Concentrations of plasma copper and zinc and blood selenium in ewes and lambs of Merino, Dohne Merino and SA Mutton Merino sheep

F.E. van Niekerk* and C.H. van Niekerk

Department of Human and Animal Physiology, University of Stellenbosch, Stellenbosch 7600, Republic of South Africa

E.W.P. Heine

Regional Veterinary Laboratory, Directorate of Veterinary Services, Department of Agriculture, Economics and Marketing, Private Bag X5020, Stellenbosch 7600, Republic of South Africa

J. Coetzee

Department of Sheep and Wool Science, Faculty of Agricultural Sciences, University of Stellenbosch, Stellenbosch 7600, Republic of South Africa

Received 14 June 1988; accepted 20 February 1989

Concentrations of plasma copper and zinc as well as blood selenium were determined in single and twin lambs and their dams for a period of 120 days from the birth of the lambs, using Merino, Dohne Merino and SA Mutton Merino sheep. The ewes and their lambs were kept on the same pastures and received the same feed supplementation. Plasma concentrations of copper and zinc were respectively 50% lower and 40% higher in new-born lambs, but both returned to adult concentrations within the next 14 days. Concentrations of selenium in lambs throughout the preweaning period were lower than those of the ewes. Blood concentrations of selenium in lambs of all three breeds declined rapidly during the first eight weeks after birth. At the age of six months, Merino ram lambs displayed higher ($P \le 0.05$) plasma copper and blood selenium concentrations but lower ($P \le 0.05$) concentrations of plasma zinc than did Dohne Merino or SA Mutton Merino ram lambs.

Plasmakoper-, plasmasink- asook bloedseleniumkonsentrasies is by ooie en hul enkel- of tweelinglammers vanaf geboorte tot op 'n ouderdom van 120 dae bepaal. Merino-, Dohne Merino- en SA Vleismerino-skape is gebruik. Ooie en lammers van al drie rasse is op dieselfde tipe weiding met dieselfde aanvullende voeding gehou. By geboorte was die plasmakoperkonsentrasies van die lammers ongeveer 50% laer, en die plasmasinkkonsentrasies ongeveer 40% hoër as dié van die ooie. Hierdie konsentrasies by die lammers het egter binne 14 dae verander tot normale konsentrasies van volwasse skape. Bloedseleniumkonsentrasies van lammers was tydens die voorspeense periode deurgaans laer in vergelyking met dié van ooie. Bloedseleniumkonsentrasies van lammers van al drie rasse het 'n skerp daling tydens die eerste agt weke na geboorte getoon. Merino-ramlammers het op 'n ouderdom van ses maande hoër ($P \le 0.05$) plasmakoper- en bloedseleniumkonsentrasies, maar laer ($P \le 0.05$) plasmasinkkonsentrasies as Dohne Merino- en SA Vleismerino-ramlammers gehad.

Keywords: Breed, ewes, lactation, lambs, trace elements.

* To whom correspondence should be addressed at present address: Elsenburg Agricultural Centre, Private Bag, Elsenburg 7607, Republic of South Africa.

Introduction

Trace-element adequacy of new-born lambs is of great importance to ensure a high survival and growth rate (Suttle, 1988; Van Niekerk & Van Niekerk, 1989b; Williams, McDonald & Bremner, 1978). Possible differences in the metabolism and requirements of trace elements between lambs and adult sheep of different breeds are important, especially in the diagnosis of trace-element deficiencies of lambs at risk (Suttle, 1988). Copper deficiency in the ewe is reflected by the birth of lambs with nervous and/or bone disorders (Grace, 1983). When lambs contract enzoötic ataxia after birth, many die within three to four days, whereas older lambs nearly always survive (Grace, 1983). The metabolism of copper is complex and the dietary copper requirement can vary according to the presence of antagonists (Underwood, 1977). Concentrations of copper and zinc in the liver are more reliable than concentrations of these minerals in blood in assessing the status thereof in sheep (Grace, 1983). Van Niekerk & Van Niekerk (1989a) discussed limitations in determining the biologically active concentration of copper in plasma under dietary conditions of high molybdenum and sulphate intakes. It is, however, impractical to repeatedly obtain large numbers of liver samples from new-born and young lambs by means of liver biopsies.

