CHAPTER 2

2.0 THE EFFECT OF HIGH BROILER LITTER DIETS AS SURVIVAL RATION ON THE HEALTH OF SHEEP

2.1 INTRODUCTION

Drought occurs frequently in southern Africa and devastating veld fires often destroy vast areas of grassland during the dry winter periods. Consequently, livestock farmers often have to resort to emergency feeding measures to sustain their animals, utilising whatever feed is available. Such conditions have prompted farmers to resort to feeding poultry manure/litter (Fourie *et al.*, 1991) as the sole feed for their livestock.

Sugarcane molasses is an available energy source for the rumen micro-organisms (Yan *et al.*, 1996), readily accessible in many parts of Southern Africa and not consumed by humans. It is often mixed with broiler litter to complement the high nitrogen content of litter with available energy in a ruminant's diet.

Although poultry manure / litter has been used successfully as a ration ingredient in ruminant diets (Bosman, 1973; Kargaard & Van Niekerk, 1977), many potential problems exist. Problems of nutrients imbalances and the effects of potentially toxic component such as pathogens, dietary copper, medicinal compounds, mycotoxins, ionophores and botulinum toxin (Fontenot, 1991; Fourie *et al.*, 1991) will be accentuated when the product is fed as a sole feed. Silanikove & Tiomkin (1992) reported liver damage in beef cows when overwintered on high poultry litter diets in Israel. The authors attributed the

damage to high ruminal ammonia concentrations due to fermentation of the litter in the rumen. In South Africa, this report of liver damage seems to have prompted warnings against the use of poultry litter as a ruminant feed at any rate of inclusion. However, liver damage is not usually observed in cattle when poultry litter is fed, except when it contains high concentrations of copper (Rankins *et al.,* 1993). The objective of the present study was to investigate the effect of high levels of broiler litter on the health of sheep. Since broiler litter contains excessive levels of non-protein nitrogen, improving the dietary nitrogen to energy balance to improve the product as a survival feed was evaluated as well.

2.2 MATERIALS AND METHODS

2.2.1 Experimental procedure

Eighteen South African Mutton Merino wethers (*ca.* 2 years old and *ca.* 42 kg body mass) were assigned randomly to 3 treatments, namely pure broiler litter and broiler litter mixed with 7.5 or 15.0 percent molasses. It would have been preferred to have a negative control, however, this was not possible due limited availability of facilities. Sun-dried broiler litter with wood shavings as bedding material was used. The litter was sifted to remove lumps and foreign material. Before the onset of the trial the sheep were vaccinated twice against botulism and dosed with a broad-spectrum anthelmintic. During a 14-day adaptation period the sheep received broiler litter and grass hay. The sheep were fed individually in slatted-floor feeding pens with free access to water. Feed refusal was measured daily, the sheep weighed once a month. Feed samples were collected

throughout the trial for chemical analyses. Blood samples collected (before and during experimental period) by venipuncture in heparinised vacutainers and spot urine samples were obtained 4 times during the experimental period. A spot urine sample was collected in a clean plastic bag tied to the stomach of the wether. The urine was preserved in 10% HCl.

After an average of 83 days the wethers were slaughtered over a period of 10 days. The warm carcasses, livers and kidneys were weighed. Liver, heart and kidney samples were taken and preserved in 10% buffered formalin for histopathological examination. Liver samples were also dried at 80^0C for Cu analysis. Backfat thickness at the 13^{th} rib was measured on the cold carcass.

2.2.2 Laboratory Analyses

The concentrations of haemoglobin in whole blood, glucose and bilirubin in plasma, and the activities of the following plasma enzymes: aspartate aminotransferase (AST, EC 2.6.1.1), creatine kinase (CK, EC 2.7.3.2), alanine aminotransferase (ALT, EC 2.6.1.2) S-glutamyltransferase (SGT, EC 2.3.2.2) and sorbitol dehydrogenase (SDH, EC 1.1.1.14) were measured using Boehringer Mannheim analytical kits (Boehringer Mannheim GmbH Diagnostica, Germany). An analytical kit was used to determine the concentration of Beta hydroxybutyrate in plasma (Sigma Diagnostics). Packed cell volume in whole blood was determined by microhaematocrit. The method described by De Villiers *et al.* (1977) was used to determine free fatty acid concentration in plasma, that of Tietz (1976) for plasma albumin and the Bertelot method (Anonymous, 1974) for plasma urea nitrogen

concentrations. Total allantoin concentration in urine was measured (Borchers, 1977) to estimate urinary purine concentration. The creatine concentration in urine was read on an auto-analyser using the alkaline picrate method.

