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Effect of zinc oxide treatment for facial eczema on the copper status of Romney sheep grazing ryegrass pastures

J.R. ROUNCE, S.O. KNOWLES, N.D. GRACE AND J. LEE

AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand

ABSTRACT

The copper (Cu) status of grazing Romney sheep was monitored following treatment with zinc oxide (ZnO). In a series of experiments sheep of various ages were treated with ZnO as either an oral drench weekly for one year, or as an intraruminal controlled release bolus (Time Capsule™, AgResearch) once (on day 0) or twice (on day 0 and 40), in two consecutive years. Measurements of Cu status included Cu concentrations in liver, kidney and muscle in all animals, and liver metallothionein (MT) in sheep dosed with ZnO orally. Drenching of two-year old wethers with 12 g ZnO/week for 52 weeks did not significantly change Cu liver concentration compared to untreated control animals (mean ± SD, 1.93 ± 0.88 and 1.67 ± 0.87mmol cu/kg FW, respectively). However, administering a single 42-day intraruminal ZnO bolus to six-month old lambs resulted in decreased liver Cu concentrations after 40 days (0.96 ± 0.06 compared to 1.33 ± 0.07 mmol Cu/kg FW in controls; P<0.001). When lambs which were growing at 100g/day were treated with a second ZnO bolus and evaluated after an additional 40 days, Cu liver concentrations were reduced by a further 20% but this change was not statistically significant (P=0.07). The variable effects of different forms of Zn treatment on liver Cu concentration are discussed in terms of duration of the dose, age of the animals, DM intake, initial Cu pool size in the liver, and Cu/Zn-binding MT. Under most conditions the benefits of ZnO as an effective facial eczema control are likely to outweigh any short term reduction in liver Cu.

Keywords: ZnO, Cu and Zn interactions, metallothionein, Cu absorption, ovine

INTRODUCTION

Pharmacological doses of zinc (Zn) as ZnO are an effective and accepted treatment for the prevention of facial eczema, a mycotic disease of grazing ruminants (Munday *et al*., 1993). Doses for sheep of about 1g Zn/day are typically administered as an oral drench or as an intraruminal controlled release capsule. Some studies have shown that excess dietary Zn can reduce Cu absorption and decrease the Cu concentrations in plasma and liver, but reports describing this Cu-Zn antagonism are often inconsistent, indicating variable effects in a range of animal species (Bremner *et al*., 1976; O'Dell, 1989). To date, most studies have been relatively short term, and the long term effects of Cu-Zn interaction in ruminants are not well characterised. The mechanism for the interaction is thought to be mediated through Zn induction of MT, a high cysteine-containing protein which binds both Cu and Zn (Bremner, 1991; Lee *et al*., 1994a,b). Direct toxicity of Zn at high doses to ruminants has been reported (Allworth *et al*., 1985; Allen *et al*., 1986).

Previously, we have described the effect of high and sustained dietary ZnO supplements on the elemental composition of wool and fleece characteristics (Grace and Lee, 1992), on trace element metabolism (Lee *et al*., 1991), and on the expression of MT mRNA and protein (Lee *et al*., 1994a,b). We have also documented reduced Cd accumulation in the liver and kidney tissues of ZnO treated sheep (Lee *et al*., 1996a). In this report we present new data on the effect of short and long term treatment of Romney wethers and lambs with two forms of ZnO on Cu concentrations in liver, kidney and muscle tissue.

MATERIALS AND METHODS

Experimental protocols, animal management, and tissue sampling procedures for ZnO drenching trials have been described in detail previously (Grace and Lee, 1992; Lee *et al*., 1994a). Briefly, two groups of 15 Romney wethers matched for age and weight (14 months old, 55 kg) received for at least 52 weeks either a weekly oral dose of 12 g ZnO packed into gelatine capsules or no treatment. The sheep were grazed together on ryegrass/white clover pasture. Effectiveness of the ZnO treatment was monitored by measuring plasma Zn concentration (Grace and Lee, 1992).