Concentrations of plasma zinc are not only affected by dietary zinc intake but also by a number of normal physiological events (Grace, 1983). In cattle, concentrations of plasma zinc fall at parturition, returning to normal within the next few days (Dufty, Bingley, & Cove, 1977). Profound changes also occur during various disease states (e.g. liver damage, chronic and acute infections, acute tissue injury) and under stress (Corrigall, Dalgarno, Ewan & Williams, 1976). *Inter alia* major reductions in concentrations of plasma zinc reflect a redistribution of zinc within the body; notably a major movement of zinc to the liver (Beisel, 1977).

Selenium plays an important role in the growth and development of the foetus as well as that of young lambs (Hartley & Grant, 1961). A selenium deficiency critically affects a young lamb at three different stages of growth. Lambs may be stillborn or die within 24 h after birth (congenital white muscle disease). Otherwise,

lambs of 3 to 6 weeks of age may contract a condition known as delayed white muscle disease although hoggets of 9 to 12 months may also be affected. Older lambs of 9 to 12 months may show poor growth (hogget ill thrift). The symptoms as well as pathology of these three conditions differ as described by Hartley & Grant (1961).

It was the objective of this study to determine whether lactation and the number of lambs born would have any influence on the concentrations of plasma copper and zinc and blood selenium in ewes and lambs. This was done for three different breeds of sheep and possible breed differences regarding these trace minerals are discussed.

Materials and Methods

Merino (n = 74), Dohne Merino (n = 119) and SA Mutton Merino (n = 124) ewes and their lambs which were kept on the experimental farm of the University of Stellenbosch, were used. Ewes of the three breeds were mated at the same time every year. During pregnancy. all the ewes of all three breeds used in Phase 1 received the same feeding and treatment. Ewes grazed on wheat stubble from three weeks after mating untill four weeks prior to lambing, when they were transferred to kikuyu pastures. From six weeks prior to lambing, all the ewes received 0,5 kg supplementary feeding daily. This was increased by 0,1 kg per week up to lambing. The diet consisted of 55% oaten hay, 35,6% barley, 4% fish meal, 3% Rumevite stud concentrate, 1% bentonite, 0,4% NaHCO₃, 0,2% MgO, 0,36% Na₂S₂O₃ and 0,41% NaCl. After lambing, ewes received a daily supplement (1,5-2,0 kg) consisting of 53% lucerne, 40,3% maize, 3% fish meal, 0,35% CaCO₃, 2% bentonite, 0,4% NaHCO₃, 0,2% MgO, 0,29% Na₂S₂O₃ and 0,45% NaCl while grazing on pastures consisting of kikuyu, oats, rye grass, clover and lucerne.

Phase 1

During the 1987 lambing season, experimental animals were selected one week after lambing, commencing from among those that had given birth within the previous 48 h. Initial selection resulted in groups of seven ewes with single lambs and seven with twin lambs from each breed. Owing to lamb mortality, only results from five ewes with single lambs and five with twin lambs both of the Merino and the SA Mutton Merino breeds, were analysed in this study. In the case of the Dohne Merino, four ewes with single lambs and six with twin lambs were included.

The first blood samples were taken from both the ewes and their lambs within 48 h after birth and this was repeated at 14-day intervals until weaning at *ca*. 120 days. Lambs were weighed at birth, at 100 days and again at the age of 6 months. During Phase 1, the selected experimental animals of all three breeds were kept as a single flock.

