The effect of zinc deficiency on wool growth and skin and wool follicle histology of male Merino lambs

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The aims of this work were to quantify the requirements of Zn for wool growth in growing male Merino lambs, and to describe the histological lesions of Zn deficiency in skin and wool follicles. Four groups of male Merino lambs (*n* 4) weighing 22 kg were fed *ad lib.* for 96 d on diets that contained 4 (basal diet), 10, 17 or 27 mg Zn/kg. Sheep in a fifth group were fed on the diet containing 27 mg Zn/kg, but were pair-fed to sheep on the 4 mg Zn/kg diet. Zn was added to the basal diet as $ZnSO_4$ to give the respective treatment concentrations. Sheep fed on the diet containing 4 mg Zn/kg showed clinical signs of Zn deficiency and lower feed intakes and wool growth than sheep in the other groups. Their wool fibres were improperly keratinized and the wool follicles contained a higher proportion of apoptotic bodies than other groups. There was no evidence of parakeratosis and the rate of bulb-cell production was not affected. Sheep from other groups showed no clinical signs of Zn deficiency, and mean feed intakes and growth rates did not differ significantly between sheep fed on diets containing 10, 17 or 27 mg Zn/kg. However, wool growth was reduced in sheep fed on the diet containing 10 mg Zn/kg compared with those fed on diets containing 17 or 27 mg/kg. The mean concentration of Zn in the plasma at which wool growth was 90% of maximum was 0·5 mg/l. The equivalent value for the diet was 12 mg/kg, with 95% confidence intervals of 8 to 16 mg/kg. The results suggest that Zn deficiency reduces wool growth through a specific mechanism, perhaps involving impaired protein synthesis.

Zinc: Wool: Skin: Sheep

Immature Merino wethers grazing spring pastures have a net Zn requirement of approximately 8 mg/d (Standing Committee on Agriculture, 1990). Of this, between 20 and 30% is incorporated into wool, assuming an average Zn concentration in clean wool of 110 mg/kg and a clean wool growth rate of 20 g/d (Standing Committee on Agriculture, 1990). This deposition in wool represents a significant irreversible loss of Zn available for body tissues.

A severe dietary Zn deficiency (less than 3 mg Zn/kg diet) causes cessation of wool growth in sheep (Ott *et al.* 1964, 1965; Mills *et al.* 1967; Underwood & Somers, 1969; Masters *et al.* 1985), yet the minimum requirement of Zn for wool growth has not been established. Masters (1984) showed that Merino wethers fed on a diet containing 8·8 mg Zn/kg grew less wool than sheep fed on diets containing 26·5 mg Zn/kg. In contrast, Lush & Hynd (1988) were unable to show any differences in wool growth between Merino wethers fed diets containing 10 or 27 mg Zn/kg.

It is of practical importance to know how much Zn is required for normal wool

production because dry summer pastures grazed by sheep in parts of Australia contain low concentrations of Zn (below 20 mg/kg) for several months (Masters & Somers, 1980; White *et al.* 1991). Reproductive and growth responses to Zn supplements have been reported in sheep under natural grazing conditions (Egan, 1972; Masters & Fels, 1980), indicating that low concentrations of Zn in pasture can restrict animal productivity.

The aims of this work were to quantify the requirements of Zn for wool growth in growing Merino ram lambs, and to describe the histological lesions of Zn deficiency in skin and wool follicles. The effects of Zn deficiency on reproductive endocrinology and testicular growth have been reported elsewhere (Martin & White, 1992).

