AN INVESTIGATION INTO THE QUALITY OF WATER FOR ANIMAL PRODUCTION

by

NH Casey
JA Meyer
Christél Coetzee
WA van Niekerk

Department of Animal and Wildlife Sciences
University of Pretoria

Report to the Water Research Commission on the Project:

"An investigation into the quality of water for animal production"

Head of Department	:	Prof NH Casey
Project Leader	:	Prof NH Casey
Principal Researcher	:	JA Meyer
Researcher	:	Christél Coetzee
Associate Researcher	:	WA van Niekerk

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Mr DS van der Merwe	Water Research Commission (Chairman)
Prof NH Casey	University of Pretoria
Dr TS Kellerman	Agricultural Research Council
Dr R Kfir	CSIR
Prof C Maree	University of Pretoria
Mr JA Meyer	University of Pretoria
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Mr WA van Niekerk	University of Pretoria
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Mr J Veenstra	Department of Agriculture
Mr P Smit	Water Research Commission (Secretary)
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Dr F Siebrits	Agricultural Research Council

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Dr JDJ Ungerer	Faculty of Medicine
	University of Pretoria
Atomic Energy Corporation -	Environmental Affairs

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SUMMARY

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N.H.Casey, J.A.Meyer, Christél Coetzee and W.A.van Niekerk

The validity of the guidelines presently in use in southern Africa in assessing the quality of water for livestock production has been questioned. International guideline variables and the levels for specific variables differ and highlight the need for each country to have own relevant guidelines. The guidelines in use in South Africa are based largely on international guidelines and have few locally established variable guidelines.

A total of 2293 data sets of borehole water samples from non-hydrogauging stations from the NW & NE Transvaal and NW Cape were reviewed and the variables fluoride (F), total dissolved salts (TDS), chloride (Cl) and sulphate (SO_4) were found to be the variables of major importance to livestock production based on the incidence of potential toxicity assessed according to international guidelines. High risk areas were identified and selected farms were visited in the NW Cape and NW Transvaal regions. On the basis of the results on the data reviewed and the observations from the interviews conducted, research emphasis was placed on F and the palatability effects of primarily Cl, SO_4 and TDS. Similar results were obtained from a review of data from the Atomic Energy Corporation data base and recent data (1990-1994) from the Department of Water Affairs and Forestry, with nitrate being an addition to the list of water quality variables with high research priority.

The effects of five different levels of F in the drinking water (< 1 mg/l, 6 mg/l, 10 mg/l, 14 mg/l and 20 mg/l) on the growth and health of South African Mutton Merino wethers to market weight was investigated. No significant treatment effects were observed on growth or health. Thyroid gland weight was significantly affected by the treatment in some of the groups with a rise in thyroid gland weight with increasing levels of F in the drinking water. It was concluded that although there were no clinical symptoms or histopathological lesions found that F had a significant physiological impact on the sheep

(hypothyroidism) and fluorosis would have developed with time. It was further concluded that an ingestion of 96 mg F/ sheep/ d of F (25 kg live weight) and 122 mg F/ sheep /d of F (42 kg live weight) could be recommended for SAMM wethers for growth to market weight without any adverse effects on growth or health occurring. A similar finding was concluded in a second trial with a level of 15 mg/l F in the drinking water not resulting in any significant effect on growth or health of SAMM wethers to market weight.

Fluoride levels of up to 20 mg/l in the drinking water or at an ingestion rate of up to 3.206 mgF/bird/day, had no negative effects on production characteristics of Ross broilers.

No significant differences between NaF treatments regarding all major production characteristics were found in Silver Grey Hy-line layers at F levels of up to 20 mg/l or an ingestion rate of up to 4.453 mgF/day/bird over a 74 week period.

Similar findings to those made with SAMM wethers were made with Bonsmara steers exposed to NaF in the drinking water to a level of 20 mg/l F, with a resultant ingestion of 350 mg F/ steer/ /day during the initial growth phase and an ingestion of 600 mg F/ steer/ day during the final growth phase to market weight.

The effect of Cl and SO_4 on the palatability of water was investigated at varying TDS levels and ratios of Cl:TDS and SO_4:TDS. It was found that both variables had a significant adverse effect on the palatability of the water, judged by a decrease in the water intake for both variables and a decrease in the feed intake for the Cl variable. Sulphate appeared to have a negative effect on palatability at a lower level than for Cl. No significant treatment effects were found on growth to market weight or health (clinical observations, and kidney and liver histopathology).

A "zone of preference" in terms of water intake was identified in Friesland steers exposed to various Cl:TDS:SO_4 treatments in the drinking water. The response indicted that water intake may possibly be predicted by establishing the location of a "zone of preference", which aids in assessing the relative importance of water quality variables in terms of toxicological, palatability and adaptation factors. All these factors are important in assessing the effect and thus acceptance of a water source.

Saline water was found to significantly alter the bone [F] in SAMM wethers, compared to fresh water, with a significant negative correlation between salinity and bone [F] being found. A TDS concentration of 3000 - 6000 mg/l appeared to have a beneficial effect on hot carcass weight in Bonsmara steers exposed to F in the drinking water.

A need for an index system to assess the suitability of water for livestock production was identified as the present system does not fulfil this role satisfactorily. The index system should be based on the assessment of water intake for (i) toxic variables - to determine the levels of ingestion of the variable concerned, and (ii) palatability variables - to assess the impact on the variables on water requirement and feed intake. These will then be combined to form a water quality index (WQI).

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ABBREVIATIONS / ACRONYMS / UNITS OF MEASURE

ADG	average daily gain
AFI	average feed intake
Cl	chloride
DM	dry matter
EPA	Environmental Protection Agency
F	fluoride
FI	feed intake
LSMEANS	least squares means
MWI	mean water intake
NO_3	nitrate
PTV	potentially toxic variables
SO_4	sulphate
T	temperature
TAL	total alkalinity
TDS	total dissolved solids
TRT	treatment
WQI	water quality index
WI	water intake
WQV	water quality variable
WQGIS	water quality guideline index system

l	litre
kg	kilogram
mg/l	milligrams per litre
µ	microgrammes per litre

PREFACE

The structure of this report was determined by the research findings at the completion of the various phases of the project. These phases are outlined fully in the executive summary of this project (WRC Report No: 301/2/94). To assist the reader the format of this report is briefly explained.

Firstly literature dealing with water quality for livestock and data of subterranean water samples was reviewed to ascertain the need for research in the field of water quality for livestock production and to identify high priority areas. The first chapter is an introduction to water quality guidelines in general and the basic principles concerning the physiological role of water in livestock. The second chapter deals with the review of subterranean water quality in southern Africa.

Based on the results of that phase, the next phase commenced, the first being biological trials investigating the effect of the water quality variable found to have the highest incidence of potential toxicity for livestock, namely fluoride. Research was conducted using sheep and this is dealt with in Chapter 3. Chapter 4 presents research (using the same breed of sheep) concerning primarily the palatability effect of the other water quality variables found to have a high incidence of potential toxicity, namely total dissolved solids, chloride, and sulphate. Based on the results of Chapter 3, research was conducted on broilers (Chapter 5) and layers (Chapter 6) testing the same hypotheses. The same hypotheses and incorporated findings of Chapter 4 were tested in another ruminant model, beef cattle, and is dealt with in Chapter 7. Further palatability research on dairy cattle using the results from Chapter 4 to modify treatment programmes was carried out, and this is addressed in Chapter 8.

Chapter 9 introduces the resultant philosophy which emanated from all the research findings, and Chapter 10 indicates proposed areas of future research and general conclusions.

CHAPTER 1 AN INTRODUCTION TO WATER QUALITY GUIDELINES FOR LIVESTOCK, GROUND WATER AND FACTORS INFLUENCING ANIMAL WATER INTAKE

1.1 INTERNATIONAL WATER QUALITY GUIDELINES FOR LIVESTOCK

Guideline levels for water quality variables and the specific variables considered to be relevant differ between countries. This is largely due to environmental differences and indicates the need for each country to have own relevant variables and levels of acceptability. The Water Research Commission requested that further investigation into the levels of acceptability for livestock drinking water be undertaken. The main reason for this is that the present standards are based largely on assumptions as yet untested in the South African context owing to a lack of locally established variables.

The Summarised Water Quality Variables for Livestock Watering as published by the Department of Environmental Affairs (Kempster, Hattingh and Van Vliet, 1985) and the guidelines reported by Smith (1988), differ considerably from the guidelines published by Adelaar (1974) (Marcowitz and Conradie, 1985). The latter guidelines are presently in use by Namibia and the Department of Agriculture, Transvaal Region in the testing of water for livestock use. Table 1.1 presents a summary of international and local guidelines, and Tables 1.2 and 1.3 present the Australian and Canadian guidelines for salinity.

The aim of the proposed project was thus to determine the levels of acceptability (minimum and maximum) of water quality variables for livestock production applicable to South African conditions. Verification of these standards of water quality are self-explanatory in terms of animal health and performance.