Phase 2

Lambs of all the ewes of the three breeds, including the lambs used in Phase 1, were weaned at an approximate

age of 120 days. At this stage, all the lambs that were unattractive representatives of the pure breed, were culled. After culling, all the remaining ram lambs comprising 25 Merinos, 38 Dohne Merinos and 37 SA Mutton Merinos, were included in Phase 2. On reaching the age of 6 months, blood samples were taken from all the ram lambs. Ram lambs from these three breeds were kept as one flock under identical feeding conditions and grazed on pastures consisting of oats, rye grass, clover, lucerne and kikuyu. The diet was supplemented with 1 kg of a pelleted feed daily. This feed had a Cu, Zn and Se content of 5,8, 25 and 0,09 μ g/g, respectively, on a DM basis.

Blood samples (10 ml) were taken from the vena jugularis with 18 G needles into heparinized vacuum tubes (Vac-U-Test, Radem Laboratories). A sample of 2 ml blood was kept at -20° C to determine the selenium concentration while the remaining blood was centrifuged within 3 h after collection and the plasma removed and stored at -20° C for the determination of copper and zinc. Extreme care was taken to avoid haemolysis of blood samples.

Concentrations of plasma copper and plasma zinc were determined by atomic absorption spectrophotometry according to the methods used by Van Niekerk & Van Niekerk (1989a). Blood selenium was determined by the fluorometric method as described by Koh & Benson (1983).

Results were analysed according to standard one-way analysis of variance procedures using the P1V program of the BMDP statistical packet (Engelman, 1981). Differences between treatment means were tested by *t*-test procedures (Snedecor & Cochran, 1980).

Results and Discussion

The mean concentrations of plasma copper in the lambs and ewes of the three breeds are shown in Figure 1.

At birth (week 0), the mean concentrations of plasma copper in the lambs, irrespective of birth status and breed, were ca. 50% less than those of adult ewes (Figure 1). At two weeks of age, concentrations of plasma copper of lambs increased to concentrations similar to those of adult ewes. These low concentrations of plasma copper at birth are caused by very low concentrations of the copper-containing protein, caeruloplasmin, in the plasma of the foetus and newborn lamb (McCosker, 1968), but attain normal values within 3 to 5 days after birth if the copper intake of the lamb is adequate. Lambs born from ewes which suffered from a copper deficiency during pregnancy displayed total plasma copper concentrations of 10 to 15 µg/dl at birth which remained low for several weeks after birth (Van Niekerk & Van Niekerk, 1989b). Number of lambs born per ewe had no influence on the total plasma copper concentrations of the lambs throughout the experimental period.

The mean concentration of plasma copper was compared between breeds (Table 1), but no consistent significant differences were found during lactation,

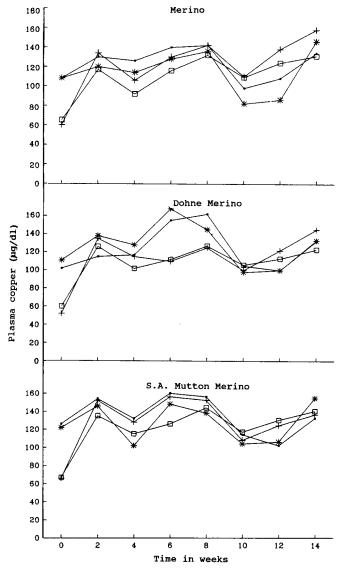


Figure 1 Mean concentrations of plasma copper $(\mu g/dl)$ of ewes with single and twin lambs, as well as of the lambs, measured over a period of 14 weeks from the birth of the lambs. Ewes with single lambs (-----), ewes with twin lambs (--------), single lambs (-----------).

Table 1 Comparison of the mean concentrations $(\mu g/dl)$ of plasma copper of adult Merino, Dohne Merino and SA Mutton Merino ewes during lactation

Time (weeks)	Merino	Dohne Merino	SA Mutton Merino
0	108 a	108 a	124 b
2	125 a	129 a	150 b
4	120 a	124 a	117ª
6	134 a	163 ^b	154 ab
8	139 a	152 a	147 a
10	90 a	101 ^{ab}	109 ^b
12	97 a	100 a	104 a
14	140 a	133 a	143 a

a,b Values in the same row with different headings differ significantly $(P \le 0.05)$.

irrespective of the number of lambs that the ewes had given birth to.