MATERIALS AND METHODS
Animals and treatments

Twenty 16-week-old male Merino lambs, weighing on average 22 kg at the start of treatment, were allocated to five treatment groups each of four sheep. The first four treatments consisted of feeding *ad lib.* on diets ranging in Zn concentration from deficient to adequate: 4 (deficient), 10, 17 and 27 mg/kg (*ad lib.* control). The fifth treatment (pair-fed control) consisted of feeding each of the sheep on an amount of the 27 mg Zn/kg diet equal to that eaten by its Zn-deficient pair (4 mg Zn/kg) the previous day. This technique offered the means of controlling for effects of reduced feed intake in the deficient sheep. The composition of the basal diet is shown in Table 1. $ZnSO_4.7H_2O$ was added to the basal diet at 26·5, 57·5 and 115 mg/kg to give the desired treatment concentrations of Zn. The experimental period lasted 96 d and was preceded by a 3-week adjustment period when all sheep were given the control diet containing 27 mg Zn/kg.

Sheep were kept in individual pens made of plastic-coated iron railings (not galvanized) and with slatted wooden floors. Feed bins were made of fibreglass and plastic, and the deionized watering nipples of stainless steel. The experiment was carried out under the Code of Practice for the Care and Use of Animals for Experimental Purposes (National Health and Medical Research Council, 1985).

Wool sampling and analysis

A 100 cm² area of skin on the right mid-side of the sheep was marked with an indelible pen on day 5 of treatment and closely clipped using model A5 Oster small-animal clippers fitted with size 40 cutters. Wool was harvested from the patch at subsequent 14-d intervals. Wool samples were equilibrated to 12% moisture and weighed. Samples from day 89 were analysed for wax, suint and yield.

Wool samples for Zn and Cu analysis were cleaned using a modified method of Hemsley & Marshall (1983). Samples of approximately 0·8 g were weighed and placed into weighed glass syringes, dried for 30 min in a 90° chamber containing N_2, and the syringe plus wool was then reweighed. Wax was extracted using five 30 s washes in 2 ml Shell X2 solvent/g wool. Excess solvent was removed under compressed air and the wool and syringe were dried under N_2 at 90° for 30 min and reweighed to give a measurement of wax content. Suint was then extracted by washing the wool twice with 30 ml distilled water at 60°. The syringe plus wool was dried overnight in a 90° oven, cooled in a desiccator and weighed before digestion in acid and analysis for Zn and Cu.

Measurement of mitotic rate

Colchicine-treated skin biopsy samples were taken on day 83 of treatment from the upper mid-side of the sheep using the method of Hynd *et al.* (1986). Bulbs (300 to 600/sample) were scored for mitotic figures for each sample. All bulbs present in a section were counted.

Table 1. *Composition (g/kg) of the basal diet*

Ingredient	
Oat straw*	400
Wheat starch	250
Sucrose	150
Spray-dried egg white	75
Vegetable oil (mixed, soya-bean based)	20
Urea*	20
Vitamins A and vitamin D_3 (150 mg/g and 2·5 mg/g respectively: Rovimix)	0·01
Vitamin E (500 mg α-tocopherol/g; Rovimix)	0·1
Tetrapotassium pyrophosphate*	30
$CaCO_3$*	25·9
$CaSO_4$ (Gypsum)*	6·2
$MgCO_3$ (light)*	2·15
KOH*	11·9
NaOH*	8·4
Trace element premix†	0·265

* The oat straw was treated with alkali, macro-elements and urea to make alkali-treated straw. To each 100 kg dry straw was added a solution containing 13·3 l distilled water, 2·86 kg KOH and 2·0 kg NaOH. The macro-minerals and urea were then added and the 'alkalage' allowed to sit in bags for at least 2 weeks before mixing with the remaining dietary ingredients. The basal diet contained (by analysis, mg/kg DM (SE)): Zn 3·9 (1), Cu 7·0 (1·4), Fe 290, Mo 1·7 (0·2). The untreated straw contained (g/kg): S 0·8, N 5·1, Zn 3·3 mg.

