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The effect of a zinc, cobalt and selenium soluble glass bolus on trace element status and semen quality of ram lambs

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Abstract

Supplemental zinc and selenium were administered to ram lambs grazed on pastures that were not considered to be deficient in either element. The breeding season and polygamy of the ram mean that his requirements for semen production will be relatively large over a short breeding season and this may induce a localised deficiency of zinc and/or selenium, thus resulting in a decrease in semen quality and production.

Thirty-three 8-month-old ram lambs were kept at grass and fed a supplement of barley and peas, with ad libitum access to grass silage when grazing became restricted. On day 0, the rams were allocated to two groups by restricted randomisation of live weight. One group each had a zinc, cobalt and selenium soluble glass bolus (Zincosel®, Telsol) administered with the other group not receiving a bolus to act as a control. Blood samples were taken by jugular venipuncture at day 0 (prior to bolus administration) and at days 23, 44, 65 and 86. Blood samples were analysed for zinc status (plasma zinc concentration) and selenium status (erythrocyte glutathione peroxidase activity). Semen was collected once a week between days 44 and 86, by diversion during a natural mount. Semen quality was assessed by ejaculate volume, spermatocrit, sperm concentration, abnormal morphology, motility, percentage live (negrosin–eosin stain), membrane integrity (hypo-osmotic swelling test (HOS)) and seminal fluid glutathione peroxidase activity and zinc concentration. The bolused lambs had a significantly increased erythrocyte glutathione peroxidase activity ($P < 0.01$) on all samplings after bolusing and had significant increases in motility, proportion of live sperm and proportion of intact membranes indicated by the HOS. The

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bolused ram lambs had an increased selenium status and apparent improvement in semen membrane quality.

Keywords: Sheep, male reproduction; Zinc; Selenium; Supplementation; Semen quality

1. Introduction

There has been little published work in recent years on the effects of supplemental zinc and selenium on fertility in rams. However, supplementation with zinc and selenium in other species (especially human) and the intrinsic roles of both zinc and selenium in sperm production and antioxidant status suggest a role for supplemental zinc and selenium for the male ovine (Irvine, 1996; Vézina et al., 1996; Underwood and Somers, 1969).

Some sub-fertile human males have been shown to be selenium deficient and this deficiency will affect the morphology and motility of spermatozoa (Scott et al., 1998). An increase in the formation of reactive oxygen species decreases fertility, as the reactive oxygen species will attack the membranes of the spermatozoa, decreasing their viability (Irvine, 1996). Increasing the selenium status will increase the antioxidant glutathione peroxidase activity thus decreasing the reactive oxygen species and leading to an increase in male fertility (Irvine, 1996).

The production of semen necessitates extensive cell division and this requires large amounts of zinc, as zinc is involved extensively in nucleic acid and protein metabolism (Underwood, 1981) and is hence fundamental to cell differentiation and replication. Zinc is essential in the production of many of the sex hormones including testosterone and gonadotrophin releasing hormone (Hambidge et al., 1986). Zinc is important for the attachment of head to tail in spermatozoa and is required for the production of an antibacterial compound released from the prostate gland into the semen (Saaranen et al., 1987). Zinc requirements for testicular growth and development and for spermatogenesis are greater than the requirements for body growth and appetite (Underwood and Somers, 1969). Supplementation with zinc increases daily sperm production and reduces the proportion of abnormal spermatozoa (Underwood and Somers, 1969). Zinc also has antioxidative properties and may also act to reduce the reactive oxygen species and hence increase fertility (Bray et al., 1997).

The breeding season and polygamy of the ram mean that his requirements for semen production will be relatively high over a short breeding season and this may induce a localised deficiency of zinc and/or selenium resulting in a decrease in semen quality and production.

2. Materials and methods

Thirty-three entire ram lambs were split into two groups by restricted randomisation of liveweight. On day 0, one group ($n = 17$) were treated with a 33-g Zincosel® (Telsol) zinc, cobalt and selenium soluble glass bolus (15.2% w/w zinc, 0.5% w/w cobalt and

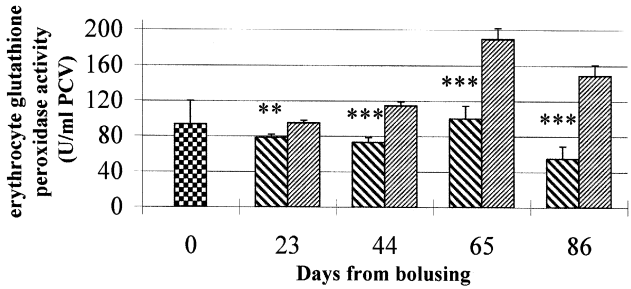

Fig. 1. Mean (+s.e.) erythrocyte glutathione peroxidase activities of the control (heavier diagonal lines) and bolused (diagonal lines) rams adjusted for day 0 covariate (checkered) (+s.d).