When mean concentrations of total plasma copper between the lambs (Table 2) of the three different breeds were compared, no consistent significant differences were found during the preweaning period.

Table 2 Comparison of the mean concentrations of plasma copper ($\mu g/dl$) of Merino, Dohne Merino and SA Mutton Merino lambs from birth until 14 weeks of age

Age (weeks)	Merino	Dohne Merino	SA Mutton Merino
0	63 a	58 ª	66 a
2	122 a	128 a	140 a
4	96 a	105 a	119 a
6	120 a	112 a	136 ^b
8	135 ab	126 a	146 ^b
10	109 a	105 a	114ª
12	128 a	115 a	128 a
14	140 a	128 a	138 a

a,b Values in the same row with different headings differ significantly $(P \le 0.05)$.

Although no significant differences were found between the lactating ewes and the lambs of the Merino, Dohne Merino and SA Mutton Merino breeds on this particular farm, it cannot be accepted that concentrations of plasma copper of all three breeds were always the same. Van Niekerk & Van Niekerk (1989c) found that Merino ewes have a slightly higher plasma copper concentration than Dohne Merino and SA Mutton Merino ewes, but all the ewes were non-pregnant during that study. When, however, the concentrations of plasma copper of 6-month-old ram lambs (Table 7) of the three breeds under discussion were compared, it was obvious that the concentrations of copper in the plasma of Merino ram lambs were significantly $(P \le 0.05)$ higher than in Dohne Merino or SA Mutton Merino ram lambs. This is in agreement with the findings of Van Niekerk & Van Niekerk (1989c).

The mean concentrations of plasma zinc in the lambs and ewes are shown in Figure 2.

At birth, the concentrations of plasma zinc in the lambs were ca. 40% higher than those of the ewes, but it declined within two weeks after birth, eventually reaching concentrations found in ewes. Concentrations of plasma zinc did not differ significantly between single and twin lambs.

In Table 3, the mean concentrations of plasma zinc in ewes of the three breeds are compared during lactation. At birth (week 0), there were no significant differences between concentrations of plasma zinc in ewes between breeds. However, fourteen days after parturition, the concentrations of plasma zinc were significantly lower $(P \le 0.05)$ in Merino ewes than in either the Dohne Merino or the SA Mutton Merino ewes, remaining so throughout lactation.

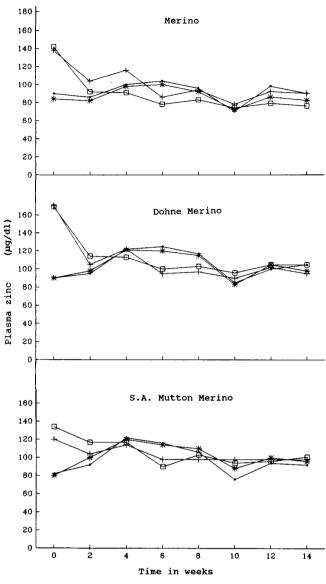


Figure 2 Mean concentrations of plasma zinc $(\mu g/dl)$ of ewes with single and twin lambs, as well as of the lambs, measured over a period of 14 weeks from the birth of the lambs. Ewes with single lambs (-+-), ewes with twin lambs (-+-), single lambs (---).

Table 3 Comparison of the mean concentrations $(\mu g/dl)$ of plasma zinc of adult Merino, Dohne Merino and SA Mutton Merino ewes during lactation

Time				
(weeks)	Merino	Dohne Merino	SA Mutton Merino	
0	87ª	90°	81 ^a	
2	84 a	97 ^b	96 ^b	
4	99 a	122 b	121 ^b	
6	102 a	122 b	115 b	
8	94 a	116 ^b	108 b	
10	71 ^a	84 ^b	82 a	
12	92 a	103 b	97 ^{ab}	
14	86 ª	101 ^в	94 ^b	

^{a,b} Values in the same row with different headings differ significantly $(P \le 0.05)$.