† The trace-element premix contained (g/kg): $Fe_2(SO_4)_3.9H_2O$ 568, $MnSO_4.H_2O$ 230, $CuSO_4.5H_2O$ 73·3, $Na_2MoO_4.2H_2O$ 19·0, $CoCl_2.6H_2O$ 1·51, Na_2SeO_3 2·49, KI 0·49, $Na_2HAsO_4.7H_2O$ 1·55, NH_4VO_3 0·86, $Na_2Cr_2O_7.2H_2O$ 1·09, $Na_2B_4O_7.10H_2O$ 100, $NiSO_4.6H_2O$ 1·70. For each 470 kg (dry matter) batch of feed, 124 g of the trace-element premix was dissolved in 500 ml concentrated HCl and made up to 5 l in distilled water. The solution was left to stand overnight and added to the feed during mixing.

The data represent the mean number of cells undergoing mitosis in the 2 h period of colchicine arrest in the average follicle bulb section.

Histological examination of skin

Skin samples were taken from the upper mid-side region with a 10 mm diameter trephine following subcutaneous administration of 0·5 ml local anaesthetic (xylocaine) on days 0, 26, 54 and 83 of treatment. The samples were fixed in buffered formalin (3·97 g KH_2PO_4 and 7·97 g K_2HPO_4/100 ml formalin (400 ml HCHO/l) made to 1 l in distilled water) embedded in paraffin, sectioned at 8 μm thickness and stained with haematoxylin, eosin and picric acid (Carter & Clarke, 1957).

Trace element determination

The Zn and Cu concentrations of feed and wool were determined using atomic absorption spectroscopy (AAS) on 0·5 g samples digested in a mixture of 7 ml HNO_3 (16 mol/l), 0·5 ml H_2SO_4 (18 mol/l) and 0·5 ml $HClO_4$ (12 mol/l). Concentrations of metal are expressed on a dry matter basis. Plasma was treated with 10 % trichloroacetic acid (w/v), centrifuged for 15 min at 1500 g and the supernatant fraction analysed for Zn using AAS.

Statistical methods

Data were analysed by analysis of variance using the micro-computer program Systat (Systat Inc., Evanston, IL, USA). Repeated measures analysis of variance was performed where appropriate. Between-treatment comparisons were made using least significant

differences (LSD; Steel & Torrie, 1980). Non-linear Mitscherlich equations were fitted to mean values for the relationship between dietary and plasma Zn concentrations and wool growth.

RESULTS

Wool growth, live weight gain and feed intake

Wool growth was rapidly and severely reduced in sheep fed on the 4 mg Zn/kg diet, and marginally reduced in those fed on the 10 mg Zn/kg diet compared with those fed *ad lib.* on the 17 or 27 mg Zn/kg diets (Fig. 1). Repeated measures analysis of variance showed significant effects of treatment ($P < 0.001$) and time ($P < 0.001$) and a significant time × treatment interaction ($P < 0.001$). Sheep given the 10 mg Zn/kg diet grew significantly less wool than those given the 17 mg Zn/kg diet, but only on days 61 and 75 of sampling ($P < 0.05$). Pair-fed control sheep grew significantly more wool than Zn-deficient sheep at all times after the first sampling at day 19, but less wool than sheep given the 17 or 27 mg Zn/kg diet *ad lib.* ($P < 0.05$). Pair-fed sheep grew less wool than those given 10 mg Zn/kg diet, but only on sampling days 75 and 89 ($P < 0.05$).

Body growth virtually ceased in both Zn-deficient and pair-fed sheep (Fig. 1), and growth rate for sheep in these two groups was significantly lower than for other groups (repeated measures analysis of variance, $P < 0.001$). However, growth was not significantly different between sheep fed *ad lib.* on diets containing 10, 17 or 27 mg Zn/kg (Fig. 1). Daily feed intakes for the five treatment groups were (mean (SE), g); 693 (72), 1316 (59), 1411 (51), 1532 (97) and 703 (67) respectively. Only the Zn-deficient and pair-fed groups had significantly reduced feed intakes and there was no significant difference between intakes for sheep fed *ad lib.* on the 10, 17 or 27 mg Zn/kg diets.

