S. Afr. J. Anim. Sci. 11, 273–278 (1981)

RETENTION OF TRACE ELEMENTS IN THE LIVERS OF SHEEP FED POULTRY MANURE AS A RATION COMPONENT

Receipt of MS 13-03-81

J.B.J. van Ryssen and H.M. Jagoe*
Department of Animal Science, University of Natal, P.O. Box 375, Pietermaritzburg, 3200

(Key words: *Poultry manure, trace elements, sheep)*
(Sleutelwoorde: *Hoendermis, spoorelemente, skape)*

OPSOMMING: RETENSIE VAN SPOORELEMENTE IN DIE LEWERS VAN SKAPE OP RANTSOENE WAT HOENDERMIS BEVAT

Gedroogde batteryhoendermis (DHM) is teen 4 peile van toevoeging (0,0, 11,6, 23,5 en 35,4% respektiewelik) in afrondingsrantsoene vir skape gebruik. Byvoeging van hoendermis het 'n verhoging veroorsaak in die konsentrasies van kalsium (Ca), fosfor (P), swawel (S), koper (Cu), sink (Zn), yster (Fe) en mangaan (Mn) van die rantsoene. 'n Verhoogde inname van DHM in die rantsoen het tot 'n afname in die retensie van Cu en Zn in die lewers van die skape gelei. Gevolglik het die Cu-inhoud van die lewers feitlik konstant gebly ongeag die hoër Cu-inname. Die gegewens dui daarop dat die verhoogde inname van sekere minerale in DHM 'n onderdrukkende uitwerking op die absorpsie en metabolisme van Cu en Zn in die liggaam het. Homeostatiese beheermeganismes kon ook 'n rol by Zn-absorpsie gespeel het. Alhoewel DHM die Fe-inname van die skape drasties verhoog het, is geen verhoging in lewerystér waargeneem nie. Die werking van homeostatiese meganismes op Fe-absorpsie is waarskynlik hiervoor verantwoordelik.

SUMMARY:

Dried poultry manure from batteries (DPM) was included at 0,0, 11,6, 23,5 and 35,4% in sheep finishing rations. An increase in the DPM content of rations caused substantial increases in the concentrations of calcium (Ca), phosphorus (P), sulphur (S), copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) in the rations. Reductions in liver Cu and Zn retention were observed with increased DPM intakes, and the Cu content of the livers remained practically constant. The high intake of minerals antagonistic to Cu and Zn metabolism in the body and present in poultry manure at high levels, probably decreased the availability of Cu and Zn to the body with increased DPM intakes. Homeostatic control mechanisms may also have contributed to the reduced availability of Zn in the body. Although DPM substantially increased Fe intakes, there was little change in the retention of Fe in the livers. Homeostatic mechanisms controlling Fe absorption were probably responsible for this lack of response to Fe intake.

Most of the minerals in poultry manure are present at levels well above the nutritional requirements of sheep (Lowman & Knight, 1970; Wilke & Van der Merwe, 1974; Van Ryssen, Channon & Stielau, 1978). Of the trace elements, copper (Cu) often occurs at concentrations potentially toxic to sheep, especially in the manure from broilers given copper sulphate as a growth stimulant in their feed (Van Ryssen *et al.*, 1978). However, even when poultry manure from birds not receiving copper sulphate is included in sheep rations, the Cu content may be elevated to potentially toxic levels (NRC, 1975).

Very high levels of iron (Fe) may also occur in poultry manure (Lowman & Knight, 1970; Jiminez, 1974; Van Ryssen *et al.*, 1978). Although the toxic levels of dietary Fe for ruminants are not clearly established (NRC, 1975), the inclusion of poultry manure in rations can elevate the Fe concentrations to levels above 1 000 mg Fe/kg feed. At Fe levels as low as 200 mg/kg some indication of Fe toxicity in livestock has been reported (Lawlor, Smith & Beeson, 1965; Standish, Ammerman, Simpson, Neal & Palmer, 1969).

At high dietary levels many minerals have antagonistic actions on the absorption and metabolism of other minerals (Hill & Matrone, 1970). The statement by Fox & Reynolds (1973) that "an excess intake of an essential nutrient over the usually recognised requirement may be protective against a toxic element", may therefore be applicable in the case of poultry manure.

In an investigation to determine the energy value of layer excreta the opportunity arose to determine the effects of different levels of poultry manure in rations on the retention of minerals in the livers of sheep.