Atomic absorption spectrophotometry was used to determine the calcium, copper, magnesium, sodium, potassium, manganese and zinc concentrations in the respective samples. The fluorometric method of Koh & Benson (*1*983) was used to determine selenium concentrations. Standard reference samples (bovine liver 1577b and peach leaves 1547; National Bureau of Standards, Gaithersburg, MD, USA) were used to verify the accuracy of the mineral assays. Crude protein, ash, phosphorus, ether extract, neutral detergent fibre and acid detergent fibre concentrations in each sample were obtained using standard laboratory techniques. The litter was screened with a thin-layer chromatography for the presence of ionophore antibiotics and quantified colorimetrically (Golab *et al.*, 1973).

Pathology

Tissues were fixed in 10% formalin, sectioned and stained with haematoxylin and eosin according to standard methods. Myocardial lesions were scored according to a histological classification system applied by Bastianello *et al.* (1995).

2.2.3 Statistical analysis

Data were subjected to analyses of variance and correlation coefficients were calculated using Minitab statistical software (Minitab, State College, Pennsylvania).

2.3 RESULTS

The DM content of the pure litter was 923 g/kg, and the addition of molasses decreased it to 917 and 870 g/kg for the 92.5 and 85 percent litter treatments respectively. The chemical composition of the experimental diets is presented in Table 2.1. The crude protein concentration of the pure litter was 182 g/kg DM. The inclusion of molasses decreases the crude protein concentration to 169 and 168 g/kg DM for the 92.5 percent and 85 percent broiler diets respectively. The copper concentration in the diets varied between 22 and 27 mg/kg DM. The calcium to phosphorus ratio in all the diets was approximately 2:1. The sheep in the 15 percent molasses group consumed significantly more feed ($P<0.01$) than the sheep on the 7.5 percent molasses and the pure litter groups (Table 2.2). This was associated with significantly higher ($P<0.01$) final body mass of the sheep in high molasses treatment compared to the other treatments. In the 15 percent molasses group, intakes reached an optimum after 3 weeks on the diet while those on the other 2 diets reached optimum intake at about 6-7 weeks. Carcass mass and fat cover at the last rib area of the carcass tended to be higher in the molasses-supplemented groups than in the control, but differences were not significant (Table 2.2).

Throughout the trial the concentrations of free fatty acid, β-hydroxybutyrate and glucose in plasma showed no differences among treatments (Table 2.3). Plasma urea nitrogen concentrations at the last collection of the trial were 298, 286, and 276 mg/l for the 100, 92.5 and 85 percent broiler litter treatment respectively, versus a quoted normal range of 80-200 mg/l (Kaneko, 1989) (Table 2.3).

Table 2.1: Chemical composition of broiler litter and litter with molasses (Dry matter basis)

Broiler litter (%):	**100**	**92.5**	**85**
Molasses (%):	**0**	**7.5**	**15**
Dry matter (g/kg)	923	917	870
Ash (g/kg)	97	110	124
Crude protein (g/kg)	182	169	168
Ether extract (g/kg)	11.2	9.4	8.2
NDF[a] (g/kg)	540	520	460
ADF[b] (g/kg)	395	355	334
Calcium (g/kg)	15.0	16.0	16.0
Phosphorus(g/kg)	8.1	8.4	7.4
Sodium (g/kg)	2.90	2.75	3.35
Potassium (g/kg)	9.9	11.9	12.7
Magnesium (g/kg)	4.71	4.79	4.77
Copper (mg/kg)	22	27	22
Manganese(mg/kg)	214	241	231
Selenium (mg/kg)	0.68	0.66	0.66

[a]NDF = neutral detergent fibre.

