The susceptibility of suckling lambs to zinc toxicity

BY N. T. DAVIES, H. S. SOLIMAN*, W. CORRIGALL AND A. FLETT

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

(*Received* 15 *March* 1977 – *Accepted* 21 *March* 1977)

1. Suckling lambs maintained for 4 weeks on a milk-substitute diet containing 407 g Toprina yeast/kg dry matter of diet exhibited poor growth, low appetite and extensive renal damage.

2. The batch of yeast used contained 2065 mg zinc/kg.

3. The kidneys of lambs offered a milk diet supplemented with the same Zn content as the yeast-fed diet (32·1 mg Zn/1000 kJ) were similarly affected.

The results reported in this paper concern the rapid development of gross kidney lesions in young lambs maintained on a milk-substitute diet using yeast as the sole protein source. Analysis of this particular batch of yeast showed it to contain an abnormally high zinc content and the results of a subsequent experiment in which lambs were offered a milk diet supplemented with the same high level of Zn strongly suggest that Zn was the toxic agent responsible. The purpose therefore of this current report is to bring to the readers' attention the dangers of high dietary Zn in suckling lambs.

MATERIALS AND METHODS

Experiment 1

Four lambs (2 male and 2 female; Suffolk × Finnish Landrace × Dorset poll) representing part of an experiment designed to evaluate the adequacy of a commercial yeast (Candida sp, Toprina; BP Proteins Ltd) as a protein source in milk substitute diets for suckling lambs, were offered from one week old a formula containing (g/kg dry weight) yeast, 407; lactose, 245; Methionine, 15; butter, 275; PEG 400 mono-oleate, 35; and vitamins and minerals to give similar contents to ewe's milk. These ingredients were thoroughly mixed with water in the ratio, 160 g dry weight: 1 l water to give a final energy content of 4184 kJ/kg liquid diet. The liquid diet was offered to the lambs via an automatic feeder for 5 mins every 3 h in eight equal feeds and the amount adjusted daily so each lamb had access to 1046 kJ gross energy/kg body-weight$^{0.75}$ per d.

After 35 d the lambs were killed and one kidney and a liver sample from each lamb were removed and stored at −20° for subsequent trace element analysis. The remaining kidney was immediately fixed in formol saline (40 g formaldehyde in 1 l saline (9 g NaCl/l)) before histological examination.

Experiment 2

Four male lambs of the same strain as described above were offered from one week old a liquid diet of reconstituted dried whole milk to give an energy content of 4184 kJ/kg liquid diet. One lamb served as a control and was offered the milk diet only, while the remaining three were offered the milk diet supplemented with Zn (as $ZnSO_4.7H_2O$) to supply 32·1 mg Zn/1000 kJ. Lambs were bottle-fed three times a day so that they received 1046 kJ/kg body-weight$^{0.75}$ per d. After 33 d the control and two experimental lambs

* Present address: Department of Animal Production, Faculty of Agriculture, Aim Shams University, Cairo, Egypt.

were killed while the remaining lamb was transferred to unsupplemented milk for a further 14 d. Samples of liver and kidney were retained for trace element analysis and kidney samples fixed, as described above, for histological examination.

Trace element analysis

Diet and tissue samples were analysed for Zn, copper, iron, lead and cadmium by atomic absorption spectrophotometry using a Varian Techtron AA5, after a wet ashing procedure (Davies & Nightingale, 1975).

Histological examination

Fixed samples of kidney were sectioned on a microtome and stained in haematoxylin/eosin. Examination of the slides was carried out without prior reference to the animal's treatment.

RESULTS AND DISCUSSION

Experiment 1

Four lambs maintained on the diet containing 'Toprina' yeast from one particular batch exhibited, to varying degrees, low appetite and poor growth rates over a four-week trial period. Their average daily weight gains varied from 15·2 to 36·4 g/d with a mean of 21·2 g/d. Average daily feed intakes were highly variable ranging from 442·2 to 801·2 kJ/kg $W^{0.75}$ per d with a mean of 550·6 kJ/kg $W^{0.75}$ per d. These values can be compared with growth rates of 150–160 g/d and daily food intakes of 1046 kJ/kg $W^{0.75}$ for lambs maintained under identical conditions on whole milk diets (H. Soliman, unpublished observations).