Procedure

Animals, treatments and procedure

Forty S.A. Mutton Merino yearling ram lambs were allocated according to body mass into 4 groups of 7

* Present address: Maybaker (SA) (Pty) Ltd, P.O. Box 1130, Port Elizabeth, 6000

lambs each, while 12 lambs were allotted to the pre-experimental group (Pr-X) so as to achieve greater representation in the initial group. The experiment was conducted according to a restricted randomised block design. The 4 rations tested contained 0,0% , 11,6% , 23,5% and 35,4% of dried poultry manure (DPM) obtained from layers housed in batteries. These rations are referred to as treatments 0, 12, 24 or 36, respectively. The rations were formulated to be isonitrogenous and approximately equal in Ca and P content. The lambs were fed individually and all feed intakes were recorded. Groups 0 and 12 were slaughtered after 54 days on feed and the remaining 2 groups (the slower gaining groups) after 60 days. The livers were removed at slaughter, placed in plastic bags and sealed. The masses of the fresh livers were recorded as soon as possible after collection and representative samples from each liver were collected and dried at 80°C. The dried samples were stored pending analyses.

Chemical and statistical analyses

The Ca and P contents of the rations were determined on an auto analyzer (Technicon Auto Analyzer II) while sulphur (S) was determined by the method of Blanchar, Rehm & Caldwell (1965). The Cu, Fe, zinc (Zn) and manganese (Mn) content of rations and livers were measured with an atomic absorption spectrophotometer after wet acid digestion. Molybdenum (Mo) was determined according to the molybdenum-iron-thiocyanate method described by Van Ryssen & Stielau (1980).

Mineral retention in the liver was calculated as: (liver mineral content at slaughter minus liver mineral content derived from the Pr-X group divided by mineral intake) x 100. The F-test and Student's t-test were used to compare differences between treatments statistically.

Results

Each increase in the amount of DPM included in a ration raised the concentration of all ration minerals which were examined, with the exception of the Mo concentration (Table 1). The latter remained virtually unchanged because the Mo content of DPM was approximately the same as that for the remaining ration ingredients. The daily mineral intakes by the sheep (except for Mo, Ca and P) increased with each additional increment of DPM present in the ration. Thus, the daily intake of Fe, Zn or S was more than doubled between the 0% and the 35,4% levels of DPM in the ration (Table 2). The DPM contained 30,2% ash, 7,26% Ca, 2,42% P, 1,1% S and had a trace element content (mg/kg) of 46 Cu, 892 Fe, 303 Zn, 293 Mn and 0,4 Mo.

A close examination of the data obtained from the pre-experimental slaughter group revealed a highly signifi-

Table 1

Mineral content of rations containing different levels of dried poultry manure (D P M)

D P M Treatment	Calcium* %	Phosphorus* %	Sulphur %	Copper mg/kg	Molybdenum mg/kg	Zinc mg/kg	Iron mg/kg	Manganese mg/kg
0	2,03	0,74	0,23	11,7	0,66	39	323	147
12	1,91	0,72	0,30	12,9	0,56	55	375	156
24	2,47	1,01	0,39	14,8	0,64	90	472	169
36	2,87	1,11	0,48	17,8	0,71	126	607	167

* Additional calcium and phosphorus added at low D P M inclusions.

Table 2

Average daily mineral intakes of sheep receiving rations containing different levels of dried poultry manure (DPM)

D P M in ration %	Calcium g	Phosphorus g	Sulphur g	Copper mg	Molybdenum mg	Zinc mg	Iron mg	Manganese mg
0	32,5	12,1	3,75	19,1	1,1	64	526	240
12	31,3	11,8	4,90	21,2	0,9	90	615	256
24	42,7	17,5	6,75	25,6	1,1	156	817	292
36	50,8	19,6	8,50	31,5	1,3	223	1074	296

Table 3

Status and retention of copper and iron in the livers of sheep receiving different levels of dried poultry manure (DPM) in their rations

DPM Treatment	Liver mineral status*				
	Concentration mg/kg DM	Copper Total mg	Retention %	Concentration mg/kg DM	Iron** Total mg
Pr-X	250[a]	29[a]	–	373[a]	42
SEM	27	3,5		26	2,7
0	413[c]	82[c]	4,90[a]	206[c]	41
SEM	27	5,0		15	4,5
12	392[c]	77[c]	4,04	222[c]	43
SEM	27	4,8		19	2,8
24	423[c]	85[c]	3,52	238[c]	48
SEM	30	4,9		19	3,4
36	443[c]	88[c]	3,06[b]	216[c]	43
SEM	30	7,5		13	3,4

* Different superscripts within columns designate differences between treatment averages;
a - b at P < 0,05; a - c at P < 0,01 levels of significance