Clean wool growth on day 89 of treatment was reduced only when dietary Zn concentration fell below 17 mg/kg, whereas plasma Zn concentration was reduced at all concentrations of dietary Zn below 27 mg/kg (Fig. 2). Pair-fed sheep had mean plasma Zn concentrations that were not significantly different from *ad lib.*-fed sheep on diets containing 17 or 27 mg Zn/kg.

Estimates of the concentrations of Zn in the diet at which wool growth was 90% of maximum (90% critical value; Ulrich, 1952) were obtained by fitting a Mitscherlich curve (Fig. 2). The critical value for wool growth was 12 mg Zn/kg in the diet, corresponding to 0.5 mg Zn/l in plasma.

Wax and suint

Zn deficiency increased the concentration of wax ($P = 0.2$) and suint ($P < 0.01$) in wool, and consequently decreased the yield ($P < 0.01$, Fig. 3). Composition and yield of wool from pair-fed control sheep were the same as from sheep fed *ad lib.* on the control diet. There were no significant differences in wax, suint or yield between the sheep fed on diets containing 10, 17 or 27 mg Zn/kg.

Mitotic rate

There was only a slight non-significant reduction in the mean number of mitoses per bulb section in the deficient sheep compared with other groups (ANOVA, $P > 0.05$; Fig. 4). There was no significant linear relationship between mitotic rate and clean wool growth (r^2 0.21, n 20, $P > 0.05$).

Although mitotic rate was not decreased by Zn deficiency, there was a change in the pattern of mitosis within the bulb brought about by a change in bulb shape. The bulbs of Zn-deficient sheep were more elongated and narrower, with metaphase nuclei apparent a large distance from the base of the bulb. It appeared that the germinative tissue of the bulb had been drawn upwards and inwards.
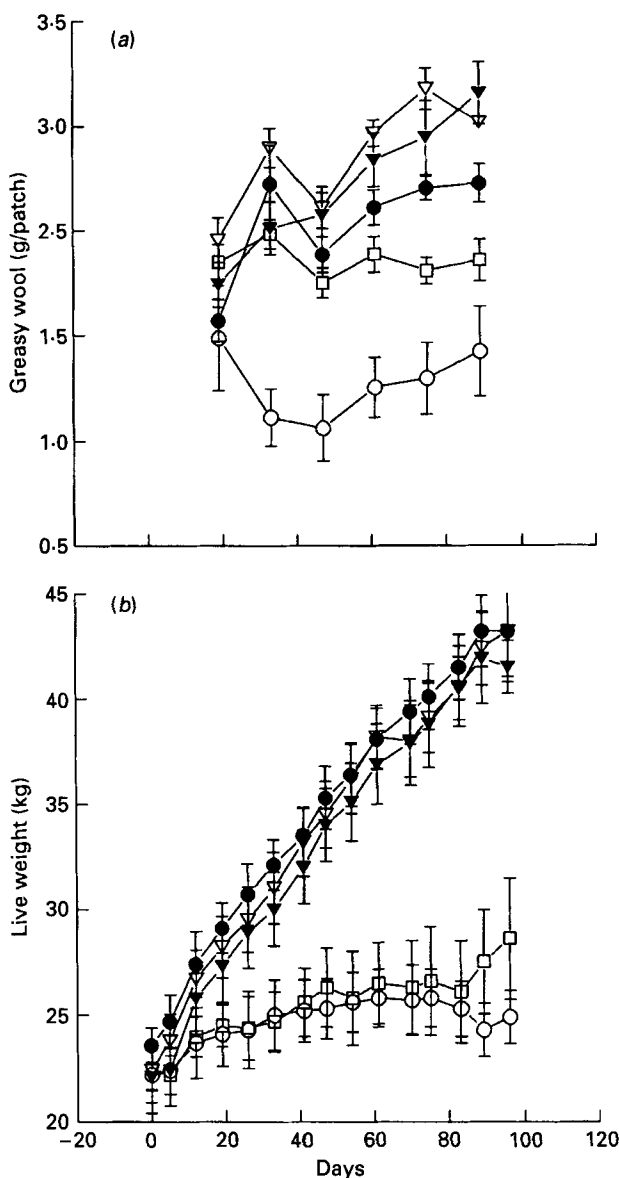
Fig. 1. The effect of dietary Zn on wool growth (a) and live-weight gain (b). Values are means with their standard errors for four sheep per group. Symbols represent dietary Zn treatments: 4 ○-○, 10 ●-●, 17 ▽-▽, 27 (ad lib.) ▼-▼ and 27 (pair-fed) mg Zn/kg □-□.