Post-mortem examination revealed obvious lesions of the kidneys. They were enlarged and very pale. Histological examination gave similar findings in all, varying in extent. There was an increase of fibrous tissue particularly in the outer cortex, but also in linear areas among the straight tubules. Some glomeruli were atrophic and there were wide Bowman's spaces. Many of the tubules were dilated and tortuous. Occasional proteinaceous and some cellular casts were seen.

Renal damage is often associated with the ingestion of heavy metals such as Pb and Cd (reviewed by Bremner, 1974) and, since it was apparent that the severity of the renal damage was greater in the lambs that ate the most diet, it seemed possible that a heavy-metal toxicity may have been responsible. Analysis of the Toprina yeast showed it to contain less than 0·02 mg Cd/kg and less than 0·5 mg Pb/kg. However, it contained 2065 mg Zn/kg which supplied 32·1 mg Zn/1000 kJ. While this Zn concentration (equivalent to 840 mg Zn/kg dry matter) was appreciably lower than that inducing Zn toxicity in post-weaning lambs (Ott, Smith, Harrington & Beeson, 1966), analysis of the kidneys revealed that Zn concentrations in the cortices ranged from 2720 to 7943 mg Zn/kg dry weight with a mean of 3887 mg Zn/kg dry weight, and the severity of the lesions was clearly related to the cortical Zn contents. These kidney cortex Zn contents can be compared with 299 and 320 mg Zn/kg dry weight for lambs of similar age maintained on a milk diet (Soliman, Davies & Ørskov, unpublished observations).

Experiment 2

This experiment was designed to elucidate whether Zn was the toxic agent responsible for the kidney lesions. One lamb served as a control and was fed unsupplemented reconstituted dried milk while the three experimental lambs received milk supplemented with $ZnSO_4.7H_2O$ to supply the same Zn content as the yeast diet (32·1 mg Zn/1000 kJ).

Unlike the situation in Expt 1 the high dietary Zn content did not affect food intake since all animals consumed the 1046 kJ/kg $W^{0.75}$ per d offered. Despite this the animals

Table 1. *Weights, percentage dry matter* (DM), *and zinc copper and iron contents* (*mg/kg* DM) *of kidneys from lambs fed basal milk diet for* 33 *d* (636), *zinc-supplemented milk for* 33 *d* (634 *and* 635) *and zinc-supplemented milk for* 33 *d followed by the basal milk diet for* 14 *d* (633)

Lamb no.	Treatment	wt (g)	% DM	Cortex Zn	Cortex Cu	Cortex Fe	Medulla Zn	Medulla Cu	Medulla Fe
636	Basal	17·3	18·4	136	24·8	292	95·0	15·6	149·0
634	Zn-supplemented	40·2	21·4	23900	149	44·6	3220	42·3	60·3
635	Zn-supplemented	45·9	19·9	19000	115	49·5	4790	37·4	69·7
633	Zn-supplemented then basal	46·4	20·2	16200	73·8	54·2	4750	35·8	77·9

receiving the high Zn milk grew at average daily rates of 96·9, 96·8 and 81·3 g/d compared with 160 g/d for the control. The lamb transferred from high Zn milk to the unsupplemented milk showed no improvement in its average daily weight gain.

Post-mortem examination of the three lambs which had consumed the high-Zn milk showed similar pathological changes to those observed in the yeast-fed lambs. The kidneys were enlarged and pale, whereas those from the control lamb appeared normal. Histological examination of the affected kidneys showed fibrous tissue increase, some glomerular atrophy and tubular distortion. Cells of the tubular epithelium had densely staining cytoplasm and pyknotic nuclei. Histological examination of kidneys from the control lamb revealed no specific lesions.

The weights, percentage dry matter and trace metal composition of the kidneys are shown in Table 1. In all lambs which received high Zn milk the Zn content of the cortex was at least two orders of magnitude greater than that of the control. All affected animals showed elevated cortical Cu contents and substantial reductions in Fe contents, although the significance of these findings in relation to the renal damage cannot be assessed. Qualitatively similar although less marked changes in metal composition were seen in the medullary tissue. These findings do not indicate any substantial loss of Zn from the kidneys of lamb 633 over the 14 d period when it received unsupplemented milk.