The mean concentrations of plasma zinc in the lambs are given in Table 4.

Table 4 Comparison of the mean concentrations of plasma zinc ($\mu g/dl$) in Merino, Dohne Merino and SA Mutton Merino lambs from birth until the age of 14 weeks

Age (weeks)	Merino	Dohne Merino	SA Mutton Merino
0	140 a	169 ^b	129 a
2	96 ª	111 ^b	112 ^b
4	99 a	115 ^b	116 b
6	80 a	99 b	93 b
8	86 a	101 ^ь	101 ^b
10	75 a	95 b	95 ^b
12	83 a	104 ^ь	96 ^b
14	80 a	102 ^ь	100 b

^{a,b} Values in the same row with different headings differ significantly $(P \le 0.05)$.

From the second week after birth, the concentrations of plasma zinc in Merino lambs were also significantly lower ($P \le 0.05$) than those of the Dohne Merino and SA Mutton Merino lambs. These results are similar to those found for adult ewes (Table 3). It is noteworthy that, while there are indications that the plasma concentrations of copper are higher in the Merino than in the Dohne Merino or SA Mutton Merino (Van Niekerk & Van Niekerk, 1989c), the plasma zinc concentrations are lower $(P \le 0.05)$ in the Merino. By inducing a copper deficiency in SA Mutton Merino ewes, Van Niekerk & Van Niekerk (1989b) found that the total plasma copper decreased while the concentrations of plasma zinc increased. It is, however, doubtful whether antagonism between copper and zinc plays any role in the equilibrium of these two minerals in the blood or plasma, but it is an aspect which must not be overlooked.

The mean concentrations of blood selenium in the ewes and their lambs of the three breeds are given in Figure 3. Similar patterns were observed in the concentrations of blood selenium in the three breeds. Concentrations of blood selenium in lambs at birth were lower than those of the ewes, whereas twin lambs displayed lower concentrations of blood selenium than single lambs. These differences between the ewes and their lambs existed throughout the preweaning period. During the first eight weeks after parturition, the concentrations of blood selenium in the Merino lambs declined from a mean of 231 to 119 ng/ml, in Dohne Merino lambs from 196 to 128 ng/ml and from 221 to 115 ng/ml in SA Mutton Merino lambs. During the last six weeks before weaning, these values remained relatively constant at these lowered concentrations. During this same period, concentrations of blood selenium in ewes declined only slightly. A possible explanation for this may lie in the weaning process. Shortly after birth, lambs have a feed conversion ratio of approximately 1:1 which increases rapidly to approximately 3—5:1 at weaning, i.e. at the

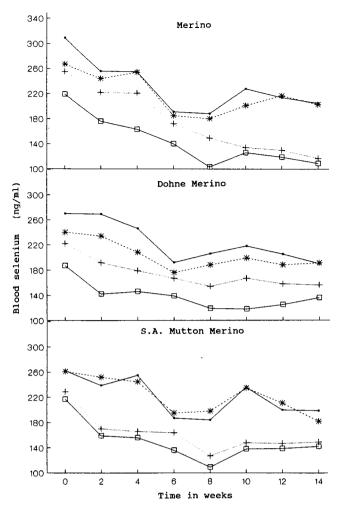


Figure 3 Mean concentrations of blood selenium (ng/ml) of ewes with single and twin lambs, as well as of the lambs, measured over a period of 14 weeks from the birth of the lambs. Ewes with single lambs (———), ewes with twin lambs (————), single lambs (—————).

age of 120 days (personal communication; Vosloo, L.P., P.O. Box 604, Uniedal, Stellenbosch 7600, 1988). During this time, the diet of the lamb changes from milk to solids which implicates a possible difference in availability of absorption. As the feed conversion ratio increases, more food is consumed and therefore more selenium might be absorbed.