In the second experiment two groups of 24 Romney lambs (six months old, weighing approximately 23 kg) were distributed among replicated plots of ryegrass/clover pasture. Treated lambs received two intraruminal boluses (Time Capsule™, AgResearch), each containing 50 g ZnO and considered effective for about 42 days, which were administered successively; one at the start of the trial and a second 40 days later. Half of the animals in the treated and control groups were slaughtered approximately 40 days after start and the remainder at 80 days. This trial was repeated 12 months later. During the first year, particularly dry conditions led to poor animal growth rates and no weight gain over the experimental period. During the second year growth rates were greater than 100g/day for the 80-day experimental period (Rounce, Lee and Grace, unpublished data).

Samples of animal tissue (20-30 g FW) were homogenised using a stainless steel Sorvall omni-mixer (Sorval Inc., USA), freeze-dried in polyethylene containers, ground to a fine mesh using a coffee grinder, and analysed for elemental content by inductively coupled plasma emission spectrometry (ICP-ES) under operating conditions described by Lee *et al*. (1994). Accuracy of the determinations were assessed by analysing in parallel with the unknown samples a certified reference material (U.S. National Bureau of Standards bovine liver reference, NBS 1577a). Metallothionein was measured in liver tissue via HPLC to assist in identifying regulatory aspects of Cu and Zn accumulation in the liver (Lee *et al*., 1994a).

Data were subjected to multivariate analysis of variance for unbalanced data using the GLM procedure of Minitab (version 11.21, Minitab Inc.), with means tested for differences by treatment (Control v +ZnO), time (40 days v 80 days) and year (1994 v 1995). Main, first and second order interactions were tested. Except where otherwise noted, level of probability is P - 0.05. Values are reported as mean ± standard deviation.

RESULTS AND DISCUSSION

The effects of weekly drenching of sheep with ZnO on Cu and Zn concentrations in liver, kidney and muscle are presented in Table 1. Concentration of Cu in the liver of treated animals was greater than controls, but this increase was not significant (P>0.05). Measured values showed considerable variation around the mean (CV 45%). Drenching also increased Cu and Zn concentrations in kidney (P<0.05). Weekly dosing with ZnO for 52 weeks did not affect Cu or Zn concentrations in samples of muscle tissue (from the hind leg). Note that we have previously shown that plasma concentrations of Cu and elements other than Zn are not changed by ZnO administration (Lee *et al*., 1991).

TABLE 1: Effect of sustained oral drenching with ZnO (12 g ZnO/week) for one year on concentrations (mean ± SD, n=15) of Cu and Zn in liver, kidney and muscle, and on metallothionein protein in liver, in grazing Romney sheep.

	Control (µmol Cu/kg FW)	+ 12 g ZnO/week	P value
Liver			
Cu	1666±867	1934±884	NS
Zn	497±102	1237±646	P<0.05
Metallothionein (n=7)	10.5±5.7	92±108	P<0.001
Cu, Zn bound to MT (n=7)			
Σ Cu, Zn	157	1373	
MT-(Cu, Zn) as % of total	7.7	40	
Kidney			
Cu	61±34	110±95	P<0.05
Zn	412±51	1938±1190	P<0.05
Muscle			
Cu	27±26	45±49	NS
Zn	319±68	378±64	NS

Increased dietary Zn resulted in a marked enhancement of liver MT, which may have bound Cu as well as Zn (Table 1). The amount of Cu and Zn bound to MT increased from 7.7% of total liver Cu and Zn in the control group to more than 40 % in the treatment group. The exact proportions of Cu and Zn among the seven metal binding sites of MT could not be measured, but we have previously shown that induction of MT in individual animals is highly variable and that MT isoforms can be identified with differing stoichiometries of Cu and Zn (Lee *et al*., 1994a). Liver MT synthesis would explain in part the observed retention of Cu in the liver, and this could have masked any inhibitory effect of Zn on Cu absorption from the gut. Isotope tracer experiments would be required to distinguish these effects.