The area incorporated in the study ranges from Venda and Lebowa to Bushmanland with the emphasis on subterranean water due to the importance of this water source in these regions. A brief synopsis of ground water and factors influencing animal water intake follow.

TABLE 1.1. Summary of international and local criteria, guidelines and standards.

DETERMINAND	UNIT	RSA Dept. Environmental Affairs ⊕			AUSTRALIAN GUIDELINES *	CANADIAN GUIDELINES *	USA (EPA)	Adelaar (1976) ●	RSA Dept. Agricultural Development ■
Aesthetic/ physical		MDS	MAC	MAC	Desired working level	Guideline	Limit	Recommended max. concentrations for nutrients	Recommended max. limits for nutrients
Conductivity	mS/m	300	400	1000					
Iron	mg/l		10		10 *				10
Radioactivity	Bq/l	0.20	0.20	0.20					
Biological									
Streptococci, faecal	Per 100ml		40			Blue green algae Pathogens and parasites *	Coliform, (ml) faecal 1000/100 Total 5000/100		
High toxicity									
Cadmium	µg/l	10		50	10	20	50		
Mercury	"	2	5	10	2	1	1		
Molybdenum	"	10	10	10	10 *	500	-		
Pest. aldrin	"		1			0.7			
Pest. chlordane	"		5			7			
Pest. dieldrin	"		1			0.7			
Pest. endrin	"		0.5			0.2			

Pest.,heptachlor	-		0.1			3			
Pest.,lindane (HCH)	-		3			4			
Pest.,methoxlor	-		3			5			
Pest.,2,4 D	-		20			100			
Pest.,2,4,5 T	-		2						
Pest.,2,4,5 TP	-		10			10			
Moderate toxicity									
Arsenic	µg/l	200	200	1000	1000	500 ▼	200		
Copper	-	100	500	5000	500 5000	1000- cattle 5000 swine ▲ poultry 500- sheep	500		
Fluoride	mg/l	1	2	2	2	2 ▲	2	4	
Lead	µg/l	50	100	500	500	100	100		
Pest.,DDT	-		50			50			
Pest.,Malathion	-		100						
Pest.,Parathion	-		100			35			
Selenium	-	20	50	50	20 ▲	50	50		
Vanadium	-	100	100	100		100	1000		
Low toxicity									

Aluminium	mg/l	5	5	5		5	5		
Chromium	"	0.05	1	5	1.5	1	1		
Cobalt	"	1	1	1		1	1		
Manganese	"		10			-	-		
Nitrate + Nitrite	"	20	45	100	90-200 (2013)	100	2013: 100 2012: 10	2013: 400	2013: 100
Pentachlorophenol	"		1			0.1			
Non-ionic									
Boron	mg/l	5	5	5		5	5		
Calcium	"	1000	1000	1000	1000 ♣	1000		1000	1000
Chloride	"		1500					9000	9000
Magnesium	mg/l	250		900	250-500 ♣			500	500
Sulphate	"	500	1000	1000	1000	1000		1000	1000
Zinc	"	20	25	25	20	50	25		
	"								
Beryllium	mg/l					0.1			
Uranium	"					0.2			
Nickel	"					1			
Total dissolved solids						9000	soluble solids - 500	5000	5000
Sodium								2000	2000

Bicarbonate (HCO3)								500	500

◊ Summarized water quality criteria Department of Environmental Affairs (Kempster, Hattingh and van Vliet, 1980).

For each constituent : the lowest criterion reported = minimum value
the most commonly reported criterion = median value
the highest criterion reported = maximum value

+ Hart *et al*., 1992

+ Smith, 1988

• Problems experienced with animals feeding on pasture irrigated with high Fe water.

♦ Less than 800 mg/l probably desirable for beef cattle especially if Mg is also present

♣ Depends on animal

• May affect animal through accumulation in pasture.

♣ May affect animal through accumulation in plants

♥ 5.0 mg/l when not added to feed.

▲ 1.0 mg/l if feed contains fluoride.

• Avoid heavy growths of blue green algae. Water of high quality should be used.

■ Adelaar, 1974. (Merkowitz and Citroniki, 1985).

▬ Borehole Water Association of South Africa, 1990

Table 1.2. Australian recommendations for TDS concentration (Smith, 1988).

Stock	Desirable max. concentration for healthy growth (mg/l)	Max. concent. at which good conditions can be expected (mg/l)	Max. concent. which may be safe for limited periods (mg/l)
Sheep, dry feed	6000	13000	Depends on type of feed
Beef cattle	4000	5000	10000
Dairy cattle	3000	4000	6000
Horses	4000	6000	7000
Pigs	2000	3000	4000
Poultry	2000	3000	4000

Table 1.3. Canadian guidelines for saline waters for livestock watering (Smith, 1988).

Total soluble salts content of water (mg/l)	Electrical conductivity	Suitability for livestock
< 1000	< 1.5	Relatively low level of salinity, excellent for all classes of livestock
1000-3000	1.5-5	Satisfactory for all classes of livestock and poultry, but some loss in productivity should be anticipated; may cause temporary and mild diarrhoea in livestock not accustomed to the water, or watery droppings in poultry
3000-5000	5-8	Satisfactory for livestock but may cause temporary diarrhoea or be refused at first by animals not accustomed to the water; poor water for poultry often causing water faeces, increased mortality and decreased growth, especially in turkeys
5000-7000	8-11	Can be used with reasonable safety for beef cattle, sheep, swine and horses; avoid use for pregnant or lactating animals, and dairy cattle, not acceptable for poultry
7000-10000	11-16	Unfit for poultry and probably for swine; considerable risk in using for pregnant or lactating cows, horses or sheep, or for the young of these species; in general, use should be avoided, although older ruminants, horses, poultry or swine may subsist on them under certain conditions
>10000	>16	Risks with these highly saline waters are so great that they cannot be recommended under any conditions

1.2 GROUND WATER

Next to air, water is the most essential requirement for life and most fresh water is located underground.

Ground water derives primarily from surface and meteoric water, but contributions are also received from mantle sources (Bowen, 1980). There is no doubt that the demand for ground water will continue to grow. In the USA, in the late 1950's, about 15% of the country's water demand was satisfied by aquifer discharge, and in 1968, 20% of the national need was provided by ground water.

According to the Borehole Water Association of Southern Africa (1990) two-thirds of South Africa is largely dependent on ground water. About 280 towns rely exclusively on ground water, while farmers are even more dependent on underground sources for stock watering - see Table 1.4. Estimates of 1800 million m^3 of present ground water consumption may increase to 5000 million m^3 in the next two to three decades.

Table 1.4 Usage of ground water by various sectors (Borehole Water Association of Southern Africa, 1990)

Sector	Percentage usage
Irrigation	78%
Stock watering	6%
Rural domestic	7%
Mining quarries	5%
Urban industrial mining	4%

Approximately 10% of all rainfall in South Africa infiltrates the ground. Some of this may become ground water. In some cases ground water may be ancient and in others just a few weeks old. In most of South African aquifers, flow velocities are usually a few meters per year. The fact that ground water is less susceptible to evaporative, contaminative and drought effects is seen as an advantage over surface water.

The State Drilling Service has existed since 1880, and is operated by the Directorate of Soil Conservation and Drilling Services of the Department of Agriculture. The state drills around 1500 boreholes per year, while the private sector drills around 60000 or more. The state operates largely in difficult areas where private contractors are not keen to work. The basic aim of the subsidy scheme offered is to improve natural grazing via irrigation, and the creation of grazing camps system by helping farmers to obtain borehole water for stock-watering and household use.

With regard to rights of use of ground water South African law vests in the owner of land the right to the use of ground water on his land with some prohibitions preventing the transfer of ground water across farm boundaries without a permit stipulated in Section 5(2) of Act No.54, 1956 (Borehole Water Association of Southern Africa, 1990). Section 28 of the Water Act No.54, 1956, authorises the Minister of Water Affairs to declare an area as a Subterranean Government Water Control Area, largely to prevent over-utilization which has occurred with progress in the geo-hydrological field.

1.3 POLLUTION OF GROUND WATER

A number of possibilities can occur which result in the pollution of ground water. Usually the introduction of sewage or industrial waste constitute a threat as they often comprise contaminants. Organic pollution is not as important as pollution due to inorganic matter, because the latter penetrate more easily down to an aquifer and are much more difficult to remove. This is because natural dilution is very slow and artificial flushing is expensive (Bowen, 1980).

Table 1.5 Causes of degradation of ground water (Bowen, 1980)

(a)	Natural degradation : Inflow of juvenile water.
(b)	Degradation associated with development programmes : Return water from irrigation. Interchange between aquifers due to factors such as improperly constructed wells. Overdraft conditions: these result from taking more than the safe yield from an aquifer (safe yield = the quantity of water which can be withdrawn annually without producing any unwanted result of a deleterious nature). Sea water intrusion. Diffusion, either upwards or laterally, of connate brines due to over-pumping.
(c)	Degradation due to mineralisation : Caused by plant transpiration and/or evaporation.