Skin histology

Skin samples only from the Zn-deficient sheep showed evidence of histopathological changes. In the samples taken on days 26, 54 and 83 there were more apoptotic bodies (Kerr et al. 1972) in the 4 mg Zn/kg group than other groups (Fig. 5a).

Wool fibres from Zn-deficient sheep were improperly keratinized as indicated by retained cell nuclei in the fibres (Fig. 5b). The partial keratinization also appeared to have proceeded more slowly in the samples taken on day 83 than at earlier times, as judged by longer
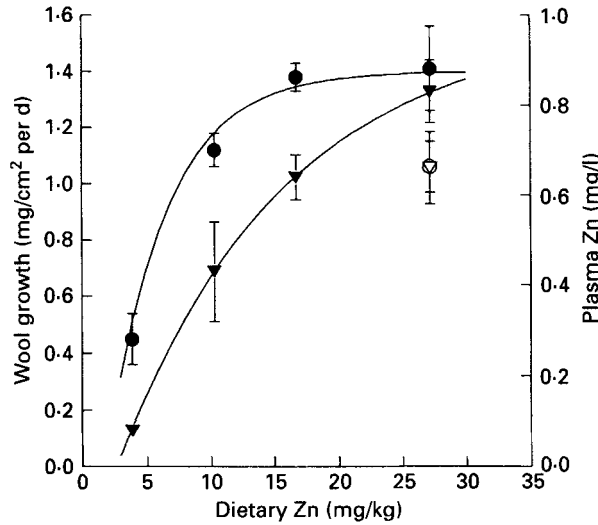
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Fig. 2. The effect of dietary Zn concentration on clean wool growth (●–●) between days 75 and 89 of treatment, and concentration of Zn in plasma (▼–▼) at day 89 of sampling. Open symbols represent values for the pair-fed control group. Values are means with their standard errors for four sheep per group. The fitted curves are described by the Mitscherlich equations $Y = 1.4 - 2.1 \exp(-0.22X)$ (r^2 0.99) for wool growth and $Y = 0.98 - 1.2 \exp(-0.076X)$ (r^2 0.99) for plasma Zn.
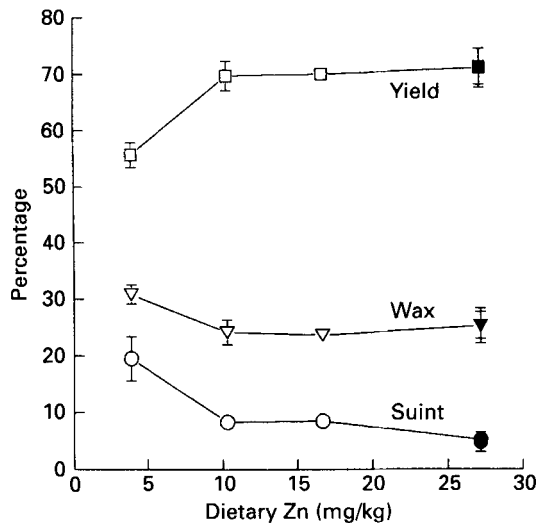

Fig. 3. The effect of dietary Zn concentration on yield (□–□), wax (▽–▽) and suint (○–○) of wool grown between days 75 and 89 of treatment. Closed symbols represent values for the pair-fed control group. Values are means with their standard errors for four sheep per group.

