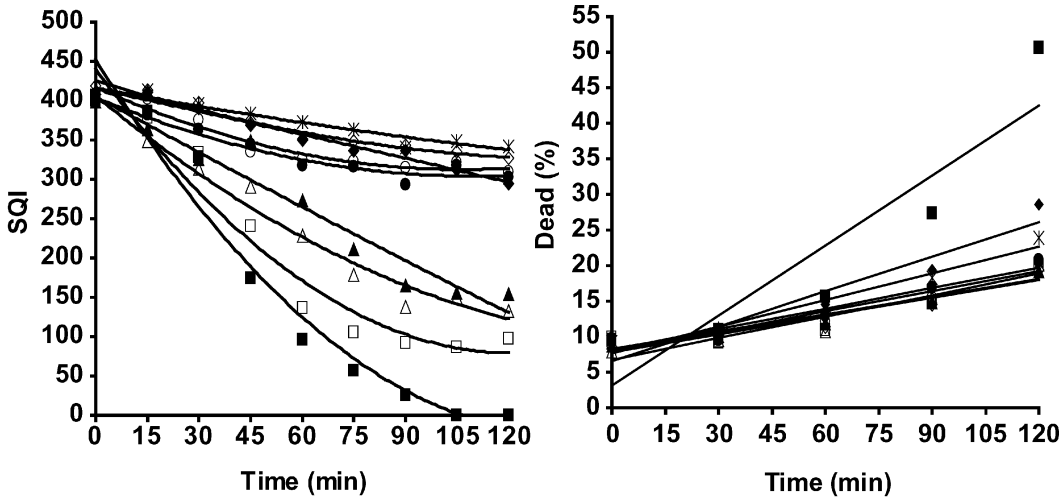
Similar to the SQI, the percentage of dead sperm increased steadily with increasing concentration of $MnSO_4$ (Figure 2). For every treatment, the percentage of dead sperm increased linearly with incubation. The rate of linear incline in the percent of dead sperm was greatest for the 57,000 and 65,000 mg/L treatments.

Manganese levels of 6,500 mg/L and greater were toxic, as indicated by avian sperm motility (SQI) and viability. Using bovine semen, Lapointe et al. (1996), found an increase (53%) in sperm motility with exposure to $MnCl_2$ (12.5 mg/L). Before conducting the current study, a pilot trial was conducted using Mn levels from 0.0065 to 6,500 mg/L that were similar to those of Lapointe et al. (1996). Results from that experiment revealed no significant effects of low Mn concentration on avian sperm motility or viability.

Experiment 3—Zinc

The SQI was depressed for semen samples exposed to Zn 180 or $ZnSO_4$ at levels higher than 65 and 6.5 mg/L, respectively, when averages over semen incubation time were examined (Figure 3). Additionally, the rate of decrease in SQI over incubation time was exacerbated by exposure of semen to the highest level of Zn 180 and $ZnSO_4$. By 105 min of incubation, the semen sample exposed to Zn 180 at 650 mg/L exhibited no motility, as evidenced by an SQI of zero.

When averaged over all incubation times, the percentage of dead sperm was increased by exposure to the highest level of Zn 180 (Figure 3). Also, this level of Zn 180 produced a more rapid linear increase in the percentage of dead sperm with increasing length of in-



	Zn Source and Level	SQI-Regression equation over incubation time	SQI Mean	% Dead-Regression equation over incubation time	% Dead Mean
✳	Control	$y = 0.0013x^2 - 0.8^{a}x + 416^{ab}$	374^{a}	$y = 0.13^{bc}x + 8^{a}$	15^{bc}
◆	Zn 180 0.650 mg/L	$y = -0.0002x^2 - 1.0^{a}x + 415^{ab}$	356^{a}	$y = 0.08^{c}x + 8^{a}$	13^{bc}
●	Zn 180 6.500 mg/L	$y = 0.0082x^2 - 1.8^{ab}x + 404^{b}$	337^{ab}	$y = 0.10^{bc}x + 8^{a}$	14^{bc}
▲	Zn 180 65.000 mg/L	$y = 0.0008x^2 - 2.4^{ab}x + 405^{b}$	266^{abc}	$y = 0.16^{b}x + 7^{a}$	16^{b}
■	Zn 180 650.000 mg/L	$y = 0.0260x^2 - 7.0^{c}x + 452^{a}$	164^{c}	$y = 0.33^{a}x + 3^{b}$	23^{a}
◇	$ZnSO_4$ 0.650 mg/L	$y = 0.0048x^2 - 1.4^{a}x + 426^{ab}$	367^{a}	$y = 0.08^{bc}x + 8^{a}$	13^{c}
○	$ZnSO_4$ 6.500 mg/L	$y = 0.0091x^2 - 2.0^{ab}x + 417^{ab}$	346^{ab}	$y = 0.10^{bc}x + 8^{a}$	14^{bc}
△	$ZnSO_4$ 65.000 mg/L	$y = 0.0104x^2 - 3.6^{abc}x + 406^{b}$	242^{bc}	$y = 0.10^{bc}x + 7^{a}$	13^{c}
□	$ZnSO_4$ 650.000 mg/L	$y = 0.0247x^2 - 6.0^{bc}x + 440^{ab}$	208^{c}	$y = 0.09^{bc}x + 8^{a}$	13^{c}