1.4 A BRIEF SYNOPSIS OF FACTORS INFLUENCING ANIMAL WATER INTAKE

Adequate water intake is essential for livestock to tolerate excess toxic substances (oxalates, ammonia, and mineral salts) (National Academy of Sciences, 1985). The exact amounts of water required by livestock species are not known. A guideline is that voluntary consumption is 2 to 3 times the dry matter intake (DMI), and increases with high protein and salt containing feeds (National Academy of Sciences, 1985).

Water functions in the body as a solvent which transports nutrients, waste products, is involved in chemical reactions (hydrolysis), copes with large changes in heat production without large changes in body temperature (high specific heat), and the high latent heat of evaporation plays a role in body temperature regulation (evapotranspiration). Water is

obtained from drinking water, feed and metabolic water (breakdown of fats, carbohydrates and proteins).

Livestock have an obligatory loss of water and salts due to evaporation and the need to excrete metabolic waste products (evaporation from lungs and skin, secretion by sweat glands, and the excretion of urine and faeces) due to the essential need for temperature regulation and the removal of potentially toxic waste products. Livestock rely on thirst for the initiation of water intake, which must compensate for the obligatory water loss relatively quickly as water is not stored in significant quantities (Grossman, 1990). Livestock (herbivores) survive on Na-deficient diets and rely on an innate salt appetite to insure a sufficient supply of Na and electrolytes (no known appetite exists for K and Mg). As early as 1821 (Rullier, 1821) thirst was scientifically investigated in France, where thirst was concluded to be an "instinctive sentiment which does not admit of any explanation". Contemporary investigators agree that thirst arises as a direct consequence of a loss of water from the cellular and/or extracellular stores of the body.

1.4.1 FEED AND WATER INTAKE CORRELATION

The interrelationship between feed and water intake has been investigated in depth (Fitzsimons and Le Magnen, 1969; Kisseleff, 1969). Forbes (1968) found that there was a significant relationship between feed and water intake expressed by the following formula :

$$TWI = 3.86DMI - 0.99$$

where TWI = total water intake
DMI = dry matter intake.

The association between feed and water intake is most striking when animals are maintained on a dry diet or pelleted ration, which suggests that water intake may be controlled to an extent by oral and oesophageal stimuli. Water needs are generated by:

(i) water needed to chew and swallow;

(ii) ingestion of feed promotes secretion of digestive juices which removes water from extracellular fluids;

(iii) many foods represent an osmotic load that depletes intracellular fluid.

Osmotic effects and urinary requirements (post ingestional factors) also play a role. Le Magnen and Tallon (1967) found that high protein diets had a higher correlation between feed and water intake as opposed to high carbohydrate diets.
Many animals drink in excess of their physiological needs.

1.4.2 ENVIRONMENTAL VARIABLES

Water intake may be influenced by the following :

(i) Ambient temperature - water losses in hot climates are greater and the need to consume more water is greater (Adolf, 1947, Epstein and Milestone, 1968). Sheep may consume 12 times as much water in hot summer months as opposed to cooler winter months (Butcher, 1970). Forbes (1968) found the following relationship exists between water intake and mean temperature above 1 ° C:

$$TWI/DMI = 0.18T + 1.25$$

where T = average temperature (°C) for each week.

(ii) Diet - exerts significant influences on water intake due to mainly the excretion of metabolites of protein-rich feeds which require more water for excretion of metabolic breakdown products and excess Na (Le Magnen and Tallon, 1967).

(iii) Availability of feed- the pattern of eating has a significant effect on the water intake and metabolism of food (Siegel and Stuckey, 1947).

(iv) Taste - improving the hedonic properties of water by addition of artificial sweeteners can increase the intake of water (Rolls, Wood and Rolls, 1980). The opposite is also true for bitter additions (Rowland, 1977).

(v) Water temperature - following deprivation rats drink significantly more water at body temperature than at a cold temperature (12 °C) (Kapatos and Gold, 1972).

1.4.3 THEORIES ON THIRST PHYSIOLOGY

Water enters the compartments via the gastrointestinal tract (absorbtion of water, nutrients and electrolytes into the blood plasma). Water then moves into the interstitial spaces (dilutes plasma) and an osmotic gradient is set up which results in the movement of water into the cellular compartment. The reverse occurs during water deprivation. In the short term the movement of water from the cells into the extracellular compartment is sufficient to prevent blood plasma depletion, and the resulting cellular dehydration is a signal for thirst and renal conservation of water.

If water deprivation continues the intracellular fluid becomes concentrated and the osmotic gradient is reduced and the resultant decrease in extracellular fluid (hypovolemia) activates neural and endocrine mechanisms which is the second signal for thirst.

The relative contribution of cellular and extracellular effects on thirst have been estimated via the administering of water to restore cellular dehydration and the administering of isotonic saline to restore extracellular volume (Ramsay, Rolls and Wood, 1977) found that 64 - 68% was the contribution of cellular dehydration to thirst, and 20-26% was the extracellular contribution.

Some species appear to rely on different cues (cellular and extracellular) for thirst stimulation (Wood, Maddison, Rolls, Rolls and Gibbs, 1980; Wood, Rolls and Rolls, 1982). Denton, (1982) as reported by Rowland and Fregly (1988), found that sheep are unlike rabbits insofar as they rapidly and accurately make up sodium losses. Only small changes in the intake of

sodium bicarbonate solutions by pregnant sheep on either low or normal sodium diets have been reported. The absence of a convincing salt appetite is curious, especially during the third trimester of pregnancy when there is an accumulation of body sodium (Rowland and Fregly, 1988). Calves exhibit an appetite for Na solutions, and like other ruminants may be able to smell NaCl over distances.

1.4.3.1 CELLULAR DEHYDRATION THIRST

Verney (1947) and later Erickson, Fernandez and Olsson, (1971) proposed that osmoreceptors were responsible for the sensing of cellular dehydration and related ADH secretion. Andersson (1978) postulated that Na receptors were responsible since the organism's principal goal must be the protection of Na concentrations in the plasma (>90% of the osmolality of extracellular fluid is due to Na), and circumventricular organs of the brain are sensitive to Na concentration of the cerebrospinal fluid (CSF). McKinley, Denton, Leksell, Tarjan and Wisinger, (1980) presented evidence that indicated that in sheep a combination of both receptors exists (hypertonic sucrose dissolved in artificial CSF elicits water drinking). Leksell, Congiu, Denton, Fei, McKinley, Tarjan and Weisinger, (1981) observed that infusions of mannitol significantly reduced drinking in water-deprived sheep. Treatments that decrease the Na content of the CSF also elicit Na appetite in sheep (Weisinger ,Considine, Denton, McKinley, Mouw, Muller and Tarjan, 1982). Thus both Na appetite and Na concentration of the CSF are implicated in sheep in thirst physiology.

In most species behavioral (drinking) and renal responses to salt loads are as a result of osmoreceptor activation. Goats and possibly sheep are the only known exceptions (Grossman, 1990), with thirst related receptors identified in the lateral preoptic region, anteroventral third ventricle and organum vasculosum of the lamina terminalis.

1.4.3.2 EXTRACELLULAR DEHYDRATION

According to Grossman (1990) the effects of extracellular hypovolemia cannot be due to cellular dehydration because loss of isotonic plasma does not result in persisting osmotic

relationships between the cellular and extracellular fluids that occur during extracellular hypovolemia. Vascular hypovolemia results in the kidney releasing renin which has the end result of A-II formation, but the continued response to extracellular thirst stimuli despite nephrectomy of pharmacological renin-AI blockage indicates that an alternate or additional system exists. It is still not clear if the renin-AI system plays a permissive or mediatory role in extracellular thirst.

CHAPTER 2 THE ASSESSMENT AND MAPPING OF BOREHOLE WATER SAMPLES FROM THE NORTH WESTERN and NORTH EASTERN TRANSVAAL and the NORTH WESTERN CAPE for LIVESTOCK PRODUCTION

2.1 INTRODUCTION

Guideline levels for water quality variables and the specific variables considered to be relevant for livestock drinking water differ between countries (Adelaar, 1974; Environmental Protection Agency; Hart, Angehrn-Bertinazzi, Campbell and Jones, 1992; Smith, 1988; Kempster, Hattingh and van Vliet, 1985). This is largely due to environmental differences and indicates the need for each country to have own relevant variables and guidelines. Research regarding water quality variable levels of acceptability for livestock drinking water for southern Africa is needed, mainly as the present levels are based largely on assumptions as yet untested in the South African context owning to a lack of locally established guidelines. The Summarised Water Quality Variables for Livestock Watering as published by the Department of Environmental Affairs (Kempster *et al.*, 1985) and the guidelines reported by Smith (1988) differ considerably from those recommended by Adelaar (1974) (Maricowitz and Conradie, 1985; Borehole Water Association of southern Africa, 1990). The latter guidelines are presently in use by Namibia, the Department of Agriculture, and the Directorate for Soil Conservation and Drilling Services, in the assessment of water for livestock drinking purposes. Verification of these guidelines are self explanatory in terms of animal health and performance, but are also of importance for the Directorate of Soil Protection and Borehole Services in declaring a borehole successful or not-successful, the result of which determines the maintenance (and subsidizing) or closure of a drilling site and therefore has implications for the livestock producers in question.