keratogenous zones in those follicles. A small percentage (< 5%) of fibres were distorted in the upper parts of the follicles and degraded within thickened outer root sheaths (Fig. 5c). This small percentage would account for the lack of any break in the wool of the Zn-deficient group. There was no obvious sign of impaired keratinization of fibres in the control groups consuming 27 mg Zn/kg in spite of the low Cu concentrations in the wool of this group (Fig. 6).
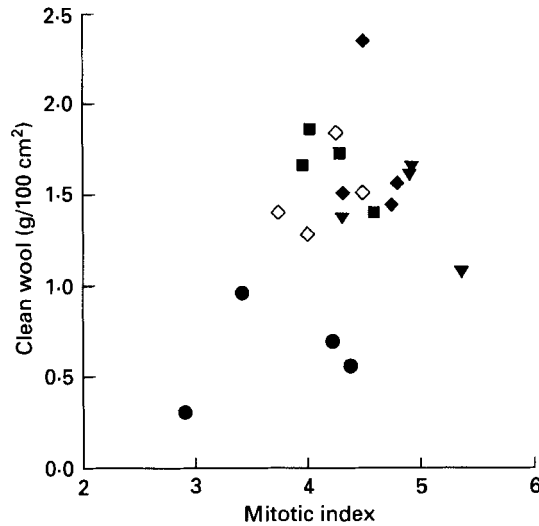
Fig. 4. The relationship between mitotic rate on day 83 and clean wool growth between days 75 and 89 of treatment. There was no significant linear relationship ($P > 0.05$). Each value represents a single sheep. Symbols represent treatments as follows: 4 ●, 10 ▼, 17 ■, 27 (*ad lib.*) ◆, and 27 (pair-fed) mg Zn/kg ◇.
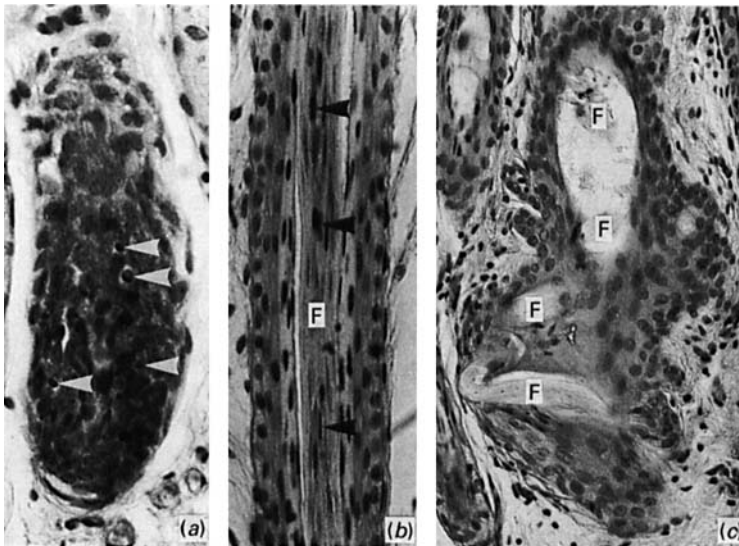

Fig. 5. Abnormal features present from day 26 onwards in skin samples from sheep fed on a Zn-deficient diet: (*a*) Apoptotic bodies (arrowheads) in follicle bulb. (*b*) Retained cell nuclei (arrowheads) in an improperly keratinized wool fibre (F). (*c*) A distorted and partly degraded wool fibre (F) in the distal part of a follicle. For details of procedures, see p. 427.