**Iron retention approximately 0

SEM = standard error of mean

Table 4

Status and retention of zinc and manganese in the liver of sheep receiving different levels of dried poultry manure (DPM) in their rations

DPM Treatment	Liver mineral status*					
	Concentration mg/kg	Zinc Total mg	Retention %	Concentration mg/kg	Manganese Total mg	Retention %
Pr–X	123[c]	14[a]	–	9,0[a]	1,04[a]	–
SEM	7,7	2,1		0,53	0,09	
0	136[b]	27[c]	0,37[a]	12,6[c]	2,47[c]	0,0111[a]
SEM	8,7	2,0		0,93	0,16	
12	143[e]	28[c]	0,30[a]	11,0	2,16[b]	0,0090[a]
SEM	7,0	1,9		1.05	0,20	
24	155[e]	31[c]	0,19[b]	10,1	2,04[c]	0,0057[b]
SEM	5,4	1,0		0,42	0,06	
36	162[e]	32[c]	0,14[b]	9,1[a]	1,81[d]	0,0043[b]
SEM	11,8	2,4		0,98	0,22	

* Different superscripts within columns designate differences between treatments:
c - e at P < 0,05 and a - b; a - c; a - e; d - e; a - d at P < 0,01 level of significance

SEM = standard error of mean

cant relationship between the amount of Cu present in the liver and the shrunken body mass of the lamb at slaugher ($Y = 2{,}424X - 32{,}76$, $n = 12$, $r = 0{,}724$, $P < 0{,}01$; where Y = total liver Cu in mg and X = shrunken body mass in kg after 18 hours fasting). The same did not hold for Fe, Zn or Mn. For this reason, the regression equation was used to estimate the initial Cu content of the livers of the experimental sheep, while the initial Fe, Zn and Mn levels were taken as simple mean values of these minerals in the livers of the pre-experimental slaughter group.

The liver Cu concentrations increased slightly at the 2 highest levels of DPM in the diet (Table 3), but the Cu retention rates decreased as the amount of DPM in the ration increased. The Cu retention rate was significantly lower for the 36 than for the 0 treatment.

A decrease in Fe concentration of the livers was observed (Table 3) between that in the Pr-X group and the treatment groups, though total liver Fe content remained constant. The result was that practically no additional Fe was retained in the livers of all treatments during the experimental periods.

The concentration, content and retention of Zn in the liver (Table 4) followed a trend very similar to that for Cu, while liver Mn content and concentration decreased with increased DPM inclusions. Hepatic Zn and Mn retention decreased with increased DPM intake. Differences were statistically significant for both Zn and Mn between the lowest DPM (0 and 12) and highest DPM (29 and 36) inclusion rates.

Discussion

When sheep consume Cu in excess of their requirements, a positive linear relationship between Cu intake and liver Cu content has been reported (Dick, 1954; Hemingway & MacPherson, 1967; Van Ryssen & Stielau, 1980). This indicates, according to Corbett, Saylor, Long & Leach (1978), very little homeostatic control over Cu absorption in sheep. The proportion of dietary Cu retained in the liver will therefore remain relatively constant, irrespective of Cu intake, if all other factors are constant (Van Ryssen & Stielau, 1980). Therefore, in the present trial, the pronounced reduction in hepatic Cu retention with increased poultry manure intakes must have beeen due to factors which interfere with Cu absorption rather than to a homeostatic mechanism. It is suggested that the reduced Cu retentions observed at higher manure intakes are the result of increased intakes of minerals present in DPM and antagonistic to Cu absorption and retention.

Suttle (1974) suggested that the impairment of Cu metabolism due to S could be expected if rations contained more than 0,2% S. Such reductions of Cu availability due to dietary S were observed by Wynne & McClymont (1956), Goodrich & Tillman (1966) and Boyazoglu, Barrett & Du Toit (1972). Kirchgessner & Grassmann (1970) reported decreased hepatic Cu retention in cattle when Ca levels increased from 5 to 15 g Ca/kg feed. The antagonistic effect of Zn on Cu metabolism was demonstrated in sheep (Dick, 1954; Bremner, Young & Mills, 1976) and in pigs (Suttle & Mills, 1966). Similarly, interactions were reported between Fe and Cu by Dick (1954). Abdellatif (1968) and Lawlor, Smith & Beeson (1965). The content of these materials in the experimental ration increased with increasing levels of manure inclusion and were present at levels at which Cu absorption could have been affected.