FIGURE 3. Effect of Zn source and level on sperm quality index (SQI) and percentage dead sperm over incubation time. [a–c]Linear slopes, y-intercepts, or means in a column with different superscripts are significantly different at $P < 0.05$. The SQI means in the table represent 2 replicates per treatment averaged over 9 incubation times (n = 18, SEM = 33.6). The percentage dead sperm means in the table represent 2 replicates per treatment averaged over 5 incubation times (n = 10, SEM = 1.0).

cubation. Through 60 min of incubation, Zn180 at the highest level yielded sperm viability results consistent with the control. After 60 min of incubation, the percentage of dead sperm for this level increased significantly as compared with all other treatments.

The findings of this experiment are in partial agreement with the research of Blesbois and Mauger (1989). Blesbois and Mauger (1989) found that Zn concentration in the seminal plasma of fowl was 1 to 3 mg/L and was unaffected by age. In the same study, Blesbois and Mauger found that Zn concentrations of 1, 3, and 9 mg/L in storage medium had no effect on sperm motility. However, in young birds, Zn reduced the fertilizing ability of stored spermatozoa at the 2 highest concentrations. All Zn concentrations reduced fertilizing ability when tested in older birds. Bakst (1985) showed that Zn could depress O_2 uptake by sperm when added to avian semen in vitro. Interestingly, at the highest level of Zn 180, during the first 60 min of incubation in the current study, sperm motility dropped dramatically, whereas viability was unaffected. Therefore, sperm became immotile before they died due to Zn exposure. This depression in motility may be due to a reduction in oxygen uptake as reported by Bakst (1985). Apparently, Zn added in vitro does not kill avian sperm until exposure is greater than 65 mg/L. However, it does decrease motility at levels greater than 0.65 mg/L.

In conclusion, Se, Mn, and Zn appear to be detrimental to overall rooster semen quality when added to semen in vitro. The beneficial effects of supplementation with these minerals as reported in earlier in vivo studies might indicate that the minerals indirectly improved semen quality by acting at the reproductive tissue level during spermatogenesis, rather than acting directly on the spermatozoa. Additional in vivo research should be conducted to determine the manner in which these minerals improve semen quality.

REFERENCES

Alabi, N. S., P. D. Whanger, and A. S. Wu. 1985. Interactive effects of organic and inorganic selenium with cadmium and mercury on spermatozoal oxygen consumption and motility in vitro. Biol. Reprod. 33:911–919.

Anke, M., and B. Groppel. 1970. Manganese deficiency and radioisotope studies on manganese metabolism. Page 133 in Trace Element Metabolism in Animals. C. F. Mills, ed. E. and S. Livingstone, Edinburgh, UK.

Anke, M., B. Groppel, W. Reissig, H. Ludke, M. Grun, and G. Dittrich. 1973. Manganese deficiency in ruminants. (3) Manganese deficiency stimulated reproductive, skeletal, and nervous disturbances in female ruminants and their offspring. Arch. Tierernahr. 23:197–211.

Apagar, J. 1985. Zinc and reproduction. Annu. Rev. Nutr. 5:43–68.

Bakst, M. R. 1985. Zinc reduces turkey sperm oxygen uptake in vitro. Poult. Sci. 64:564–566.

Bakst, M. R., and M. P. Richards. 1985. Concentrations of selected cations in turkey serum and oviductal mucosae. Poult. Sci. 64:555–563.

Bartle, J. L., P. L. Senger, and J. K. Hillers. 1980. Influences of injected selenium in dairy bulls on blood and semen selenium, glutathione peroxidase and seminal quality. Biol. Reprod. 23:1007–1013.

Bilgili, S. F., and J. A. Renden. 1984. Fluorometric determination of avian sperm viability and concentration. Poult. Sci. 63:2275–2277.

Blesbois, E., and I. Mauger. 1989. Zinc content of fowl seminal plasma and its effects on spermatozoa after storage at 4°C. Br. Poult. Sci. 30:677–685.

Calvin, H. I., F. H. Hwang, and H. Wohlrab. 1975. Localization of zinc in a dense fibre-connecting piece fraction of rat sperm tails analogous chemically to hair keratin. Biol. Reprod. 13:228–239.