More follicles remained immature longer in the Zn-deficient and 10 mg Zn/kg diet groups; 9% compared with 4% in other groups at day 83. Histological examination of cortical cells from Zn-deficient and pair-fed sheep showed that Zn deficiency caused a significant reduction in cell width (4·9 (SE 0·1) *v.* 5·6 (SE 0·2) μm; $P < 0.05$) and cell volume (546 (SE 39) *v.* 775 (SE 48) μm^3; $P < 0.01$) with no significant change in cell length
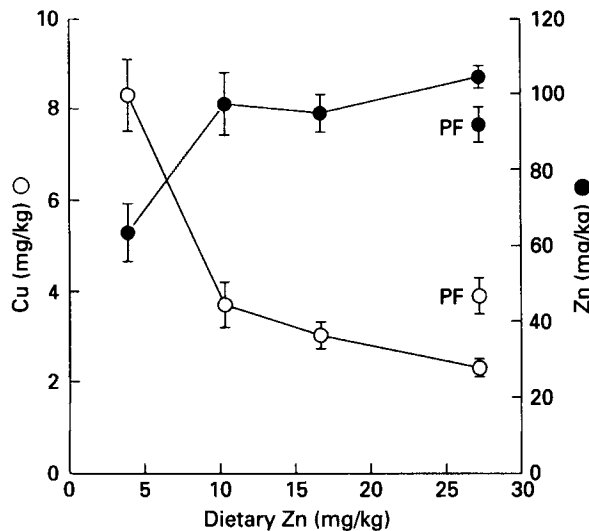
Fig. 6. The effect of dietary Zn concentration on the Zn (●–●) and Cu (○–○) concentrations in clean wool on day 89 of treatment. PF signifies values for the pair-fed control treatment. Values are means with their standard errors for four sheep per group. For details of treatments and procedures, see pp. 426–427.

(77 (SE 5) v. 86 (SE 2) μm; $P < 0.157$). The only other detected effect of Zn deficiency in the Zn-deficient group was a slight tendency towards more *stratum corneum* on the epidermis in samples taken on day 83. However, the epidermis could not be regarded as hyperkeratotic and there was no sign of parakeratosis in any of the samples.

The sizes of the sebaceous and sweat glands bore no relation to the amount of Zn ingested. Apart from slightly retarded maturation of follicles in the 10 mg Zn/kg diet group, there were no histological differences between the 10 mg Zn/kg diet and the 17 and 27 mg Zn/kg diet groups.

Zinc and copper contents of wool

The concentration of Zn in wool was only significantly reduced in the Zn-deficient group compared with other groups ($P < 0.01$; Fig. 6). However, the concentration of Cu in wool showed a progressive decline with increasing Zn concentration in the diet ($P < 0.01$). Pair-fed sheep receiving the 27 mg Zn/kg diet had a higher mean concentration of Cu than *ad lib.*-fed control sheep ($P < 0.05$) and this value was similar to that for the 10 and 17 mg Zn/kg groups.

DISCUSSION

Using the concept of critical value as described by Ulrich (1952), the results show that the concentrations of Zn in the diet and plasma at which wool growth was 90% of maximum were 12 mg/kg and 0.5 mg/l for a mean rate of wool growth of 1.4 mg/cm² per d. If the coefficient of variation of Zn requirements is the same as that for voluntary feed intake (7–16%; Minson, 1990) then the population range (mean ± 2 SD) over which Zn intakes can be considered marginal for wool growth in Merino sheep extends from approximately 8 to 16 mg/kg for the diet and 0.34 to 0.66 mg/l for plasma.

A critical value represents a minimum concentration of a nutrient under conditions where other nutrients are non-limiting. The failure of Lush & Hynd (1988) to observe a reduction in wool growth in Merino wethers fed on diets containing 10 mg Zn/kg,

compared with those of 27 mg/kg diet and above, could be explained by the fact that the rate of wool growth in their Zn-adequate sheep was low (0.7 mg/cm^2 per d). Evidence to support the effects of diet quality on Zn requirements for wool growth comes from the work of Masters (1984), who showed that increasing dietary Zn from 4.8 to 26.5 mg/kg only increased wool growth when dietary N concentration was increased from 10 to 30 g/kg, causing wool growth rate to increase from 0.74 to 1.3 mg/cm^2 per d. Under conditions where wool growth is below the genetic potential the concentration of Zn in plasma may prove to be a more useful indicator of the adequacy of Zn supply than dietary Zn concentration. Thus, a plasma Zn concentration below 0.5 mg/l may indicate an inadequate supply of Zn for wool growth, even when feed quality and wool growth rate are low.