The concentrations of Zn in the livers of the lambs receiving high Zn milk were elevated, being 2664, 2133 and 2311 mg Zn/kg dry weight respectively for lambs 633, 634 and 635 compared with 299 mg Zn/kg dry weight for the control lamb (636). In contrast to the kidney, liver Cu contents were lower than the control being 39·0, 156 and 168 mg Cu/kg dry weight for 633, 634 and 635 respectively compared with 309 mg Cu/kg dry weight for lamb 636.

The cortical Zn contents of kidneys from these milk-fed animals were much higher than those of the lambs receiving the yeast diet. However, it is not possible to make any statements regarding differences in Zn availability from these two diets even though both groups of animals were offered diets containing the same concentration of Zn (32·1 mg Zn/1000 kJ). The yeast-fed lambs never consumed all the diet that was offered to them in contrast to the milk-fed lambs. In addition the amount of food offered each day was adjusted according to the lambs' body-weight (1046 kJ/kg $W^{0.75}$ per d) and the growth rates of the milk-fed lambs were approximately four times greater than those of the lambs receiving the yeast-based diet.

It has been stated that 'zinc is relatively non-toxic to birds and mammals' and 'that rats, pigs, poultry, sheep and cattle exhibit considerable tolerance to high intakes of zinc' (Underwood, 1971). Rats maintained on diets containing 2500 mg Zn/kg exhibited no symptoms of Zn toxicity although at 5000 or 10000 mg Zn/kg young rats exhibited anorexia, subnormal growth and at the higher level, heavy mortality (Heller & Burke, 1927). These dietary Zn contents can be compared with 840 mg Zn/kg dry matter in the 'Toprina' based milk substitute used in these current experiments.

Young lambs appear to be less tolerant to high Zn intakes than rats. Ott *et al.* (1966) report that weaned lambs maintained on diets supplemented with 1500 mg Zn/kg had reduced food consumption, weight gains and decreased feed conversion efficiency although no report was made of renal damage.

The results of this current study when compared with the work of Ott *et al.* (1966) suggest that the suckling lamb is considerably more susceptible to Zn toxicity than more mature ruminating animals. This may be due to greater absorption of dietary Zn since Zn absorption has been shown to be higher in the young of many species, compared with older animals. These examples include rats (Ballou & Thompson, 1961) and cattle (Miller & Cragle, 1965). However, in all of these studies the younger animals were fed rations of different composition from those fed to the more mature animals and it has not been established how much of the difference in Zn absorption was due to age and how much to alterations in dietary composition.

In conclusion it must be emphasized that in the first experiment yeast was used as the sole protein source and it is highly unlikely that this level of inclusion (407 g/kg dry diet) would ever be used in commercial practice. Secondly, the Zn content of the batch of 'Toprina' yeast used in this study at 2065 mg Zn/kg was abnormally high. Analysis of four different batches of 'Toprina' yeast produced at the same plant at about the same time contained from 889 to 1020 mg Zn/kg and more recent batches consistently contain 500–600 mg Zn/kg. It has not yet been established whether inclusion of 'Toprina' yeast with these lower Zn contents in milk-substitute diets, or at lower levels of inclusion, would similarly produce deleterious effects in suckling animals.

The helpful discussions and advice of Drs E. R. Ørskov and C. F. Mills are gratefully acknowledged.

REFERENCES

Ballou, J. E. & Thompson, R. C. (1961). *Hlth Phys.* **6**, 6.
Bremner, I. (1974). *Q. Rev. Biophys.* **7**, 75.
Davies, N. T. & Nightingale, R. (1975). *Br. J. Nutr.* **34**, 243.
Heller, V. G. & Burke, A. D. (1927). *J. biol. Chem.* **74**, 85.
Miller, J. K. & Cragle, R. G. (1965). *J. Dairy Sci.* **48**, 370.
Ott, E. A., Smith, W. H., Harrington, R. B. & Beeson, W. M. (1966). *J. Anim. Sci.* **25**, 414.
Underwood, E. J. (1971). *Trace Elements in Human and Animal Nutrition*, 3rd edn. New York and London: Academic Press.

Printed in Great Britain