[b]ADF = acid detergent fibre.

Table 2.2: Average feed intake, body mass gain, body mass at slaughter, fresh liver, kidney and carcass mass and fat thickness at the last rib area of the carcass (means ± SD)

Broiler litter (%)	**100**	**92.5**	**85**
Molasses (%)	**0**	**7.5**	**15**
Feed intake (kg/d)	$1.3^{a*} \pm 0.08$	$1.5^{a} \pm 0.15$	$2.1^{b} \pm 0.07$
Final body mass (kg)	$44^{a} \pm 3.6$	$48^{ab} \pm 3.6$	$52^{b} \pm 2.4$
Gain/d (g)	26 ± 23	63 ± 32	101 ± 10
Carcass mass (kg)	20 ± 0.9	22 ± 1.3	23 ± 0.9
Liver:			
Mass (fresh) (g)	610 ± 40	592 ± 41	668 ± 23
as % body mass	1.4 ± 0.19	1.2 ± 0.18	1.3 ± 0.03
Kidney:			
As % carcass mass	3.0 ± 0.33	2,8 ± 0.15	2.9 ± 0.12
Kidney mass (g)	108 ± 6.0	134 ± 5.4	134 ± 6.5
Subcutaneous fat			
Fat thickness (mm)	1.8 ± 0.21	2.6 ± 0.52	2.8 ± 0.68

*Values within a row with different superscripts differ significantly at $p < 0.05$.

Table 2.3: Concentration of various plasma metabolites and activity of enzymes in plasma at the last collection (mean ± SD)

Broiler litter (%)	**100**	**92.5**	**85**	
Molasses (%)	**0**	**7.5**	**15**	**Norm***
Free fatty acids (mmol/l)	0.6 ± 0.16	0.35 ± 0.05	0.47 ± 0.08	0.55**
β-hydroxybutyrate (mmol/l)	0.3 ± 0.03	0.19 ± 0.02	0.21 ± 0.02	0.55
Glucose (mmol/l)	3.55 ± 0.1	3.35 ± 0.1	3.47 ± 0.12	2.8 – 4.4
Urea nitrogen (mg/l)	293 ± 17	267 ± 14	257 ± 16	80 – 200
AST[a] (U/l)	58 ± 3.9	56 ± 2.5	61 ± 8.5	61
δ-GT[b] (U/l)	39 ± 1.9	37 ±4.1	39 ± 3.5	20 – 50
CK[c] (U/l)	63 ± 10.6	56 ± 22.6	40 ± 3.6	8 – 13
SDH[d] (U/l)	1.6 ± 0.2	1.8 ± 0.16	1.4 ± 0.19	8 – 16
ALT[e] (U/l)	16.5 ± 1.94	13.3 ± 3.15	16.5 ± 2.3	30

[a]AST = aspartate aminotransferase.

[b]δGT = gamma glutamyltransferase.

[c]CK = creatine kinase.

[d]SDH = sorbitol dehydrogenase.

[e]ALT = alanine aminotransferase.

*Kaneko (1989)

**De Villiers *et al.* (1977)

Differences between treatments were not significant

However, high concentrations were measured from the onset of the trial. The average ± standard deviation (SD) plasma albumin (35.3±4.14 g/l), plasma globulin (24.9±4.79 g/l), bilirubin (30.4±6.2 g/l), pack cell volume (0.78±0.048) and haemoglobin (164±13.9 g/l) concentrations remain relatively constant throughout the trial, showed no treatment effects and were within the normal range for sheep (Kaneko, 1989). Copper concentrations in the livers did not differ significantly among treatment group and were 387, 383 and 338 mg/kg DM for the 100, 92.5 and 85 percent broiler litter treatment respectively.

No significant differences in the activity of any of the plasma enzymes were observed between the different group (Table 2.3). Activities were within the normal ranges for sheep with the exception of plasma CK, where the activity was higher than the expected normal values. At the final blood collection CK activities were recorded at 62.9±10.6, 56.1±22.6 and 40.0±3.6 U/l for the pure litter, 92.5 and 85 percent litter diet, respectively. However, these values were within a similar range throughout the trial. The activity of plasma AST was randomly distributed and did not show any correlation with pathological lesions in the liver or heart.