The maximum rate of wool growth of 1.4 mg/cm^2 per d in the current experiment corresponds to a total body production of 15.4 g clean wool/d (Bennett, 1973), an amount below the maximum rate of about 20 g/d reported for Merinos at pasture (Hogan *et al.* 1979). There is no appreciable photoperiod effect on wool growth in Merinos (Nagorcka, 1979) and the seasonal variation in clean wool growth of about 4 to 20 g/d is determined largely by diet quality. It is, therefore, possible that the higher wool growth in sheep at pasture compared with that in this experiment would necessitate a mean requirement of dietary Zn concentration of 16 mg/kg if Zn requirement is linearly proportional to wool growth. It is considered likely that these results can be applied to sheep grazing natural herbage because tracer studies with ruminants have shown that the absorption and retention of inorganic forms of Zn are largely indistinguishable from intrinsically labelled plant material (Neathery *et al.* 1972; Bedi & Chesters, 1982) and Zn from forage diets is highly available to sheep (Suttle *et al.* 1982).

Histological lesions in the skin and wool follicle of sheep fed on the diet containing 4 mg Zn/kg were similar to, but less extensive than, those reported in pre-ruminant lambs fed on a milk diet containing 3 mg Zn/kg dry matter (Masters *et al.* 1985). In the current experiment skin from the Zn-deficient sheep contained apoptotic bodies in follicle bulbs, impaired keratinization of fibres with retained cell nuclei and fibre distortion in the distal parts of some follicles.

The finding that Zn deficiency had little effect on mitotic activity per follicle bulb is surprising in the light of evidence that Zn is required for several enzymes controlling events during cell division and gene expression (Vallee & Falchuk, 1981). A decrease in wool output per unit area of skin with no change in the rate of wool production per bulb would require a decrease in follicle density or a decrease in cortical cell size or both. The 30% reduction in cortical cell size of Zn-deficient sheep compared with pair-fed sheep supports the contention that the keratinization (protein synthetic) process is more sensitive to reduced Zn supply than is cell division.

Defective keratinization is not specific to Zn deficiency and occurs in sheep fed on whole grain supplemented with methionine (Chapman & Reis, 1978), in preruminant folate-deficient lambs (Chapman, 1989) and in lambs fed on diets deficient in lysine (Chapman *et al.* 1983). Whether Zn is required for the structural integrity of the wool keratin proteins or whether it is required as a component of an enzyme for wool protein synthesis cannot be determined from this study. The Zn content of wool did not reflect Zn intake since there was no correlation between the concentration of Zn in the wool and the concentrations of Zn in either the diet or plasma when levels of dietary Zn were between 10 and 27 mg/kg. Only when Zn intakes became sufficiently low to cause major histological changes to the wool fibre did the concentration of Zn in wool decline. The fact that Cu concentration was increased significantly in wool only from sheep fed on the diet containing 4 mg Zn/kg further indicates a major physicochemical change in the wool fibre brought about by Zn deficiency.

In summary, the results show that for a wool growth rate of 1·4 mg/cm² per d the 90 % critical concentration of dietary Zn for wool growth was about 12 mg/kg. The equivalent concentration in plasma was 0·5 mg/l. A diet of 10 mg Zn/kg was adequate to prevent clinical signs of Zn deficiency but not to maintain a maximum rate of wool growth. A diet of 4 mg Zn/kg produced clinical signs of Zn deficiency and markedly reduced the rate of wool growth. This reduction was associated with an impaired keratinization of the wool fibre that was not due to a reduced rate of mitosis in the bulb cells. The results suggest that Zn deficiency reduces wool growth through a specific mechanism, perhaps involving protein synthesis. It was not due simply to a generalized effect of Zn deficiency on appetite or rate of cell division.

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