Effect of zinc deficiency on the pregnant ewe and developing foetus

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1. Mature Merino ewes were given either a low-zinc diet (4 mg/kg) or an adequate-Zn diet (50 mg/kg) for all or part of pregnancy.

2. The ewes consuming the low-Zn diet consumed 25% less feed than those given the adequate-Zn diet during the last 115 d of pregnancy.

3. Zn concentration in the plasma of Zn-deficient pregnant ewes declined from 0·7 to 0·3 mg/l.

4. The lambs born to Zn-deficient ewes weighed less and had reduced concentrations of Zn or less total Zn, or both, in the whole carcass, liver and pancreas.

5. A reduction in activity of alkaline phosphatase (*EC* 3.1.3.1) in the liver and a slight reduction in thymidine kinase (*EC* 2.7.1.21) activity in the thymus was also observed in Zn-deficient lambs.

6. The Zn-deficient ewes deposited approximately 63 mg Zn into each single-born lamb; this indicates that during the last third of pregnancy the developing foetuses were accumulating the equivalent of 35% of the total dietary Zn intake of the ewes.

Severe zinc deficiency affects reproduction in many animal species (see Underwood, 1977). In pregnant rats foetal death, congenital malformations, dystocia, reduced litter size and poor post-natal survival result (Hurley & Swenerton, 1966; Apgar, 1968; Williams *et al.* 1973). Little information is available on the Zn requirements of the pregnant ruminant. Masters (1981) reported that a marginal Zn intake (9 mg Zn/kg) throughout pregnancy in ewes had no effect on the maintenance of pregnancy or birth weights of lambs, although it resulted in large decreases in Zn concentrations in certain lamb tissues. Apgar and co-workers (Apgar & Travis, 1979; Apgar *et al.* 1981) induced Zn deficiency in pregnant ewes during the last third of pregnancy. Even though the deficient ewes showed a rapid deterioration in condition the number and weight of lambs born were not affected.

The pregnant ewe has an increased requirement for Zn towards the end of pregnancy. The developing foetus accumulates 1·0–2·0 mg Zn/d near term (Hansard & Mohammed 1968; Williams *et al.* 1978). If the increased Zn requirement during pregnancy must come directly from recently consumed feed and none is contributed from body stores or tissues as occurs in the rat (Hurley & Swenerton, 1971), then these demands are unlikely to be met when ewes are consuming a marginal or low-Zn diet. Two previous reports have shown that pregnancy results in depletion of Zn from grazing ewes and that Zn supplements may increase the number of lambs produced (Egan, 1972; Masters & Fels, 1980).

The experiment outlined here investigates the effect of Zn deficiency on mating and pregnancy in the ewe, development of the lamb and accumulation and distribution of Zn in lamb tissues.

EXPERIMENTAL

Animals and treatments

Forty-six mature-age Merino ewes were allocated according to body-weight and plasma Zn concentrations into two groups of twenty-three. One group was given a Zn-deficient diet containing 4 mg Zn/kg dry diet (−Zn, see Table 1); this concentration was determined by

Table 1. *Dry matter composition of basal diet (g/kg)*

Hammer-milled wheat straw	480	NaCl	10
Wheat starch	300	$CaCO_3$	10
Sodium caseinate	44	$Ca(H_2PO_4).H_2O$	25
Urea	20	$MgSO_4.7H_2O$	8
Sucrose	100	KCl	2·3
		Trace elements*	0·7

* Composition of trace element mix (g/kg): Fe_2O_3 50·0, $CuSO_4.5H_2O$ 41·5, $MnSO_4.4H_2O$ 58·0, KI 1·2, $CoCl_2.6H_2O$ 5·0, Na_2SO_4 836·0, selenious acid (H_2SeO_3) 0·4, $Na_2MoO_4.2H_2O$ 7·9.

analysis. The other group received the deficient diet supplemented with zinc sulphate to contain approximately 50 mg Zn/kg (+Zn); this is considered adequate for growth and maintenance of plasma Zn levels (Mills *et al.* 1967). For the first 35 d the ewes were given up to 0·95 kg/d, after this they were fed *ad lib.* The sheep were maintained in two groups in concrete floored pens lined with pressed hardboard; pen floors were washed twice daily. Deionized water was provided *ad lib.* in stainless-steel troughs and feed in plastic-coated troughs. Every 6 weeks the ewes were dosed orally with 75 mg retinol, 3·1 mg cholecalciferol and 87·5 mg D-α-tocopherol.