Furthermore, current guidelines do not offer solutions for areas which have inherently saline waters in excess of the recommended maximum limits, nor do they take into account the synergistic and antagonistic factors that affect the tolerance levels and subsequent production of livestock (including palatability effects). As this scenario presents itself frequently in the

arid zones of southern Africa the need to accurately assess the impact of a water source for a given environment on a given livestock production system consequently arises.

The complexity and number of factors that influence water quality, and the vast number of water quality variables involved, necessitated a review of data of southern African subterranean water samples in order to ascertain which variables pose the largest "potential" problems for livestock drinking purposes. The word "potential" has emphasis placed on it as there is uncertainty of the validity of the guidelines used to judge water quality.

The aim of this analysis was to identify specific variables that would serve as a starting point for further research, and to identify areas that have a high incidence of potentially toxic variables. These areas could then be investigated, specifically in terms of the experience of the farmers in the region as regards the response of stock to the water, the type of livestock production system and research needs could thus be formulated. The region incorporated in the study ranged from Venda and Lebowa to Bushmanland with the emphasis on subterranean water due to the importance of this water source in these regions. About 105 towns in the Republic of South Africa rely exclusively on ground water, while farmers are even more dependent on underground sources for stock watering (Borehole Water Association of Southern Africa, 1990).

The major obstacle in investigating the water quality variables of importance for livestock production, apart from the questionable validity and format of the current guidelines, is the lack of accurate water quality monitoring and hence usable data. Originally data from the Department of Agricultural Development, Transvaal Division, was obtained and reviewed. This data was found to be inadequate, mainly due to the lack of specification of the source and nature of the water sample, and the questionable sampling procedures (primarily the sampling methods, sample storage procedures, sample dispersion), and therefore excluded in the review. Due to the different sampling methods and analytical procedures used between available data bases, one data base was used in preference to a conglomeration of several. The data base found to be most suited to the purposes of the review was the chemical data base from the Department of Water Affairs and Forestry.

2.2 METHODS

A total of 2293 data sets comprising chemical analysis of borehole water from non-hydrogauging stations from the NW & NE Transvaal and the NW Cape for the period 1987 - 1990 was obtained from the Geohydrological Division of the Department of Water Affairs. The data reviewed dated no further back than 1987 in order to gain an idea of the present chemical composition of the water. Exceptions had to be made with certain drainage regions (discussed in the results) due to the absence or insufficient amount of recent data. Additions to the Department of Water Affairs and Forestry's data base during the period 1991 - 1994 were used to evaluate possible changes in water quality variable trends along with data from the Atomic Energy Corporation data base which was included for comparative purposes. Data from the Atomic Energy Corporation was used only for comparative purposes and did not form the focus of the review as the nature of the data was not suited to the purposes of the review, primarily due to the time intervals between sampling and the method of sampling (high frequency sampling on a specific site).

The water samples comprising the data set had been analyzed for TDS; Na; Mg; Ca; F; Cl; NO_3; SO_4; PO_4; TAL; Si; K; NH_4 and pH. The data was studied and classified to identify those variables that appear to pose the largest concern for livestock watering based on the incidence of potentially toxic levels. The incidence of a particular variable occurring at levels greater than the internationally recommended levels were tallied and expressed as a percentage of the total number of potentially toxic variables (PTV). The guidelines as presented in a review by Kempster *et al.*,(1985) were used in the assessment of toxicity as opposed to those proposed by Adelaar (1974) for two reasons. Firstly, they are in accordance with many international guidelines (USA, Canada and Australia) and considerably more research has been carried out to arrive at those guidelines. Secondly, there is a large difference in the levels of acceptability between the two guidelines, for example, the guideline for Cl of 3000 mg/l as proposed by Adelaar (1974) is twice that of the 1500 mg/l presented by Kempster *et al.*, (1985) and in the case of F, Adelaar (1974) recommends a level of 6 mg/l, three times that of the 2 mg/l proposed by Kempster *et al.*,(1985). By using the lower levels recommended the possibility of overlooking a potential problem, should the higher guidelines be incorrect, is lessened.

After certain "high risk" regions had been identified visits were made to selected areas and interviews conducted with the farmers in these regions.

2.3 RESULTS AND DISCUSSION

The incidence of PTV for the NW and NE Transvaal region is shown in Table 2.1. Fluoride had the highest incidence of the PTV, responsible for 48.9% of the toxic levels recorded. Total dissolved salts was second with 23.2% and Cl third with 15.6%. These three variables together comprised 87.7% of the PTV recorded.

Table 2.1 Incidence of PTV in the NW & NE Transvaal.

VARIABLES	Number of PTV	Percentage of total number of PTV
F	88	48.9
TDS	442	23.2
Cl	28	15.6
SO_4	7	3.9
Mg	7	3.9
NO_3	5	2.8
Ca	3	1.7
TOTAL	580	100

The incidence of PTV in the NW Cape are presented in Table 2.2. Fluoride was responsible for the highest number of the PTV with 36.8%, Cl second with 25.8% and TDS third with 18.6%. Sulphate contributed to 9.6% of the PTV recorded, more than twice the percentage in the NW and NE Transvaal. Compared to the NW and NE Transvaal, the recorded PTV for Mg and Ca in the NW Cape region accounted for less than half the number of the PTV recorded. Fluoride, TDS and Cl followed a similar pattern to that found for the NE and NW Transvaal region, contributing to 81.2% of the total number of the PTV recorded.

Table 2.2 Incidence of PTV in the NW Cape.

VARIABLES	Number of PTV	Percentage of total number of PTV
F	584	36.8
TDS	296	18.6
Cl	410	25.8
Na	69	4.3
SO_4	152	9.6
Mg	28	1.8
NO_3	40	2.5
Ca	9	0.6
TOTAL	1588	100

Fluoride, TDS and Cl were responsible for 81.9% of the total number of the PTV for the combined regions (Table 2.3). These variables tended to be concentrated around specific areas when in toxic levels and certain "high risk" areas were thus identified. While there were other variables that did have recordings of potentially toxic levels, these were usually scattered and not as concentrated or of as high a magnitude as F, TDS and Cl (also SO_4 in the NW Cape).

A total of 1162 samples were analyzed in the NW and NE Transvaal region. Of these a total of 180 potentially toxic levels were recorded, yielding a total of 130 potentially toxic boreholes (11.2%).

Table 2.3 Incidence of PTV in the NW & NE Transvaal and the NW Cape.

VARIABLES	Number of PTV	Percentage of total number of PTV
F	672	38
TDS	338	19.1
Cl	438	24.8
Na	69	3.9
SO_4	159	9
Mg	35	2
NO_3	45	2.5
Ca	12	0.7
TOTAL	1768	100

A total of 1131 samples were analyzed in the NW Cape region with 1768 potentially toxic levels being recorded, yielding a total of 723 boreholes with suspected unsuitable water (63.9%). In total 37% of the 2293 samples reviewed yielded 1768 potentially toxic variables and 853 potentially toxic boreholes.

Figure 2.1 illustrates the occurrence of toxic levels in the NW and NE Transvaal. The majority were recorded from three drainage regions, namely 124, 140 and 150, with over 85% of the potentially toxic levels recorded from these three regions. This is reflected in Figure 2.2 which shows the locality of the areas in which these levels occurred. A similar result was observed in the NW Cape region, but due to the method of data collection, the samples were not allocated to specific drainage regions.