Differences between concentrations of blood selenium in single and twin lambs are in agreement with the findings of Wright & Bell (1964). Although these differences in concentrations of blood selenium in single and twin lambs were consistent for all three breeds during the preweaning period, it was, however, not significant. The mean concentrations of blood selenium in the ewes are compared in Table 5. Although Merino ewes had the highest mean concentration of blood selenium just after birth, this difference was, however, not consistently maintained throughout lactation.

The mean concentrations of blood selenium in the lambs are compared in Table 6. Merino lambs displayed the highest concentrations of blood selenium immediately *post partum*. The decline in concentrations of blood selenium towards weaning was, however, more

Table 5 Comparison of the mean concentrations of blood selenium (ng/ml) of adult Merino, Dohne Merino and SA Mutton Merino ewes during lactation

Time (weeks)	Merino	Dohne Merino	SA Mutton Merino
0	288 a	252 b	262 a
2	250 a	248 a	246 a
4	254 a	223 b	250 a
6	188 a	183 a	191 ^a
8	184 a	196 a	191 a
10	214 ab	207 a	235 b
12	216 a	195 ^b	205 ab
14	204 a	190 a	191 ^a

^{a,b} Values in the same row with different headings differ significantly $(P \le 0.05)$.

Table 6 Comparison of the mean concentrations of blood selenium (ng/ml) of Merino, Dohne Merino and SA Mutton Merino lambs from birth until the age of 14 weeks

Age (weeks)	Merino	Dohne Merino	SA Mutton Merino
0	231 a	196 ^b	221 a
2	191 a	155 ^b	163 ^b
4	183 a	154 ^b	159 b
6	151 a	146 ^b	145 ^b
8	119 a	128 ª	115 a
10	129 a	130 a	141 ^a
12	123 a	133 ab	141 ^b
14	112 a	141 ^b	144 ^b

^{a,b} Values in the same row with different headings differ significantly $(P \le 0.05)$.

marked in lambs of this breed than in the other two breeds. Merino lambs therefore tended to have significantly ($P \leq 0.05$) lower concentrations of blood selenium than the Dohne Merino and SA Mutton Merino lambs. However, when adult, non-pregnant ewes were examined, Merinos tended to have the highest concentrations of blood selenium of the three breeds under discussion (unpublished results). This observation suggests that lactation might have an important influence on the concentrations of blood selenium.

The mean concentrations of plasma copper and zinc, as well as blood selenium, in the ram lambs at the age of six months are given in Table 7. These results confirmed the breed differences in concentrations of plasma copper and zinc as well as concentrations of blood selenium during Phase 1. Concentrations of plasma copper in Merino ram lambs were significantly higher ($P \le 0.05$), while concentrations of plasma zinc were significantly ($P \le 0.05$) lower, than in ram lambs of the other two breeds. Merino ram lambs also had significant higher ($P \le 0.05$) concentrations of blood selenium when compared with the other two breeds.

Table 7 Mean concentrations of plasma copper $(\mu g/dl)$ and zinc $(\mu g/dl)$ as well as blood selenium (ng/ml) of 6-months-old Merino, Dohne Merino and SA Mutton Merino ram lambs

Mineral	Merino n = 25	Dohne Merino n = 39	SA Mutton Merino n = 37
Copper	92 a	78 ⁶	77 ^b
Zinc	$70^{\rm a}$	88 ^b	86 ^b
Selenium	100 a	70 ^ь	74 ^b

^{a,b} Values in the same row with different headings differ significantly $(P \le 0.05)$.

Conclusions

Concentrations of plasma copper in new-born lambs were found to be approximately 50% less than those of adult sheep, while concentrations of plasma zinc were approximately 40% higher in new-born lambs. At the age of fourteen days, both the concentrations of plasma copper and zinc of lambs were similar to those of adult ewes. The number of lambs born per ewe had no influence on plasma concentrations of these trace minerals in lambs.