The purine:creatinine $(W^{0.75})^{-1}$ (PD:C) ratios during the trial are presented in Table 2.4. At 28 days after the onset of the trial these ratios were lower than at other stages, although differences were not statistically significant.

Mineral concentrations in the plasma did not reflect any treatment effect. Average concentrations ± SD (mmol/l) over the experimental period were: 2.15±0.12 calcium; 2.48±0.394 inorganic phosphorus; 12.6±2.49 copper; 0.987±0.076 magnesium; 145±3.6 sodium; 5.2±0.29 potassium; 16.8±1.66 zinc. The calcium to phosphorus ratio in urine varied widely and did not show any consistent pattern. However, in many of the sheep, irrespective of treatment, the concentration of phosphorus in urine was higher than its respective calcium concentration.

Myocardial pathology was mild and similar for all the treatment groups (Table 2.5). The hepatic pathology was mild and consisted of hyaline degeneration and single-cell necrosis, considered to be none specific, and triaditis, an incidental finding usually associated with parasites. Renal pathology was restricted to one case of tubular degeneration and 11 intertubular congestion. When myocardial pathology was observed, the litter was screened for ionophores. Narasin, at a concentration of 10 mg/kg, was measured in the broiler litter.

Table 2.4: Purine: creatine ratio ($W^{0.75})^{-1}$ in urine from spot samples taken during the trial (mean ± SD)*

	Day of collection			
Litter (%)	**1**	**28**	**63**	**81**
100	27 ± 10.7	16 ± 4.8	24 ± 6.5	24 ± 7.2
92.5	28 ±24.6	13 ± 6.1	27 ± 6.0	25 ± 5.7
85	31 ± 29.0	24 ± 9.5	32 ± 4.3	26 ± 3.2

*Differences among means not significant.

Table 2.5: Number of sheep fed broiler litter with or without molasses with histopathological lesions in the myocardium (n = 6/treatment)

Broiler litter (%)	**100**	**92.5**	**85**
Molasses (%)	**0**	**7.5**	**15**
Myofibre hypertrophy	5	3	4
Myofibre atrophy	5	3	4
Hyaline degeneration	3	3	4
Hyaline necrosis	3	2	4
Attempted regeneration	3	3	4
Interstitial fibrosis	1	2	4
Incidental lesions:			
Sarcocysts	4	1	3
Congestion	2	2	5

2.4 DISCUSSION

The crude protein concentration of 180 g/kg DM, although less than average South African value, was well within the levels of crude protein in litter fed to animals. However, this was substantially lower than 300 g dietary protein/kg feed that reportedly caused the liver damage in cows (Silanikove & Tiomkin, 1992). A direct comparison of results is therefore not possible. The high selenium concentrations of the diets indicate a potential selenium oversupply if selenium in broiler litter is available. The plasma urea nitrogen in the present trial is well above the normal range of 80-200 mg/l (Kaneko, 1989), indicating that excess ammonia must have been absorbed from the rumen. The high plasma urea nitrogen observed in the first collection was probably due to feeding of litter during the preliminary period. The normal concentrations of plasma albumin, bilirubin and hepatic enzyme activity in plasma indicate negligible hepatic pathology. Rankins *et al.* (1993) questioned the observation of Silanikove & Tiomkin (1992), and suggested that the observed liver damage might have been due to copper toxicity. Unfortunately Silanikove & Tiomkin (1992) did not report the copper concentrations in the broiler litter used or in the livers of their cows. In the present study the copper concentration in the litter was 22 mg/kg DM. Copper concentration in the livers of our sheep (< 400 mg/kg) was well below toxic levels (Howell *et al.*, 1987). Van Ryssen *et al.* (1993) concluded from the survey of mineral concentrations in poultry manure that copper sulphate is at present not included in broiler rations in South Africa. Consequently, in South Africa, copper toxicosis due to ingestion of litter is unlikely, contrary to the situation in the USA (Rankins *et al.*, 1993). Angus *et al.* (1978) reported

serious liver damage in sheep consuming diets containing up to 60 percent manure from laying hens. Their findings included an accumulation of fluid in the abdomen and marked histological changes in the liver. No explanation could be given and the presence of a toxic substance was suggested. They also described kidney damage in sheep on high intakes of broiler litter, again of unknown cause (Angus *et al.*, 1978). The possibility of copper toxicity was considered and ruled out. The causes of the above lesions were obviously not present in our experimental diets.