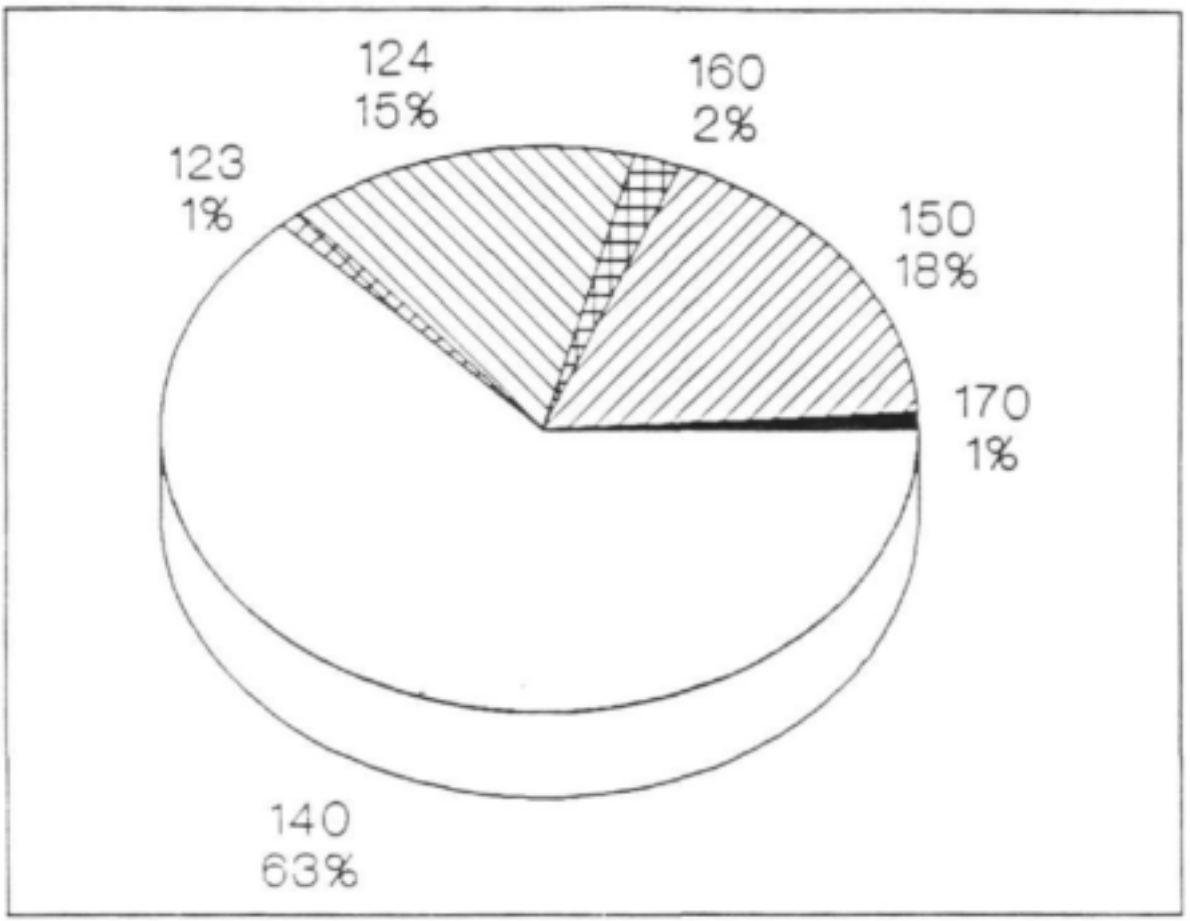


Figure 2.1 Occurrence of potentially toxic variables in the NW & NE Transvaal drainage regions 124, 140 etc.

The distribution of the values reviewed (Figure 2.3) show that many fall between the lower levels presented by Kempster *et al.*, (1985) and the higher levels recommended by Adelaar. Therefore, by using the lower levels recommended in the assessment of toxicity the possibility of overlooking a potential problem is lessened. The large number of samples between the two guidelines also highlights the need to verify which of the two guidelines are in fact correct and should thus be used to assess water quality for livestock.

Figure 2.3 further indicates that research must be directed to finding solutions for the presence of the high levels that occur. This need stems from the incidence of values far above the higher standards of Adelaar, for example, values of up to 32000 mg/l for Cl, 67000 mg/l TDS and 16 mg/l for F. Values even higher than this may well occur in the drinking trough due to the effects of evaporation and bad drinking trough management.

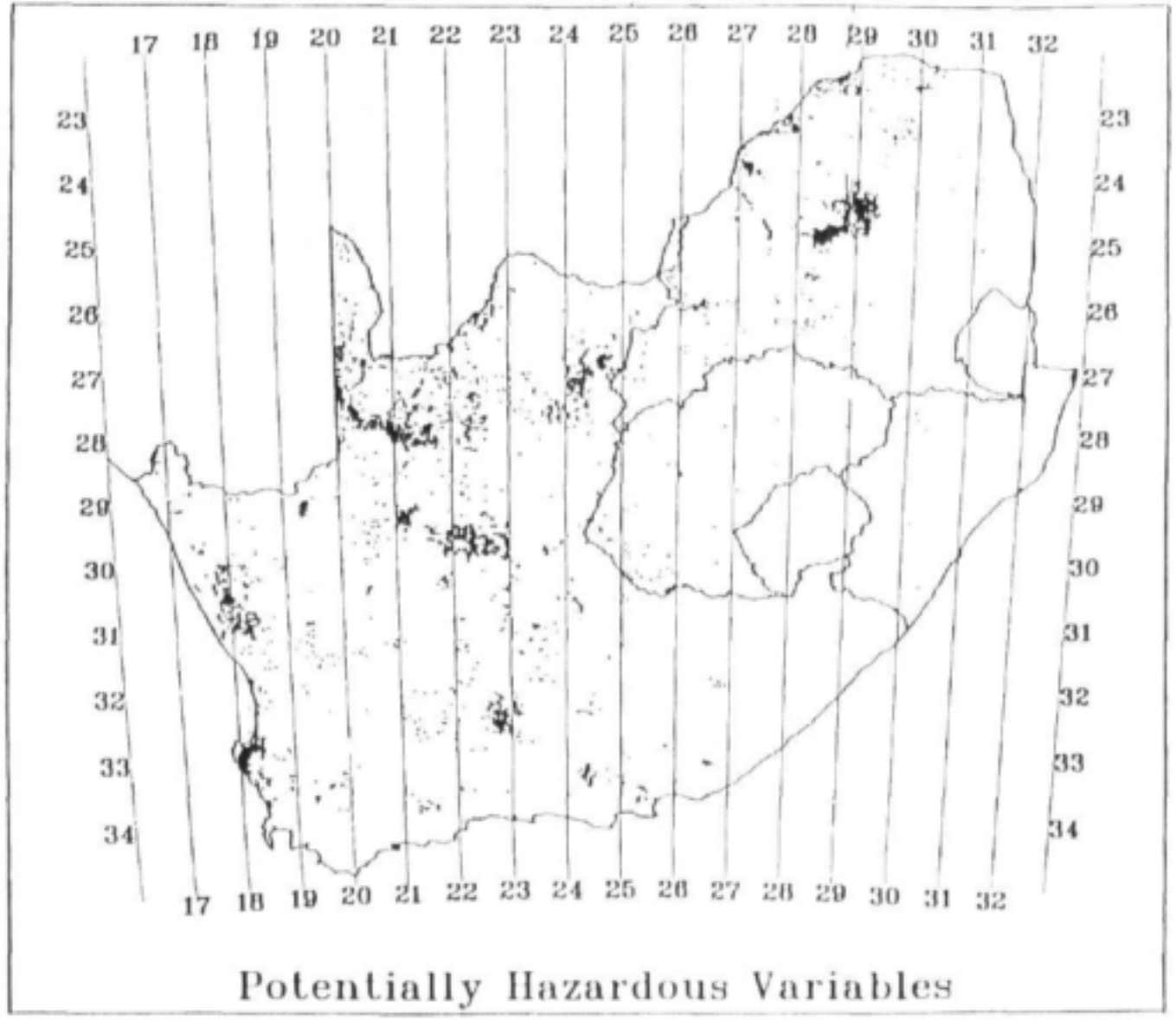
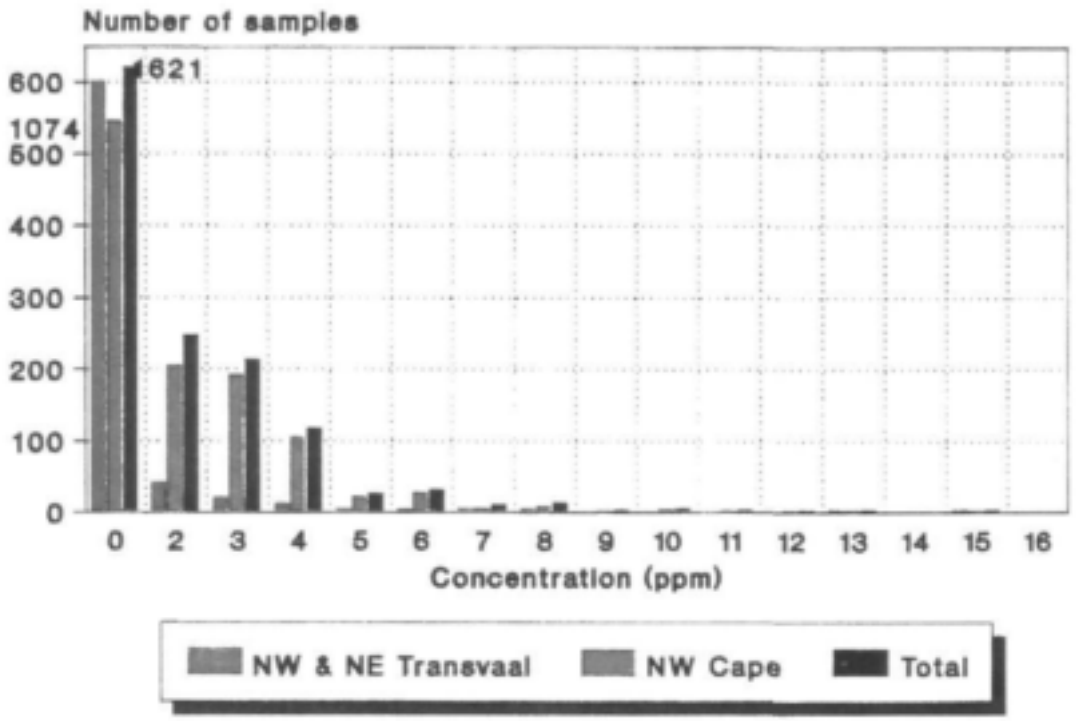