0.15% w/w selenium) with the other group left unbolused to act as controls ($n = 16$). The rams were kept in one group outside at grass, with ad libitum access to good grass silage when grazing was not available ad libitum. The rams were also fed a barley and pea based concentrate to maintain body condition.

The rams were blood sampled by jugular venipuncture to assess zinc and selenium status on days 0, 23, 44, 65 and 86. Zinc status was assessed by plasma zinc concentration measured by atomic absorption spectrophotometry (Kendall et al., 1997a) and selenium status assessed by erythrocyte glutathione peroxidase activity which was colorimetrically determined using an autoanalyser (Cobas Mira, Roche) based on the method of Paglia and Valentine (1967). Collection of semen samples in mid to late breeding season were attempted from all rams by natural mount and diversion into an artificial vagina on days 44, 52, 58, 65, 73, 79 and 86 after bolus administration (7th January to 18th February). Semen samples were assessed for spermatocrit, total sperm count, motility, proportion of live sperm (negrosin–eosin stain) and abnormalities (total, coiled tail, no tail, two tails, tail droplet and other). The functional integrity of the sperm's plasma membrane was assessed using the hypo-osmotic swelling test (HOS) (Jeyendran et al., 1992; Correa et al., 1997). Seminal plasma zinc concentration and glutathione peroxidase activity were determined using the same methodology as for blood. Statistical analysis of blood parameters was carried out by ANOVA using day 0 as a covariate using GLM on MINITAB 11, the ram lambs not producing a valid semen

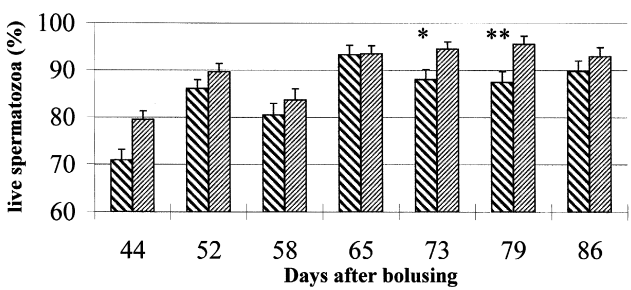

Fig. 2. Mean (+s.e.) proportion of live sperm for the control (heavier diagonal lines) and bolused (diagonal lines) rams ($^{*}P < 0.05$, $^{**}P < 0.01$).

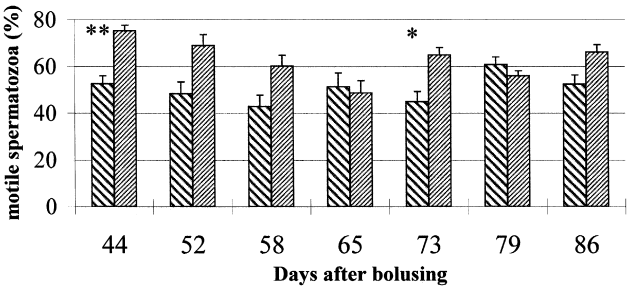

Fig. 3. Mean (+s.e.) proportion of motile sperm for the control (heavier diagonal lines) and bolused (diagonal lines) rams ($^{*}P < 0.05$, $^{**}P < 0.01$).

sample were excluded from this analysis. Statistical analysis of semen parameters was by ANOVA using GLM on MINITAB 11, however, prior to analysis proportionality data (spermatocrit, percent live, HOS, motility) were transformed using the arcsine transformation and abnormality data transformed using the square root transformation with adjustment to allow for zero values.

3. Results

Throughout the semen collections, seven control and five bolused rams failed to give a valid sample on any collection day and were excluded from all statistical analysis for the whole trial. There were no apparent differences between the rams giving semen samples and those giving none in terms of zinc or selenium status.

The bolused rams had a significantly higher selenium status than the control rams throughout the trial (day 23, $P < 0.01$; days 44, 65, 86, $P < 0.001$) (Fig. 1). The selenium status of all the rams was in the normal range (> 40 U/ml PCV). There were no significant differences between the groups in terms of zinc status with both groups having mean plasma zinc concentrations of at least 7.0 μM throughout the trial, apart from the day 0 (covariate) mean of 6.1 (s.d. ± 1.01).

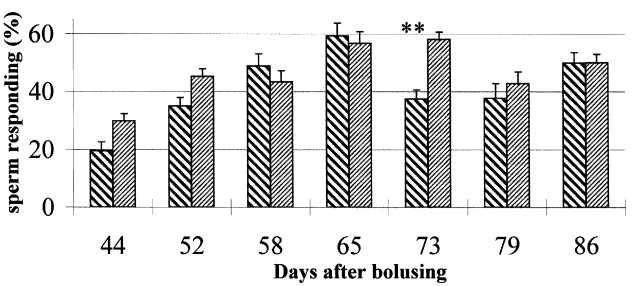

Fig. 4. Mean (+s.e.) results of the HOS for the control (heavier diagonal lines) and bolused (diagonal lines) rams ($^{**}P < 0.01$).