The elevated plasma CK activity may indicate muscle damage. However, elevated activity was measured throughout the trial and may be unrelated to the broiler litter treatments. Although the myocardial lesions resembled those of ionophore-associated litter toxicity, as described by Bastianello *et al.* (1995), they were considered very mild in this batch of sheep. The ionophore antibiotic, narasin, which was present in the broiler litter, is not registered in South Africa as an ionophore for ruminants. However, our findings suggest that the level of narasin in the feed (10 mg/kg) was not high enough to induce severe myocardial pathology or influence the animals' ability to survive on the particular diet. Since the purpose of feeding high levels of litter was to keep the animals alive, i.e. at a maintenance or even sub-maintenance level of nutrition, these histological findings were probably of minor significance.

The inclusion of molasses in the diets resulted in a significant increase in feed intake. This increased intake was reflected in improved daily mass gains and a higher final body mass in the respective groups. The higher body mass in the molasses-fed sheep must have

been due partly to higher gut content in these groups, because differences in carcass mass between treatments were less pronounced. If the objective is to keep the sheep alive, the inclusion of molasses may be unnecessary, except if the intake of the pure litter is unsatisfactory. In fact, lower litter intakes than recorded in the present trial should be sufficient for the survival of the sheep. Where litter with a crude protein concentration higher than that in the present study is used, the addition of molasses may have a more pronounced effect, e.g. in detoxifying ammonia.

The ratio of PD:C in urine can be used as an indication of relative rumen microbial protein synthesis in the animal (Chen *et al.*, 1995). Although this ratio increased slightly with an increase in molasses inclusion in the diet, variations within treatments were wide and differences were not significant. From urinary PD:C ratios, Chen *et al.* (1995) estimated rumen microbial nitrogen productions in sheep of less than 5 to more than 20 g nitrogen /d. At PD:C ratios of between 15 and 30, Chen *et al.* (1995) estimated a rumen microbial nitrogen production of 6 – 12 g/d. In the current trial a PD:C ratio of *ca.* 23 was measured, indicating a relatively low microbial production of less than 12 g/d. The low PD:C ratios observed at 28 days may reflect incomplete adaptation of the microbes to the diets at that stage. This was evident in relatively low initial DM intakes which showed an increase for the first 6 weeks in the 100% litter diet. It should be noted however, that uric acid not digested may influence the purine content of urine thus inflating the rumen microbial nitrogen production estimated through the PD:C ratios. Further studies are required to confirm this apparent long adaptation period required by the sheep or the rumen micro-organisms before they can utilise broiler litter effectively.

The concentrations of minerals in the broiler litter were lower than the means for South African samples, but well within the range of variation measured (Van Ryssen *et al.*, 1993). The cows used by Silanikove & Tiomkin (1992) were hypocalcaemic and hyperphosphataemic when they consumed broiler litter and reacted positively to calcium borogluconate injections. The authors suggested that the apparent imbalances in calcium and phosphorus metabolism were due to the severe liver damage in the cows. Although the dietary calcium to phosphorus ratio in the present study was normal at 2:1, the phosphorus was well above requirement for sheep (NRC, 1985). The high concentration of phosphorus in urine relative to that of calcium signifies that the high phosphorus intake through litter could cause urinary calculi in wethers.

It is concluded that the feeding of dry broiler litter of the quality used in the present investigation did not affect the health of the sheep when fed for a period of *ca.* 80 days. Therefore, in an emergency feeding situation of limited duration, it seems feasible to feed broiler litter to ruminants, even at restricted levels of intake. However, the necessary precautions must be taken because broiler litter has many potential problems as an animal feed (Fontenot, 1991; Fourie *et al.*, 1991).