Figure 2.2 Location of potentially toxic boreholes in southern Africa (Department of Water Affairs and Forestry, 1987 - 1994)



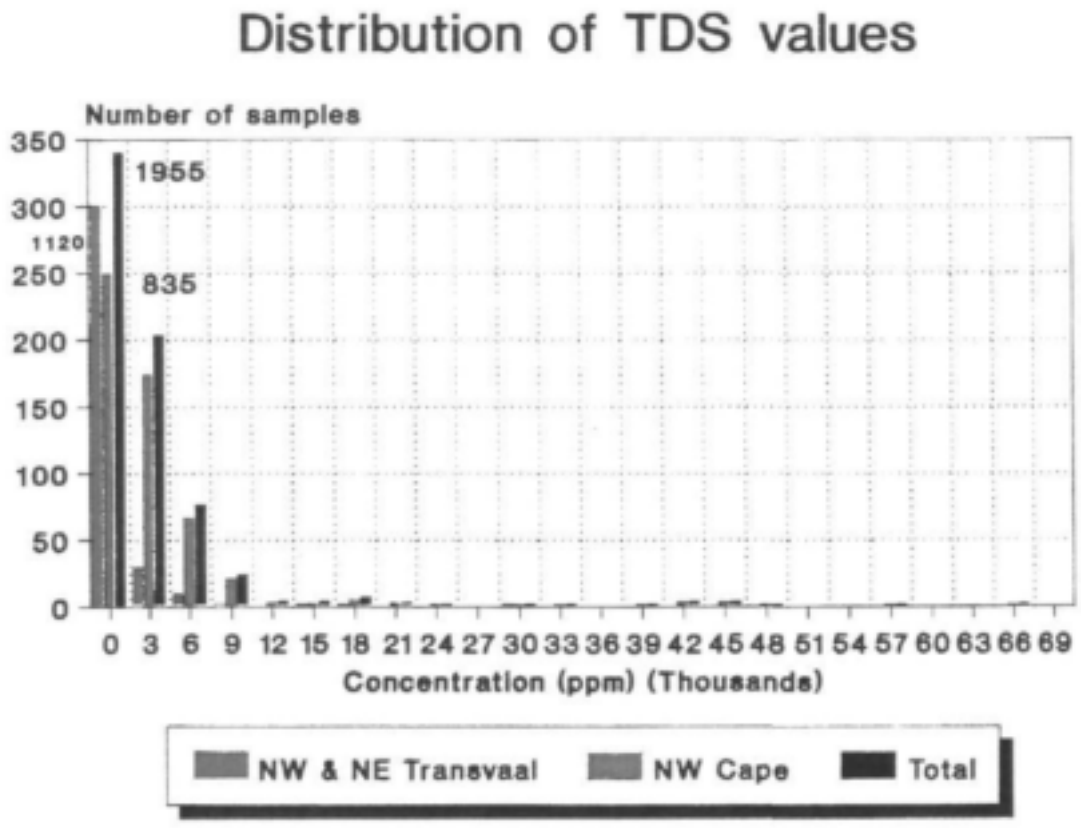


Figure 2.3 (a+b) Distribution of F+TDS values .

Similar correlations between F, TDS and Ca were found as reported by Hem (1970). The same factors were apparent in the review of data from the Atomic Energy Corporation with respect to the distribution of the values.

A different picture may well have emerged were samples taken from the water reservoir or drinking trough. Although this may not be indicative of the borehole water quality, it would be a more realistic view of what the animals are exposed to. A high number of samples are shown in Figure 2.3 to fall below the international maximum permissible levels, the same is also true for the higher local guidelines but to a lesser extent. Many of these samples may well be classed as toxic were the samples representative of the levels the livestock are exposed too. This is illustrated by the recordings of 23 samples in the NW Cape having F concentrations of between 5 - 6 mg/l. This point is further illustrated in Table 2.4 which shows three water samples, taken from the borehole, reservoir and drinking trough, from an underground water source in the NW Cape in the Kenhardt district. The first sample has only one water quality variable that is above the recommended guidelines (international guidelines), namely nitrate. According to the guidelines proposed by Adelaar (1974) there are no variables above the recommended guideline levels. The variables Cl, SO_4, NO_3 and TDS in the second and third samples are all above the maximum permissable levels recommended by the international guidelines. According to Adelaar (1974), SO_4 and TDS are above the recommended levels, with Na and Cl approaching the same status. Nitrate is above the recommended levels in the third sample according to the guidelines used by the Department of Agriculture, Adelaar (1974) and those quoted by the Borehole Water Association of Southern Africa (1990).

Table 2.4 Change in the concentrations (mg/l) of water quality variables from three different sampling sites in the NW Cape (Kenhardt district).

VARIABLES	Borehole	Reservoir	Drinking trough
Fluoride	0.00	0.00	0.00
Nitrite	0.00	0.00	0.00
Nitrate	94.2	161.2	210.2
Chloride	1194.9	2416.8	2446.0
Sulphate	668.2	1319.5	1480.3
Carbonate	0.0	0.0	0.0
Bicarbonate	286.7	292.8	292.8
Sodium	649.7	1672.7	1698.1
Potassium	4.2	7.1	7.6
Calcium	292.0	470.0	540.0
Magnesium	150.8	267.5	291.8
Iron	0.0	0.0	0.0
TDS	3197.4	6461.2	6820.4

In some cases exceptions had to be made with regard to using recent data. This occurred in some blocks in the N Cape region, resulting in the use of data from 1986 - 1990, and in drainage region 124 (NW Transvaal - Crocodile river drainage region) where there was a lack of recent data resulting in data from 1980-1986 being used. Although this region did not have as high an incidence of F toxicity as would have been expected based on the occurrence of fluorspar deposits in this region, a different picture may well have emerged were recent data available from this region. The occurrence of these deposits offer an explanation as to the origin of the high F concentrations (Crocker, Martini and Shonge, 1988).

The review of data from the Department of Water Affairs and Forestry from 1991 - 1994 shows a similar pattern (Table 2.5) , with the variable Na an exception increasing from 3.9% (for both Transvaal and Cape regions) to 10.7%.

Table 2.5 Incidence of PTV in data bases from the Atomic Energy Corporation and the Department of Water Affairs and Forestry (1991-1994).

Water Quality Variable	Atomic Energy Corporation[a] Percentage of PTV	Water quality variable	Department of Water Affairs and Forestry[b] Percentage of PTV
F	56.5	F	44.6
NO_3	18.06	Cl	16
Cl	10.48	SO_4	15.2
TDS	5.6	TDS	10.76
SO_4	5.6	Na	10.7
Ca	2.5	NO_3	1.68
Mg	0.7	Ca	0.55
Na	0.39	Mg	0.27
TOTAL percentage of water samples classed as unfit = 52.88%		TOTAL percentage of boreholes classed as unfit = 14%	

[a] Atomic Energy Corporation data base - whole southern African region (18834 samples)

[b] Department of Water Affairs and Forestry data base (1991 - 1994) - same regions as 1987 - 1990 region (5094 samples)

The lack of evidence from the data reviewed of nitrate posing a potential problem should not be taken as representative of the degree to which nitrate plays a role in ground water. As mentioned the data used has its shortcomings, but is reliable enough to ascertain that in the areas investigated, for the time period investigated, nitrate does not appear to be of as much concern as the variables F, TDS Cl and SO_4. However, due to the current international trend in the increasing levels of nitrates in ground water, the importance of nitrates in ground water should increase.

The review of the data from the Atomic Energy Corporation does in fact show NO_3 to have a high incidence of potential toxicity, although the incidence is not a true reflection of the overall incidence as concentrated sampling was carried out in specific areas with high NO_3 values which form a large part of the data base reviewed, hence the overall contribution of NO_3 to the PTV recorded is skewed, favouring a high incidence of NO_3. The variables F, Cl, TDS and SO_4 followed similar patterns to those found in the Department of Water Affairs and Forestry data base (Table 2.5).

No attempts were made to identify correlations or trends in the data reviewed. Detection of long term trends using only two or three years of data should not be expected (Sanders, Ward, Loftis, Steele, Adrian and Yevjevich, 1987).