Cao, S., C. Chen, and X. Chen. 1990. Effects of dietary manganese on lactic dehydrogenase activity and sperm concentration in male fowls. J. Shanghai Agric. College 8:93–100.

Chesters, J. K. 1978. Biochemical functions of zinc in animals. World Rev. Nutr. Diet. 32:135–164.

Corah, L. R., and S. Ives. 1992. Trace minerals in cow herd nutrition programs. Agri-Practice 13:5–7.

Edens, F. W., and A. E. Sefton. 2002. Selenomethionine supplementation to diets of broiler breeders improves performance. Poult. Sci. 81:91. (Abstr.)

Flohe, L., R. Brigelius-Flohe, M. Maiorino, A. Roveri, J. Wissing, F. Ursini, and D. L. Hatfield. 2001. Selenium and male reproduction. Pages 273–281 in Selenium: Its Molecular Biology and Role in Human Health. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Heimann, E. D., M. F. Smith, J. S. Morris, T. J. Gall, R. G. Elmore, and R. E. Morrow. 1983. Relationships among spermatozoal abnormalities and the selenium concentration of blood plasma, semen, and reproductive tissues in young bulls. Anim. Reprod. Sci. 7:315–321.

Hidiroglou, M. 1979. Trace element deficiencies and fertility in ruminants: A review. J. Dairy Sci. 62:1195–1206.

Hidiroglou, M., and J. E. Knipfel. 1984. Zinc in mammalian sperm: A review. J. Dairy Sci. 67:1147–1156.

Hurley, W. L., and R. M. Doane. 1989. Recent developments in the roles of vitamins and minerals in reproduction. J. Dairy Sci. 72:784–804.

Kaur, R., and V. R. Parshad. 1994. Effects of dietary selenium on differentiation, morphology and functions of spermatozoa of the house rat, Rattus rattus L. Mutat. Res. 309:29–35.

Lapointe, S., I. Ahmad, M. M. Buhr, and M. A. Sirard. 1996. Modulation of postthaw motility, survival, and calcium uptake, and fertility of bovine sperm by magnesium and manganese. J. Dairy Sci. 79:2163–2169.

Leonhard, M. S. 2000. Why do trace elements have an influence on fertility? Tierarztl. Prax. Ausg. G, Grosstiere Nutztiere 28:60–65.

Olson, K. 1995. Improving beef cattle nutrition for higher profits. Utah Sci. 56:7.

Marin-Guzman, J., D. C. Mahan, and R. Whitmoyer. 2000. Effect of dietary selenium and vitamin E on the ultrastructure and ATP concentration of boar spermatozoa, and the efficacy of added sodium selenite in extended semen on sperm motility. J. Anim. Sci. 78:1544–1550.

McDaniel, C. D., J. L. Hannah, H. M. Parker, T. W. Smith, C. D. Schultz, and C. D. Zumwalt. 1998. Use of a sperm analyzer for evaluating broiler breeder males. 1. Effects of altering sperm quality and quantity on the sperm motility index. Poult. Sci. 77:888–893.

Millar, M. J., M. I. Fischer, P. V. Elcoate, and C. A. Mawson. 1958. The effects of dietary zinc deficiency on the reproductive system of male rats. Can. J. Biochem. Physiol. 36:557–569.

Rojas, M. A., I. A. Dyer, and W. A. Cassatt. 1963. Manganese deficiency in the bovine. J. Anim. Sci. 24:664–667.

Segerson, E. C., and B. H. Johnson. 1979. Selenium/vitamin E and reproductive function in Angus bulls. J. Anim. Sci. 48(Suppl. 1):336. (Abstr.)

Smith, O. B., and O. O. Akinbamijo. 2000. Micronutrients and reproduction in farm animals. Anim. Reprod. Sci. 60-61:549–560.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics. A Biometrical Approach, 2nd ed. McGraw Hill Book Co., Inc., New York, NY.

Surai, P. F. 2000. Organic selenium: Benefits to animals and humans, a biochemist's view. Pages 205–260 in Biotechnology in the Feed Industry. Nottingham University Press, Nottingham, UK.

Swarup, D., and H. Sekhon. 1976. Correlation of vitamin A and zinc concentration of seminal plasma to fertility of bovine semen. Nutr. Rep. Int. 13:37–42.

Underwood, E. J. 1977. Manganese. Page 170 in Trace Elements in Human and Animal Nutrition. 4th ed. Academic Press, New York, NY.

Watanabe, T., and A. Endo. 1991. Effects of selenium deficiency on sperm morphology and spermatocyte chromosomes in mice. Mutat. Res. 262:93–99.