Relatively short term administration of ZnO to 6-month old lambs using a intraruminal controlled release bolus altered Cu and Zn concentrations in liver and kidney. As there were no significant differences in tissue Cu and Zn concentrations between days 40 and 80, and between years (1994 v 1995), except for Cu in liver and Zn in muscle, the tissue Cu and Zn data summarised in Table 2 are pooled for control and treated animals. After 40 days, Zn bolus treatment significantly reduced liver Cu content compared with controls. That decrease was more marked in the second year trial than in the first year trial (-32% and -25%, respectively). This response may have been caused by higher DM intakes, and therefore a greater amount of Cu absorbed by animals in the second trial. Liver Cu concentration was reduced another 20% by a successive ZnO bolus, when measured after a further 40 days (P<0.07, n=11). However this decrease was observed only in the second year. Concentration of Cu in liver of 40-day v 80-day animals was unchanged in the first year experiment. Treatment significantly raised the Zn concentration in liver, as well as Zn and Cu concentrations in kidney, and this was not affected by dosing frequency or trial year. As in the ZnO drenching experiment, the high kidney Zn concentrations observed here would likely have induced MT synthesis, and this MT could have bound and retained substantial Cu.

TABLE 2: Effect of ZnO bolus (Time Capsule™) treatment, over 40 or 80 days, on concentrations of Cu and Zn in liver, kidney and muscle tissue of grazing Romney lambs repeated within the same time period in two separate years. Data are the covariate adjusted means (±SD) from the ANOVA using year as the covariate.

			Significance of effects		
	Control	ZnO bolus (µmol/kg FW)	Treat -ment	40 v 80 d	Year
Liver					
Cu	1426±56 (46)[1]	959±54 (49)	<0.001	(0.1)	<0.001
Zn	620±108 (45)	1356±105 (48)	<0.001	NS	NS
Kidney					
Cu	42.5±4.9 (46)	68.1±5.1 (43)	<0.001	NS	NS
Zn	359±126 (46)	1167±130 (43)	<0.001	NS	NS
Muscle					
Cu	17.1±0.80 (31)	16.1±0.76 (34)	NS	- [2]	NS
Zn	446±19 (31)	445±18 (34)	NS	-	<0.001

[1] overall number per treatment group

[2] Muscle not sampled for the 80 d period

The two experiments differed by route of ZnO administration, duration, season and age of the sheep. Mean concentrations of Cu and Zn in liver and kidney of controls were similar in the experiments, but animal-to-animal variation was considerably greater during the drenching experiment. Age may also account for the differences in accumulation of Cu in hind leg muscle tissue, which in 24 month old sheep was nearly twice that measured in the lambs. The two experiments were independent of each other and therefore it was not possible to directly compare the route of administration of the ZnO (slow release and drench) for animals of similar age. While it is recognised that absorption, retention and possibly the metabolism of Cu in the lambs and mature animals could differ, it is likely that the main differences in the effects of Zn on Cu between the two experiments arose from the much longer duration of the Zn administration in the drench experiment.

CONCLUSIONS

Zinc oxide preventative treatment for facial eczema administered as either an oral drench or as an intraruminal controlled release capsule changed the Cu status of grazing Romney sheep. Response varied with duration of treatment (52 weeks or 80 days) and with age of the animals (14 months or six months). Regardless of treatment, liver Cu concentrations of all animals observed in this study were well above the lower Cu concentration (95µmole Cu/kg FW) recognised as indicating an inadequate Cu reserve for sheep (Lee and Grace, 1997). The effects of increased dietary Zn on Cu concentration in liver (and in kidney) are partly related to the rate and extent of MT induction and initial Cu pool size in the tissue. The duration of the Zn dose, age of animal, level of DM intake, and grazing conditions are also probable influences. Treatment with ZnO may increase the risk of Cu deficiency in animals which already have limited Cu reserves, as might occur when Cu absorption has been suppressed by high dietary intakes of molybdenum. Under most conditions, however, the benefits of ZnO as an effective facial eczema control are likely to outweigh any short term reduction in liver Cu.

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