2.4 OBSERVATIONS

High risk areas were identified by the mapping of those boreholes with suspected unsuitable water, and some of these areas were subsequently visited. The following are the main points that emerged :

(i) The quality of boreholes merely 30m apart can differ widely;

(ii) Water that has been designated as unpotable for livestock according to present guidelines is being drunk without any noticeable adverse effects and even support certain aquatic organisms and fish;

(iii) Water that has been designated as potable for livestock was refused or being drunk but resulted in loss of condition and was clearly unpalatable to the livestock;

(iv) Water quality varies largely with wet and dry seasons;

(v) Animals brought in from other regions have been able to adapt to poorer waters;

(vi) Climatic conditions influence water intake by stock and water evaporation in reservoirs and drinking troughs; and

(vii) Water quality changes drastically from the pump to the reservoir to the drinking trough, becoming more concentrated because of evaporation; some farmers clean the troughs regularly while others are carelessly lax.

2.5 A BRIEF SYNOPSIS OF HEAVY METALS AND NITRATES IN WATER QUALITY AND THE OCCURRENCE OF FLUORSPAR DEPOSITS

2.5.1 HEAVY METALS

Maximum concentrations for heavy metals in livestock drinking waters have been recommended after consideration of all relevant animal toxicology data, the potential for metal accumulation in edible tissue, the average daily intake of water by the animal, the 'normal' concentrations of the heavy metals in livestock drinking waters, and where possible, any information on synergistic or antagonistic interactions between metals.

Animals may also be exposed to toxic metals in their feed. Selenium, is an example of a heavy metal that is more likely to be present in high concentrations in the feed than in the water. In these cases the maximum safe concentration in the waters are usually reduced. The Australian recommended guidelines for heavy metals are presented in Table 2.6.

Table 2.6 Recommended Australian water quality variables for heavy metals (Hart, 1982).

Heavy metal	Livestock limit (µg.l)	Typical concentration in unpolluted waters (fresh)
Arsenic	500	< 10
Cadmium	10	0.10 - 0.4
Chromium	1000	0.1 - 1.5
Copper	500-2000	< 5
Lead	500	0.3 - 3
Mercury	2	0.01 - 0.06
Nickel	5000	1 - 10
Selenium	20	0.01 - 0.35
Zinc	20000	1 - 20

Natural processes, such as weathering and volcanic activity, continually add heavy metals to the aquatic environment (Forstner and Wittman, 1981). In addition, there are now increasingly large quantities being added by man's activities. Urban stormwater is regarded as a serious diffuse pollution source (AWRC, 1981). For many of the heavy metals, the amounts contributed globally from anthropogenic sources now exceed those from natural sources (Table 2.7). The ranges of heavy metals in sewage sludge produced in the United Kingdom are (μg/g dry weight): Cd 2-70; Cu 93-2210; Cr 5-4200; Hg 0.1-30; Pb 84-1430; Ni 10-946; Zn 350-8490 (Jones, 1978).

There are a considerable number of industrial sources of heavy metals (Leland *et al.*, 1974). This is particularly so in the case of lead, copper and zinc.

Table 2.7 Comparison of natural and man-induced mobilisation of heavy metal on a global scale (Ketchum, 1972).

Metal	Natural rate (1000 ton/yr)	Anthropogenic rate (1000 ton/yr)
Lead	180	2330
Copper	375	4460
Zinc	370	3930
Mercury	3	7
Nickel	300	385

2.5.2 NITRATES

A growing world population and diminishing feed-producing land resources per head have led to the increased use of nitrogen fertilizers. Rarely is more than half the applied nitrogen usefully recovered in the crop, yet little is known about the fate of the unrecovered residue. With the emphasis on environmental quality, it is essential that fertilizer-nitrogen management is directed towards maximum crop utilization efficiency (Arora *et al.*, 1978).

Rising nitrate concentrations in certain ground and surface waters have been associated with the intensive use of fertilizers. Filipovic and Stevanovic (1978) found that nitrate levels were higher in surface waters that were adjacent to fertilized land as opposed to those that were not. Krishnappa and Shinde (1978) found that in an experiment involving the fate of labelled urea fertilizer under conditions of tropical flooded-rice culture, the total crop recovery accounted for 23.4% of the added fertilizer nitrogen. This is illustrated in Table 2.8.

Rawitz *et al.*, (1978) stated that the trends of nitrate concentration with time leave little doubt that unless remedial action is in fact taken, the geographical extent of polluted ground water will grow and concentrations in the ground water will reach intolerable levels by the end of the century.

Table 2.8 Total excess N applied to soils of coastal plain annually and not accounted for by plant uptake, based on data for 1967 - 1970 ('Groundwater contamination in Israel', Ronen, 1974)

Source	N input (tons/a)
Fertilizer and manure	13700
Sewage	4000
Feedlots	2300
Irrigation water	1800
Rainwater	1500
Solid waste	600
TOTAL	23900

In many instances municipal and industrial waste waters are used to irrigate agricultural land. These waste waters generally undergo treatments to eliminate suspended solids and, sometimes, toxic components before they are employed in agriculture (Schalscha and Vergara, 1980). Concern with health hazards is usually traced back to Comly, who observed symptoms of methaemoglobinaemia in two infants given feeds made with water containing 90 and 140 mg/l of NO_3-N (Wild and Cameron, 1978).

The trend of increasing nitrate levels in water appear to be more rapid in ground water than surface waters. In the United Kingdom the systems of nitrate removal from water which appear the most feasible and are receiving the most attention are ion exchange, especially for borehole sites.

2.5.3 FLUORSPAR DEPOSITS IN THE REPUBLIC OF SOUTH AFRICA

The term fluorspar is widely used for ores of the mineral fluorite (CaF_2). It is the most important source of fluorine for the steel, aluminium and fluorochemical industries.

In terms of production capacity South Africa ranks second only to Mexico. The demonstrated reserves of the Republic of South Africa are the largest in the world and this country may therefore become the foremost supplier of fluorite to the West (Crocker, Martini and Sohnge, 1988). Fluorspar is found in a great variety of geological environments and has formed under a wide range of physical and chemical conditions. Any simple classification based on rock association must allow for considerable overlap and intergrading of the various classes (Worl *et al.*, 1973).

The central Bushveld Complex and the Zeerust Fluorite Field account for 99% of the total of all fluorite produced in South Africa. The relatively insignificant contribution from the alkali and carbonatite complexes and from the northern Cape Province makes up the remainder.

2.6 CONCLUSION

The variables F, TDS, Cl and SO_4 were identified as the variables of main priority and research is thus directed concerning them. The importance of nitrate in livestock watering is not made clear in the data reviewed for reasons mentioned. It is foreseeable that nitrate will play an incresing role in ground water quality data available, methods and guidelines for assessing the suitability of water for livestock use, and the need for a water quality monitoring system and an effective system is made clear.

No attempts were made to identify correlations or trends in the data reviewed. Detection of longterm trends using only two or three years of data should not be expected (Sanders, Ward, Loftis, Steele, Adrian and Yevjevich, 1987).

A large percentage of the boreholes thus classed as unfit for livestock drinking and then declared unsuccessful could possibly be used for livestock production without any adverse effects. Many of the water sources in the extensive regions exceed the recommended guidelines (particularly the international guidelines) but are the only water source available. The guidelines do not offer solutions to these scenarios or predictions of the extent of adverse effect that might occur, or the prospects of adapting stock to the waters. In practice this occurs as farmers allow the stock access to the water, but no quantification of the effect (adverse or not) is predicted and thus managed for.

CHAPTER 3 THE PHYSIOLOGICAL IMPACT OF FLUORIDE IN THE DRINKING WATER ON LIVESTOCK PRODUCTION

3.1 INTRODUCTION TO FLUORIDE

3.1.1 FLUORIDE (F)

Biological interest in F was at first confined to its toxic effects. Chronic endemic fluorosis in man and farm animals was first identified in several countries in 1937, although severe fluorosis in cattle during and after volcanic eruptions in Iceland has a longer history (Underwood, 1981).

Industrial contamination of herbage and the growing use of F containing phosphates as mineral supplements introduced further F hazards to livestock, and this stimulated research with respect to F.

Nearly 60 years ago it was discovered that F-free water was associated with a significantly higher incidence of dental cavies in humans than water that contained 1.0 - 1.5mg/l F. This focused attention on the beneficial effects as well as the toxic effects of F. Dental caries do not present a health problem in farm animals, as it does in man, although severe dental effects occur in domestic livestock as a part of the fluorosis problem.

Fluoride has long been recognized as a constant constituent of bones, teeth and the soft tissues and fluids of the body but there is no unequivocal evidence that it performs any essential function. There is evidence that F can enhance the intestinal absorption of iron in rats. It is possible that a dietary requirement for F exists but there is insufficient evidence for any recommendation to be made (Agricultural, 1981). The primary methods used for analyses of F are the selective ion electrode and potentiometric titration methods (Methods for the examination of water and associated materials, 1982).

3.1.2 FLUOROSIS

Drinking water is not normally a major source of minerals to livestock, although there are exceptions of which F is one. For example, in most incidences of fluorosis the water is the source of F and not the feed (Underwood, 1981).