Oestrus was synchronized using medoxyprogesterone acetate sponges (Repromap; Upjohn Veterinary Products, NSW, Australia). After a minimum of 8 d on the experimental diets the ewes were allowed to mate naturally with two rams and were then hand-mated to a third. Rams were given a high-Zn ration daily and were rotated between the groups of ewes every 3 d. Pregnancy status was assessed on the basis of returns to service, plasma progesterone concentrations (see Short, 1972) and laparoscopic examination of the uterus (Sawyer, 1978).

For the final 115 d of gestation, ten pregnant ewes that conceived during the first oestrus cycle were selected at random from each group. These were transferred to individual metabolism cages made of stainless-steel and high-density polyethylene. The availability of twenty suitable metabolism cages required that not all the ewes to conceive during the first cycle were continued in the experiment. The twenty selected ewes were allocated to the following dietary treatments: (1) +Zn+Zn, five ewes given the +Zn diet throughout mating and pregnancy; (2) +Zn−Zn, five ewes given the +Zn diet up to day 35 of pregnancy and then the −Zn diet until parturition (day 150); (3) −Zn−Zn, five ewes given the −Zn diet throughout mating and pregnancy; (4), −Zn+Zn, five ewes given the −Zn diet up to day 35 of pregnancy and then the +Zn diet until parturition. These treatments allowed comparison of the effects of Zn deficiency on mating, implantation and early embryonic growth with its effects on later development and growth of the foetus. Day 35 was specifically chosen to re-allocate treatments because at this stage accurate diagnosis of pregnancy could be made by laparoscopic examination.

Measurements and sampling

Ewes were weighed every 3 weeks and feed intakes were calculated weekly. Plasma was collected weekly, newly grown wool was clipped from the same patches on the mid-side every 8 weeks and colostrum was collected immediately after parturition. Lambs were weighed at birth and then slaughtered by exsanguination before they had sucked. Samples of liver and thymus were removed for thymidine kinase (*EC* 2.7.1.21) and alkaline phosphatase (*EC* 3.1.3.1) assays. Whole lamb carcasses and samples of plasma, liver, kidney, thymus and pancreas were stored at −20° for later estimation of Zn content.

Assays

Tissue samples were analysed for Zn by atomic absorption spectrophotometry after lyophilization and wet digestion in nitric acid–sulphuric acid–perchloric acid (10·0:0·5:0·5, by vol.). Lamb carcasses were minced and three 5-g samples were digested and analysed as described previously. Colostrum was similarly digested and analysed.

Thymidine kinase activities were measured by the procedure described by Duncan & Hurley (1978) with some modifications. Tissue homogenates were prepared as described by Witschi (1970). The reaction mixture in a final volume of 0·6 or 0·525 ml contained: 0·25 M-Tris-hydrochloride buffer (pH 8·0), 5·1 μmol ATP, 6·3 μmol 3-phosphoglyceric acid, 5·2 μmol $MgCl_2 . 6H_2O$, 1·6 μCi methyl [^{3}H]thymidine (0·24 μmol, 6·7 Ci/mmol; New England Nuclear, Boston, Mass.) and either 0·1 ml liver supernatant fraction or 0·025 ml thymus supernatant fraction. The mixture was incubated at 37° for 15 min and the reaction terminated by spotting 40 μl reaction mixture on to a disk (20 mm) of DEAE cellulose filter paper (Whatman DE81 chromatography paper). The disks were then washed in 1·0 mM-ammonium formate and deionized water, allowed to drain and placed in scintillation vials containing 1·0 ml 0·1M-hydrochloric acid–0·2 M-potassium chloride. After mixing for 15 min, 10 ml scintillant (toluene (technical grade) containing 4 g PPO and 0·2 g bis-MBS/l mixed with Triton X-100 (2:1, by vol.)) was added and radioactivity measured using an Isocap 300 liquid-scintillation counter (Searle, High Wycombe, Bucks).

Alkaline phosphatase activity was estimated colorimetrically using sodium *p*-nitrophenyl-phosphate as substrate. Protein concentration in the tissue supernatant fractions was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Statistical analysis

Comparisons among the four groups of ewes were made using Duncan's multiple range test. Only ewes that continued pregnancy to term and gave birth to live lambs were included in the analysis.