Table 1
Seminal plasma glutathione peroxidase activity (GSHPx) and zinc concentration, and total sperm count; mean, s.e.

Day	Seminal plasma zinc (μM)				Seminal plasma GSHPx (U/ml)				Total sperm count ($\times 10^6$/ml)			
	Control		Bolused		Control		Bolused		Control		Bolused	
44	65.5	6.0	55.3	4.8	2.19	0.66	3.66	0.52	43.3	8.1	51.7	6.4
52	51.1	4.9	47.8	4.6	2.49	0.65	3.08	0.60	55.9	6.0	65.6	5.6
58	47.9	10.6	50.6	9.3	2.48	0.47	2.64	0.44	66.7	9.2	64.8	8.6
65	41.0	6.2	50.4	5.5	2.32	0.56	2.77	0.50	98.6*	4.8	66.0*	4.6
73	56.4*	5.3	40.6*	3.7	2.37	0.49	1.89	0.36	56.4	9.6	58.0	6.8
79	39.8	6.0	44.4	4.6	1.62	0.44	1.88	0.35	67.2	12.1	76.8	9.2
86	38.0	5.5	38.9	4.2	1.99	0.49	2.11	0.39	54.2	6.9	52.1	5.6

* Indicates significance ($P < 0.05$).

The percentage of live spermatozoa (Fig. 2) was higher for the bolused rams when compared to the control group on all sampling days. This was significant on days 73 ($P < 0.05$) and 79 ($P < 0.01$). Fig. 3 illustrates the significant increases in motility on days 44 and 73 ($P < 0.01$ and $P < 0.05$, respectively) for the bolused rams. There was a significantly improved HOS result for the bolused rams compared to the controls on day 73 ($P < 0.01$) (Fig. 4). There were no significant differences in spermatocrit and ejaculate volume between the groups. However, the control group total sperm count was significantly higher on day 65 ($P < 0.05$) and the control group seminal plasma zinc concentration was significantly higher on day 73 ($P < 0.05$) (Table 1). The seminal plasma glutathione peroxidase activity was higher for six of the seven samplings, although no statistical significance was observed. There were no significant differences between the bolused and control rams for any semen abnormality on any day.

4. Discussion

The bolus significantly increased the selenium status of the rams throughout the trial, however, the selenium status of all of the rams including the control group was adequate (> 40 U GSHPx/ml PCV) throughout. The increase in selenium status even though the sheep are at a level considered to be adequate has previously been consistently seen with this type of bolus (Kendall et al., 1997a,b) and with the copper, cobalt and selenium soluble glass bolus (Cosecure) (Kendall et al., 1999, 2000). Apart from day 0, the mean group plasma zinc concentrations were maintained above 7.0 μM throughout the trial. In previous trials with this type of bolus plasma zinc concentrations have been found to increase when the sheep were zinc deficient (Kendall et al., 1997a) or immune challenged (Kendall et al., 1997b). The lack of any increase in plasma zinc concentration and the relatively high values suggest that both groups were adequate for zinc status. Plasma zinc concentrations are very difficult to increase, unless the animal is deficient, due to the very strong homeostatic mechanisms involved in zinc absorption and metabolism (Cousins, 1986). Although the bolus also contained cobalt, there have been

no reports identified that link cobalt status (vitamin B_{12}) to male fertility or semen quality and therefore cobalt was not considered within this paper.

The increased motility, live sperm and HOS were probably due to the increase seminal plasma glutathione peroxidase that, although not significant, was consistently higher for the bolused group (6 of 7 samplings). This is in agreement with the findings of Vézina et al. (1996) in humans. Wu et al. (1973) found selenium supplementation to decrease the numbers of sperm with breaks in the flagellum and hence cause increased motility and subsequently found that the number of sperm with breaks was proportional to selenium status in rats (Wu et al., 1979). However, this mechanism was not the cause of increased motility in this trial due to the control group not being deficient in selenium and there being no significant difference between the groups in tail abnormalities. The additional selenium did increase the antioxidant status as indicated by the increased glutathione peroxidase activities, which will give protection against spontaneous lipid peroxidation. Alvarez and Storey (1984) showed spontaneous lipid peroxidation to cause the plasma membrane to lose its ability to act as a permeability barrier, leading to the loss of cytosolic enzymes and substrates and a decrease in sperm motility and survival. The HOS is an assay to determine plasma membrane permeability and has also been shown to correlate with the numbers of sperm undergoing capacitation (Jeyendran et al., 1984). This mechanism explains the significant increases in motility, percentage live and the HOS in the treated rams. The observed increases found in semen quality may possibly result in increased fertility in the field; however, this would need to be verified with further work. The results suggest that supplementation with zinc and selenium may result in an increased semen quality in the field, even in rams with apparently normal zinc and selenium status.

5. Conclusions

The bolus increased the selenium status of the rams and gave an increase in sperm motility, percentage of live sperm and sperm responding to the HOS.

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