From depletion-repletion trials on sheep Suttle & Price (1976) concluded that the Cu in poultry manure was as available to sheep as the Cu in $CuSO_4$. In that trial, poultry manure was included in the ration at a 5% level. With such a low level of inclusion, the other minerals in manure cannot change the mineral content of the ration significantly – thus their influence on Cu absorption would be slight. This is also the case in the present trial where an inclusion of 12% DPM resulted in slight and probably biologically insignificant increases in the mineral content of the ration.

Poultry manure inclusion rates of up to 30% in livestock rations have often been found to give satisfactory performance (Bosman, 1973; Van der Merwe, Pretorius & du Toit, 1975). From the present trial it is clear that at these levels of inclusion the risk of Cu toxicity in sheep is very small. However, this does not exclude the possibility of Cu accumulation at higher levels of poultry manure inclusion or when the manure is derived from broilers fed copper sulphate as a growth stimulant.

No accumulation of Fe in the liver took place in the present trial. In fact, liver Fe concentrations decreased between the onset of the trial and the final stage, though total Fe content remained constant. Very effective homeostatic mechanisms are known to operate in Fe metabolism in the body (Thomas, 1970; Miller, 1979). These probably maintained Fe absorption at a low level in the present trial. Unfortuntely no blood analyses were undertaken to support this conclusion. Lawlor *et al.* (1965) suggested that evidence of Fe toxicity at relatively low Fe intakes may be the result of lowered absorption of other trace minerals present at marginal levels of requirement. High Fe intakes due to high poultry manure intakes may therefore be of greater importance in relation to interactions with other minerals than on the Fe status of the body *per se.*

The Zn content of the liver is considered to be a poor indicator of the Zn status of the ruminant (Miller, 1970) However, at a concentration of 600 mg Zn/kg DM Kincaid, Miller, Gentry, Neathery & Hampton (1976 observed that Zn accumulated in the livers, kidneys and pancreas of calves. These authors suggested that a breakdown in homeostatic control took place in the calves at high Zn intakes. In the present trial Zn levels were not as high as those used by Kincaid *et al.* (1976). It is therefore not clear to what extent homeostatic mechanisms were responsible for the reduced Zn retentions in the livers and whether the high mineral intakes contributed to this. At high dietary levels, minerals such as Ca, P, Mg, Cu and Cd are known to interact with Zn during absorption and metabolism (Miller, 1970) and this interaction may be partly responsible for the lower Zn retentions at higher DPM inclusion rates.

Tissue concentrations of Mn are characteristically very low but respond positively to increased Mn intakes (Thomas, 1970; Miller, 1979; Ivan & Hidiroglou, 1980). The measured levels of Mn in the liver correspond very well with published values (Doyle & Pfander, 1975; Ivan & Hidiroglou 1980). From the available data it is difficult to explain the numerically small though statistically significant decrease in liver Mn concentration and content in the present trial. However, the pronounced increase in Fe, Ca and P intakes with DPM inclusion may have contributed to this decrease because of the negative interaction between Mn and these minerals in the body (Thomas, 1970, Miller, 1979).

Acknowledgements

The authors wish to thank Voermol Products Ltd for financial assistance and the donation of the dried poultry manure and Epol (Pty) Ltd for mixing and pelleting the experimental rations.

References

ABDELLATIF, A.M.M., 1968. Conditioned hypocuprosis : some effects of diet on copper storage in ruminants. *Verslagen van Landbouwkundige Onderzoekingen* No. 709. Wageningen.

BLANCHAR, R.W., REHM, J. & CALDWELL, A.C., 1965. Sulphur in plant material by digestion with nitric and perchloric acid. *Proc. Soil Sci. Soc. Am.* 29, 71.

BOSMAN, S.W., 1973. Chicken litter in fattening rations for cattle and sheep. *S. Afr. J. Anim. Sci.* 3, 57.

BOYAZOGLU, P.A., BARRETT, E.L. & DU TOIT, J. De V., 1972. Sulphur containing licks and their effect on the mineral-vitamin balance of sheep. *S. Afr. J. Anim. Sci.* 2, 65.

BREMNER, I., YOUNG, B.W. & MILLS, C.F., 1976. Protective effect of zinc supplementation against copper toxicosis in sheep. *Br. J. Nutr.* 36, 551.

CORBETT, W.S., SAYLOR, W.W., LONG, T.A. & LEACH, R.M., 1978. Intracellular distribution of the hepatic copper in normal and copper-loaded sheep. *J. Anim. Sci.* 47, 1174.

DICK, A.T., 1954. Studies on the assimilation and storage of copper in crossbred sheep. *Aust. J. agric. Res.* 5, 511.