The lambs were grouped according to the final dietary Zn treatment of the ewe, therefore, lambs from +Zn+Zn or −Zn+Zn ewes were grouped as high Zn and those from −Zn−Zn and +Zn−Zn as low Zn. The two groups were then compared using Student's *t* test. Logarithmic transformation was applied to sets of values when variances were not homogenous. When the values for twin lambs were significantly different from those of single-born lambs, comparisons between the groups were made using single-born lambs only.

RESULTS

Fifteen of the twenty ewes maintained pregnancy to term and delivered live young. Two of the ewes given adequate Zn (−Zn+Zn or +Zn+Zn) required assistance during parturition compared with three Zn-deficient ewes (+Zn−Zn or −Zn−Zn). Of the ewes that were not continued in the experiment to term, one resorbed its foetus on approximately day 80–100 (−Zn+Zn) and three were removed from the experiment or died after refusing to eat (one each from +Zn−Zn, −Zn−Zn, −Zn+Zn). A fifth animal was removed due to injury (+Zn+Zn). Two of the animals that refused to eat were found to have large caseous lymphadenitis infections obstructing the oesophagus and trachea.

Ewes given the Zn-supplemented diet throughout pregnancy (+Zn+Zn) had significantly higher feed intakes for the last 115 d of pregnancy than any of the other groups ($P < 0·05$). These ewes also weighed consistently (though not significantly) more than the sheep in the other groups (Table 2). Plasma Zn concentrations in the ewes also rapidly responded to dietary Zn intake (Fig. 1). By the end of the experiment, ewes consuming the low-Zn diet had 30–40% less Zn in the plasma than the other groups. Neither wool nor colostrum Zn concentrations were significantly reduced by Zn deprivation.

Table 2. *Effect of dietary zinc intake on the pregnant ewe*

(Mean values with their standard errors; no. of ewes in parentheses)

Group†...	+Zn+Zn		−Zn+Zn		−Zn−Zn		+Zn−Zn	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Food intake (kg/d)								
Weeks 5–11	0·93	0·11 (4)	0·71*	0·03 (3)	0·69*	0·04 (4)	0·71*	0·05 (4)
Weeks 12–19	0·87	0·05 (4)	0·59**	0·03 (3)	0·55**	0·06 (4)	0·59**	0·05 (4)
Live wt (kg)								
Week 18	44·0	0·89 (4)	39·8	1·09 (3)	39·6	1·82 (4)	41·3	1·64 (4)
Wool Zn (mg/kg)								
Week 20	95·0	4·4 (4)	84·0	12·2 (3)	79·0	9·0 (4)	87·5	10·5 (4)

† +Zn+Zn, Zn-supplemented diet throughout pregnancy; −Zn+Zn, Zn-deficient diet up to day 35 of pregnancy and then Zn-supplemented diet until parturition; −Zn−Zn, Zn-deficient diet throughout pregnancy; +Zn−Zn, Zn-supplemented diet up to day 35 of pregnancy and then Zn-deficient diet until parturition.

Mean values significantly different from those for +Zn+Zn using Duncan's multiple range test: *$P < 0·05$, **$P < 0·01$.