Concentrations of blood selenium in new-born lambs were lower than those of ewes and declined sharply during the first eight weeks *post partum*. The change in diet from milk to solids, as well as the availability of selenium from these substances, might contribute to this decline in the concentration of blood selenium.

According to present results, it may be concluded that the Merino breed maintains higher $(P \leq 0.05)$ plasma copper and blood selenium concentrations than either the Dohne Merino or SA Mutton Merino under conditions where no deficiencies prevail. On the other hand, both the Dohne Merino and SA Mutton Merino breeds maintain higher $(P \leq 0.05)$ concentrations of plasma zinc than the Merino. These breed differences in the concentrations of blood and plasma trace elements might be indicative of differences in the nutritional need for these trace minerals. Different absorption rates from the digestive tract in these breeds might be a contributing factor and should be investigated.

References

- BEISEL, W.R., 1977. Zinc metabolism in infection. In: Zinc metabolism: Current aspects in health and disease. Alan R. Liss Inc. New York, New York. pp. 155—176.
- CORRIGALL, W., DALGARNO, A.C., EWAN, L.A. & WILLIAMS, R.B., 1976. Modulation of plasma copper and zinc concentrations by disease states in ruminants. *Vet. Rec.* 99, 396.
- DUFTY, J.H., BINGLEY, J.B. & COVE, L.Y., 1977. The plasma zinc concentration of non-pregnant, pregnant and parturient Hereford cattle. *Aust. Vet. J.* 53, 519.
- ENGELMAN, L., 1981. One-way analysis of variance and covariance. In: BMDP Statistical Software (1981 edn.) Ed. Dixon, W.J., Univ. California Press, USA.
- GRACE, N.D., 1983. The mineral requirements of grazing ruminants. NZ Soc. Anim. Prod., Occ. publ. no. 9.
- HARTLEY, W.J. & GRANT, A.B., 1961. A review of selenium responsive diseases of New Zealand livestock. *Fed. Proc.* 20, 679.
- KOH, T.S. & BENSON, T.H., 1983. Critical re-appraisal of fluorometric method for determination of selenium in biological material. *J. Assoc. Off. Anal. Chem.* 66, 918.
- McCOSKER, P.J., 1968. Observations on blood copper in the sheep. I. Normal copper status and variations induced by different conditions. *Res. Vet. Sci.* 9, 91.
- SNEDECOR, G.W. & COCHRAN, W.G., 1980. Statistical methods (7th edn.). Iowa State University Press, Ames, USA.
- SUTTLE, N.F., 1988. Predicting the risk of mineral deficiencies in grazing animals. S. Afr. J. Anim. Sci. 18, 15.
- UNDERWOOD, E.J., 1977. Trace elements in human and animal nutrition (4th edn.). Academic Press, New York.
- VAN NIEKERK, F.E. & VAN NIEKERK, C.H., 1989a. Effect of high levels of dietary molybdenum and sulphate on SA Mutton Merino sheep. I. Mineral status and haematological parameters. S. Afr. J. Anim. Sci. 19, 107.
- VAN NIEKERK, F.E. & VAN NIEKERK, C.H., 1989b. Effect of high levels of dietary molybdenum and sulphate on SA Mutton Merino sheep. II. Certain aspects of the oestrous cycle and pregnancy. S. Afr. J. Anim. Sci. 19, 114.
- VAN NIEKERK, F.E. & VAN NIEKERK, C.H., 1989c. Effect of haemoglobin type on the concentration of plasma copper in Merino, Dohne Merino and SA Mutton Merino ewes. S. Afr. J. Anim. Sci. 19, 131.
- WILLIAMS, R.B., McDONALD, I. & BREMNER, I., 1978. The accretion of copper and of zinc by the foetuses of prolific ewes. *Br. J. Nutr.* 40, 377.
- WRIGHT, P.L. & BELL, M.C., 1964. Selenium-75 metabolism in the gestating ewe and foetal lamb: Effects of dietary tocopherol and selenium. *J. Nutr.* 84, 49.