DOYLE, J.J. & PFANDER, W.H., 1975. Interactions of cadmium with copper, iron, zinc and manganese in ovine tissues. *J. Nutr.* 105, 599.

FOX, M.R.S. & REYNOLDS, H., 1973. Trace minerals in food: Nutritional-toxicological problems. *Feedstuffs.* 45, (13), 30.

GOODRICH, R.D. & TILLMAN, A.D., 1966. Effects of sulphur and nitrogen sources and copper levels in the metabolism of certain minerals by sheep. *J. Anim. Sci.* 25, 484.

HEMINGWAY, R.G. & MacPHERSON, A., 1967. The accumulation of copper in the liver of housed blackface lambs. *Vet. Rec.* 81, 695.

HILL, C.H. & MATRONE, G., 1970. Chemical parameters in the study of *in vivo* and *in vitro* interactions of transition elements. *Fed. Am. Soc. exp. Biol.* 29, 1474.

IVAN, M. & HIDIROGLOU, M., 1980. Effect of dietary manganese on growth and manganese metabolism in sheep. *J. Diary Sci.* 63, 385.

JIMINEZ, A.A., 1974. Feeding pelletted dried poultry litter to Holstein steers. *Feedstuffs.* 46, (47), 29.

KINCAID, R.L., MILLER, W.J., GENTRY, R.P., NEATHERY, M.W. & HAMPTON, D.L., 1976. Intracellular distribution of zinc-65 in calves receiving high but non toxic amounts of zinc. *J. Dairy Sci.* 59, 552.

KIRCHGESSNER, M. & GRASSMANN, E., 1970. The dynamics of copper absorption In: *Trace Element Metabolism in Animals*, pg. 277. Ed. Mills, C.T. Edinburgh: E. & S. Livingstone.

LAWLOR, M.J., SMITH, W.H. & BEESON, W.M., 1965. Iron requirements of the growing lamb. *J. Anim. Sci.* 24, 742.

LOWMAN, B.G. & KNIGHT, D.W., 1970. A note on the apparent digestibility of energy and protein in dried poultry excreta. *Anim. Prod.* 12, 525.

MILLER, W.J., 1970. Zinc nutrition of cattle : A review. *J. Dairy Sci.* 53, 1123.

MILLER, W.J., 1979. Dairy cattle feeding and nutrition. New York : Academic Press.

N R C, 1975. Nutrient requirements of domestic animals. Nutrient requirements of sheep. National Academy of Sciences, Washington, D.C.

STANDISH, J.F., AMMERMAN, C.B., SIMPSON, C.F., NEAL, F.C. & PALMER, A.Z., 1969. Influence of graded levels of dietary iron, as ferrous sulphate on performance and tissue mineral composition of steers. *J. Anim. Sci.* 29, 496.

SUTTLE, N.F., 1974. Effects of organic and inorganic sulphur on the availability of diet copper to sheep. *Br. J. Nutr.* 32, 559.

SUTTLE, N.F. & MILLS, C.F., 1966. Studies of the toxicity of copper to pigs. 1. Effects of oral supplements of zinc and iron salts on the development of copper toxicosis. *Br. J. Nutr.* 20, 135.

SUTTLE, N.F. & PRICE, J., 1976. The potential toxicity of copper-rich animal excreta to sheep. *Anim. Prod.* 23, 233.

THOMAS, J.W., 1970. Metabolism of iron and manganese. *J. Dairy Sci.* 53, 1107.

VAN DER MERWE, H.J., PRETORIUS, P.S. & DU TOIT, J.E.J., 1975. Benutting van rantsoene met battery-hoendermis as proteïenbron. *S. Afr. Tydskr. Veek.* 5, 17.

VAN RYSSEN, J.B.J., CHANNON, P. & STIELAU, W.J., 1977. Minerals and nitrogen in poultry manure. *S. Afr. J. Anim. Sci.* 7, 195.

VAN RYSSEN, J.B.J. & STIELAU, W.J., 1980. The effect of various levels of dietary copper and molybdenum on copper and molybdenum metabolism in sheep. *S. Afr. J. Anim. Sci.* 10, 37.

WILKE, P.I. & VAN DER MERWE, F.J., 1974. The amino acid composition and mineral content of chicken litter and laying-hen manure. *Fort Hare Papers.* 6 (1), 21.

WYNNE, K.N. & McCLYMONT, G.L., 1956. Copper-molybdenum-sulphate interaction in induction of ovine hypocupraemia and hypocuprosis. *Aust. J. agric. Res.* 7, 45.