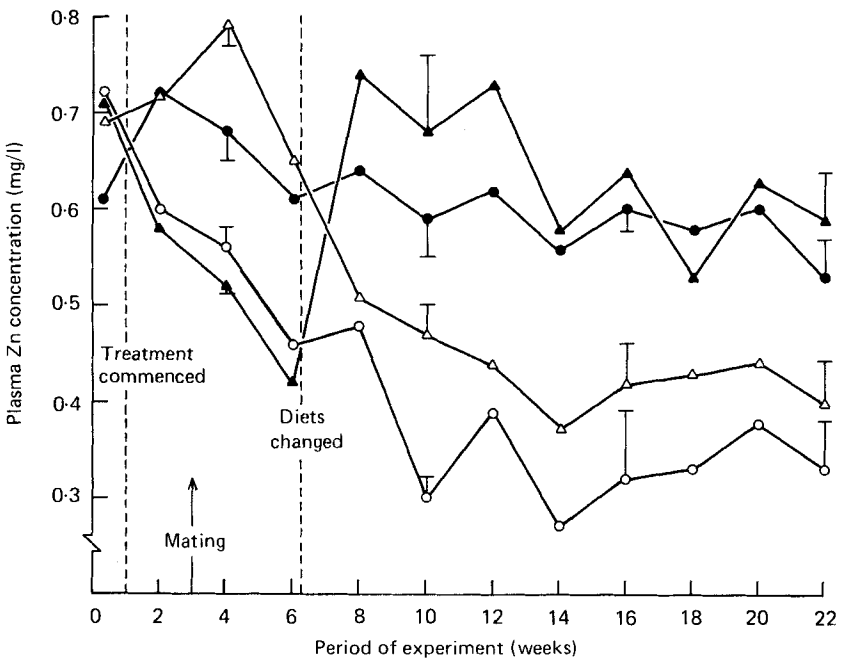

Fig. 1. Effect of dietary zinc intake on the concentration of Zn in plasma of pregnant ewes. (●), +Zn+Zn, Zn-supplemented (+Zn) diet throughout pregnancy; (▲), −Zn+Zn, Zn-deficient (−Zn) diet up to day 35 of pregnancy and +Zn diet until parturition; (△), +Zn−Zn, Zn-supplemented (+Zn) diet up to day 35 of pregnancy and −Zn diet until parturition; (○), −Zn−Zn, Zn-deficient (−Zn) diet throughout pregnancy.

Ewes supplemented with Zn (+Zn+Zn and −Zn+Zn) produced heavier single-born lambs than Zn-depleted ewes ($P < 0·05$). The high-Zn lambs also had higher liver weights and more Zn in the liver, pancreas and whole carcass ($P < 0·05$) (Table 3), higher liver alkaline phosphatase activity ($P = 0·05$) and slightly higher activity of thymidine kinase in the thymus ($P = 0·07$) than the low-Zn lambs (Table 4). A large variation within groups was also observed, particularly with respect to enzyme activities.

Table 3. *Effect of maternal zinc intake on the birth weight, organ weights, organ Zn content and organ Zn concentrations (mg/kg dry tissue) of the newborn lamb*

(Mean values with their standard errors; no. of lambs in parentheses)

Group† . . .	High Zn (+Zn+Zn and −Zn−Zn)		Low Zn (−Zn−Zn and +Zn−Zn)	
	Mean	SE	Mean	SE
Lamb wt‡ (kg)	4·57	0·28 (5)	3·81*	0·17 (5)
Liver wt (g fresh wt)‡	103·0	5·33 (5)	67·8***	4·33 (5)
Liver wt (% birth wt)	1·94	0·10 (9)	1·75	0·08 (11)
Kidney wt (g fresh wt)‡	10·9	0·44 (5)	10·1	0·66 (5)
Carcass Zn (mg)‡	81·8	4·4 (5)	62·5*	5·70 (5)
Carcass Zn (mg/kg)§	19·3	0·73 (9)	15·4**	0·80 (11)
Liver Zn (mg)	2·77	0·19 (9)	1·21***	0·30 (11)
Liver Zn (mg/kg)	187·7	34·2 (9)	81·8*	16·5 (11)
Kidney Zn (μg)‡	113·4	6·28 (5)	98·6	5·73 (5)
Kidney Zn (mg/kg)	57·6	2·80 (9)	51·5	2·42 (11)
Thymus Zn (mg/kg)	89·4	8·72 (9)	78·4	2·61 (11)
Pancreas Zn (mg/kg)	209·3	15·2 (9)	132·7*	14·0 (11)
Plasma Zn (mg/l)	1·26	0·14 (9)	1·00	0·15 (11)

† +Zn+Zn, Zn-supplemented diet throughout pregnancy; −Zn+Zn Zn-deficient diet up to day 35 of pregnancy and then Zn-supplemented diet until parturition; −Zn−Zn, Zn-deficient diet throughout pregnancy; +Zn−Zn, Zn-supplemented diet up to day 35 of pregnancy and then Zn-deficient diet until parturition.

Mean values significantly different from those for high-Zn group using Student's *t*-test: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

‡ Comparison between single lambs only, twins were excluded when they were significantly different from singles.

§ Wet weight basis.

Table 4. *Effect of maternal zinc intake on the activities of alkaline phosphatase* (EC *3.1.3.1*) *and thymidine kinase* (EC *2.7.1.21*) *in the newborn lamb*

(Mean values with their standard errors; no. of lambs in parentheses)

Group† . . .	High Zn (+Zn+Zn and −Zn+Zn)		Low Zn (−Zn−Zn and +Zn−Zn)		Statistical significance of difference (Student's *t* test)
	Mean	SE	Mean	SE	
Alkaline phosphatase ‡					
Liver	32·2	3·76 (9)	24·8	1·53 (11)	$P = 0{\cdot}05$
Thymus	113·7	8·20 (9)	134·3	15·70 (11)	NS
Plasma	625·7	103·0 (9)	450·9	72·9 (11)	NS
Thymidine kinase§					
Liver	17·9	4·5 (9)	15·5	1·25 (11)	NS
Thymus	429·9	107·2 (9)	251·9	56·1 (11)	$P = 0{\cdot}07$

† +Zn+Zn, Zn-supplemented diet throughout pregnancy; −Zn+Zn, Zn-deficient diet up to day 35 of pregnancy and then Zn-supplemented diet until parturition; −Zn−Zn, Zn-deficient diet throughout pregnancy; +Zn−Zn, Zn-supplemented diet up to day 35 of pregnancy and then Zn-deficient diet until parturition.

‡ Liver and thymus in mU/mg protein, plasma in mU/l. One unit is the enzyme activity that causes 1 μmol substrate to react in 1 min at 25°.

§ pmol [^{3}H]thymidine incorporated into thymidine monophosphate/h per mg protein.

NS, not significant.

DISCUSSION

The Zn-deficient ewes had significantly reduced feed intakes and slightly reduced live weights compared with the ewes that were supplemented with Zn throughout pregnancy. No other external signs of Zn deficiency were noted, no sheep developed parakeratosis and the heavy salivation reported when egg-white is used as a dietary protein source (Underwood & Somers, 1969; Apgar & Travis, 1979) did not occur. Therefore, although the ewes had low Zn intakes with associated reduced plasma Zn concentrations and inappetence, no obvious external signs of Zn deficiency existed. In grazing ruminants, this lack of external lesions means that Zn deficiency could easily pass undetected. That such Zn responsive situations may occur has been previously demonstrated (Masters & Fels, 1980; Mayland *et al.* 1980). The decline in food intake is a well documented characteristic of Zn deficiency (Underwood, 1977). The Zn-deficient ewes consumed 25% less dry matter than those given Zn throughout pregnancy. Unlike other experiments with other animal species (Mills *et al.* 1969) and sheep (Underwood & Somers, 1969) however, the −Zn+Zn group did not increase feed consumption when repleted with Zn despite rapid increases in plasma Zn concentration. Although there is no apparent reason for this, it may be related to the dry and dusty nature of the diet. Repletion with Zn may not have sufficiently increased the palatability of the diet.

Zn content of the diet did not significantly influence the ability of ewes to become pregnant or maintain pregnancy. There was no evidence indicating that Zn deficiency, which has been shown to cause dystocia in rats (Apgar, 1968) has a similar effect in ewes. Feeding the Zn-deficient diet to ewes resulted in decreased lamb birth weights. This may be a direct result of Zn deficiency *per se*, an indirect effect associated with decreased food intake of the ewes or a combination of both. Reduced birth weights resulting from Zn deficiency have been reported in rats (Williams *et al.* 1973; Hurley & Cosens, 1974) and grazing sheep (Masters & Fels, 1980) but not peviously with penned sheep (Apgar & Travis, 1979). Apgar & Travis (1979) induced Zn deficiency in penned sheep during the last third of pregnancy. In the current experiment Zn deficiency was initiated either before mating or during the first third of gestation. This difference in the length of deficiency may be responsible for the different results. Reduced lamb birth weights caused by decreased maternal food intake have also been reported previously (Schinckel, 1963). In the current experiment it is possible that the reduced intake by Zn-deficient ewes may have contributed to reduced lamb birth weights.

The mean total amount of Zn accumulated in each single-born lamb was 81·8 and 62·5 mg for the high- and low-Zn lambs respectively. In comparison, 140-d-old twin, triplet or quadruplet foetuses not deprived of Zn have been reported to contain 68–81 mg Zn (Hansard & Mohammed, 1968; Williams *et al.* 1978). Another report (Williams & Bremner, 1976) indicated that at least 85% of the total foetal Zn burden is accumulated after day 80 of gestation and that on day 80 the foetus contains only 7–8 mg of Zn. If this rate of accumulation is applied to the current results then the high-Zn foetus accrued approximately 74 mg Zn or between 2 and 4% of total maternal intake, and the low-Zn foetus 55 mg or approximately 35% of total maternal Zn intake between days 80 and 150.

It is well established that the efficiency of Zn absorption in rats increases during Zn deficiency (Davies & Williams, 1977). There is also evidence in cattle and sheep that Zn status/intake affects Zn absorption (Kirchgessner *et al.* 1978, Suttle *et al.* 1982). The results of this current experiment indicate that a combination of Zn deficiency and pregnancy in the ewe leads to highly efficient utilization of ingested Zn. The Zn-deficient ewes consumed daily 0·57 kg of a diet containing 4 mg Zn/kg, and total Zn intake was therefore approximately 2·3 mg/d. If Zn absorption was as low as 13% (Hansard & Mohammed, 1968) then the Zn-deficient ewes would have utilized only 0·3 mg Zn/d from the diet. This

would probably not be sufficient for even the maintenance requirement of a non-pregnant ewe and, as the Zn-deficient ewes deposited an average of 0·80 mg Zn/d into the single foetuses during the last 70 d of gestation, absorption must have been greatly enhanced during this period.

Even with complete absorption and retention of the 2·3 mg Zn ingested daily it is difficult to see how the demands of pregnancy were met. During this last half of pregnancy no skin lesions or other visible signs of deficiency appeared and plasma Zn levels did not fall to the extremely low levels reported in previous experiments (Mills *et al.* 1967; Masters & Moir, 1980). The only source of Zn available to the ewes other than that coming directly from the diet was Zn mobilized during tissue breakdown. All the ewes in the +Zn−Zn and −Zn−Zn groups had a net body-weight loss and catabolism of tissue such as muscle would mobilize Zn. For example, the −Zn−Zn ewes had a mean weight loss of 0·9 kg and, during this time, the ewe produced a 3·8 kg lamb accompanied by approximately 1·5 kg fluids and membranes (Schinckel, 1963). The total loss of body-weight by the ewe was then 6·2 kg. As muscle contains approximately 24 mg (Zn/kg (Suttle, 1979), then the total amount of Zn mobilized may be as much as 150 mg. Although this may be an overestimate due to some of the weight loss being fat and containing less Zn, the amount of Zn mobilized by tissue catabolism is still substantial. It is reasonable to assume that this Zn is utilized by the ewe and deposited in the foetus.

This release and utilization of large amounts of Zn from tissues by the ewe is not normally found in the rat (Hurley & Swenerton, 1971) but is apparent in that animal when tissue or bone catabolism is induced by a concurrent Zn and protein or Zn and calcium deficiency (Hurley & Tao, 1972; Hurley *et al.* 1973). This difference between species may be related to the size of the concepta in proportion to the adult animal and the length of gestation (Apgar *et al.* 1981). The ewe produces a foetus approximately 10% of its own weight in 150 d compared with the rat that produces a litter approximately 25–30% of its own weight in 22 d. The current experiment shows that the ewe may have a total weight loss exceeding the size of the foetus by 50% and still maintain pregnancy and produce a lamb. A comparable weight loss in the rat would be 40–45% of total body-weight, this would have severe consequences on health and survival. The ewes were mature and had no Zn requirement for growth. A younger growing ewe with a higher total Zn requirement may be less able to provide Zn by tissue catabolism and therefore may become more severely Zn deficient.

Zn concentrations in the liver and pancreas of the lambs as well as the whole carcass were significantly reduced by the low-Zn diet. Liver of high-Zn lambs contained a total of 2·8 mg Zn and those of the low-Zn lambs 1·2 mg. These levels are equivalent to 3·4 and 1·9% of total carcass Zn in the high- and low-Zn lambs respectively. This is less than the 5·6–8·8 mg Zn found in the livers of 140-d-old foetuses by other workers (Hansard & Mohammed, 1968; Williams *et al.* 1978). It is evident from the current experiment that lambs from Zn-depleted ewes have very small stores of Zn in the liver and even lambs from Zn-supplemented ewes do not have a substantial store.

Maternal Zn deficiency affected some enzymatic activities as well as tissue Zn concentrations. Alkaline phosphatase was reduced by 23% in the liver and thymidine kinase by 41% in the thymus of Zn-deprived lambs. It should be mentioned that alkaline phosphatase activity and thymic growth may be influenced by inanition as well as Zn deficiency (Luecke *et al.* 1968, 1978). Therefore, these changes may be related to feed consumption of the ewes. However, the decline in feed intake by the Zn-depleted ewes occurred as a direct result of Zn deficiency and therefore the responses observed are a consequence of reduced Zn intake. In grazing sheep both the direct and indirect effects of Zn deficiency may severely disrupt production and reproduction.

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