Chronic fluorosis is enzootic in sheep, cattle, goats and horses in parts of India, Australia and Africa as a consequence of consuming water derived from deep wells or bores. Surface waters from such fluorosis areas commonly contain less than 1mg/l F, whereas the bore waters may contain 5 - 15mg/l and as much as 40 mg/l when evaporation has occurred in troughs or bore drains before consumption by livestock (Harvey, 1952).

In parts of North Africa a chronic fluorosis, known as "darmous", occurs from the contamination of herbage and water supplies with high-fluoride phosphatic dusts blown from rock phosphate deposits and quarries. The industrial processing of phosphates, aluminium reduction, brick and tile production and steel manufacturing constitute the major sources of F contamination in areas adjacent to these industries.

Pastures are often treated with phosphatic fertilizers and phosphate licks are provided to livestock to ensure an adequate phosphorus supply to the animal, but certain sources of phosphate, such as ground rock phosphate are usually too high in F for safe use. Although relatively unpalatable it is a cheap source (Bowen, 1980).

The occurrence of chronic fluorosis in animals receiving mineral supplements is influenced thus by the nature and source of the minerals used, the amount of F intake and continuity of the intakes. Continental sources of rock phosphate contain 3 - 4 % F, whereas the Pacific and Indian Ocean island deposits usually contain about half that concentration. The high fluoride rock phosphates can be injurious to livestock when used over long periods in the amounts ordinarily required as calcium and phosphorus supplements (Phillips, Hart and Bohstedt, 1934).

As a rule mineral toxicity is less of a problem than mineral deficiencies (Church, 1979). Fluoride is an exception, and together with nitrates and salts of various heavy metals may be toxic with little, if any, effect on palatability.

During the latent period the animal is protected by the operation of two physiological mechanisms - F excretion in the urine and F deposition in the bones. A rise in urinary F excretion follows directly on increased dietary intake, but soon reaches an upper limit which cannot readily be exceeded. Fluoride deposition in the skeleton proceeds rapidly at first and then more slowly as the concentrations in the bones rise until a saturation stage is reached. This saturation point is around 15000 - 20000mg/l, or 30 - 40 times the F content of normal bone. Beyond this "flooding" occurs of the susceptible soft tissues, metabolic breakdown takes place and death ensues.

A severe anorexia accompanies the later stages of fluorosis and the effects of starvation are imposed on those of F toxicosis (Phillips and Suttie, 1960). Inappetence is not necessarily a serious feature of fluorosis during the latent period and milk production is little affected (Suttie and Kolstad, 1977a). The digestibility and utilization of protein and energy of the ration are also not significantly depressed at this stage.

In young animals exposed to excess F before the eruption of the permanent teeth, the teeth become modified in shape, colour, size, orientation and structure. The incisors become pitted, the molars abraded and there may be exposure of the pulp cavities due to fracture or wear.

Such dental defects do not occur in animals whose first exposure to excess F occurs after the eruption of the permanent teeth. Exostoses of the jaw and long bones develop in animals of any age and the joints become thickened and alkalised. Growth may be subnormal and weight losses may occur, together with a reduction in milk production and fertility. The impairment of these processes is mostly, but not entirely, secondary to the reduced feed consumption brought about by the dental lesions and joint abnormalities and the consequent inability to and unwillingness to gather and masticate fodder.

Animals are also reluctant to drink cool water because it pains them to do so (Cunha, *et al.*, 1973). The manifestations of fluorosis are therefore more prominent in young than in old animals, and in animals grazing sparse, coarse herbage than in pen-fed animals receiving more concentrated rations. The poor calf and lamb crop characteristic of fluorosis areas arise primarily from mortality of the newborn due to impoverished condition of the mothers, rather than failure of the reproductive process itself (Harvey, 1952).

Direct signs of fluorosis in newborn or suckling animals are rare because placental and mammary transfer of F is limited. However, F is readily transferred to the eggs of hens consuming high-fluoride diets so that hatchability may be reduced.

In chronic fluorosis the skeletal tissues are characterized by increased F concentrations, decreased endochondral bone growth, overproduction of osteoid, periosteal hyperostosis, formation of exostoses and decreased or imperfect mineralization, especially of the exostoses (Weatherell and Weidmann, 1959). The affected bones appear rough, porous and chalky white when compared with smooth lustre of normal bones. The Ca:P ratio of fluorotic bone is normal, the carbonate content decreased, and the magnesium content increased. This indicates that the F ion replaces carbonate but not phosphate in the bone salt and that there may be a precipitation of some F as CaF_2.

In adult animals exposed to normal F intakes the whole bones commonly contain 200 - 500mg/l F and rarely exceed 1200mg/l F on the dry fat-free basis. The teeth of these animals contain about half these concentrations, with higher concentrations in the dentin than in the metabolically less active enamel. Electron probe microanalysis of fluorotic bovine teeth has revealed marked variation in the F at different sites within the same tooth (Shearer, Kolstad and Suttie, 1978).

In chronic fluorosis the excess F is incorporated more rapidly into areas of active bone growth than in static regions, into cancellous bones (ribs, vertebrae and sternum) areas (periosteal and endosteal) of the shaft in preference to the middle regions. After calcification and eruption the normal enamel of the teeth possesses little metabolic activity and retains little fluorine. The differences within and among the bones are of great significance for the

sampling and analysis for the diagnosis of fluorosis.

The rise in skeletal F is accompanied by smaller increases in the F concentrations of the blood and soft tissues and by changes in the activity of several enzymes (Rao, 1977). Plasma F values change rapidly in response to changes in F intake. In a long-term study of dairy cows continuously or periodically exposed to high F intakes the plasma values of the control animals were consistently below 0.1mg/l, whereas those of the treated animals were significantly higher, with 1.0mg/l representing a high intake (Suttie, Carlson and Faltin, 1972).

Bone alkaline phosphatase activity is increased in fluorotic chicks and cows (Shupe, Miner, Harris and Greenwoord, 1962). Other enzyme changes in the fluorotic animal have been reported, resulting in an impaired ability to metabolize fat (Sivert and Phillips, 1959) and disturbances in carbohydrate metabolism (Zebrowskii, Suttie and Phillips, 1964). The decrease in liver glucose-6-phosphate dehydrogenase which accompanies the disturbed carbohydrate metabolism appears to be an indirect effect, resulting from the slower growth rate, reduced food consumption and continual "nibbling" eating pattern of the fluorotic rat.

3.1.3 DIAGNOSIS OF FLUOROSIS

Determination of the concentrations of F in the diet, water, bones and urine provide valuable supporting evidence in the diagnosis of fluorosis. According to Underwood (1981) tooth defects such as chalkiness or mottling, hypoplasia and erosion of enamel and excessive wear are also valuable aids with younger animals or older animals that have been exposed to excess F during the tooth-forming period; with cattle this means from a few months to about 2 - 2,5 years of age. Systemic evidence of fluorosis, reflected as anorexia and cachexia, usually appears at only quite high F intakes (approx. 100mg/l or more), or at lower intakes for a prolonged period (Underwood, 1981).

According to Suttie (1964) "analysis of bone F content and an estimation of the age of the animal, would perhaps give the best indication of the potential damage to an animal. This measurement would be independent of day to day variation in intake and would stress the accumulative nature of the disease process".

In dairy cattle F toxicosis is thought to be associated with values in excess of 5500mg/l in compact bone and 7000mg/l in cancellous bone, with concentrations between 4500 and 5500mg/l indicating a marginal zone (Suttie, Phillips and Miller, 1958). The toxic thresholds in bones of sheep have been placed lower, at 2000 - 3000mg/l in bulk cortical and 4000 - 6000mg/l in bulk cancellous bone (Jackson and Weidmann, 1958). Fluoride determination on tail bones obtained by biopsy provide a valuable means of measuring bone F accumulation in cattle (Burns and Allcroft, 1962: Suttie, 1964).

In sheep and cattle not exposed to excess F the urinary concentration rarely exceeds 10mg/l and is usually closer to 5 mg/l. In long-term experiments with dairy cows, normal animals excreted urine containing less than 5mg/l F, those that were on the borderline of toxicity, as judged by other variables, urine with 20 - 30mg/l F; and those with systemic signs of toxicity urine containing over 35mg/l F (Suttie, Gesteland and Phillips, 1961).

Plasma levels are related to the current rate of F ingestion. The teeth, during their formative period, are sensitive to small changes in plasma F concentration. When these concentrations reach 0.5mg/l or more, severe dental lesions appear in young cattle; at values below 0.5mg/l and above 0.2mg/l F less severe damage occurs; while at plasma values below 0.2mg/l few adverse effects occur (Suttie, 1972).

On the basis of these findings 0.2 mg/l was regarded as a critical plasma F concentration in cattle. Plasma values are difficult to determine, there is a marked diurnal variation and the values change so rapidly in response to changes in F intake that plasma samples must be taken soon after the actual ingestion of the F if they are to reflect the total daily intakes. For these reasons plasma values are of limited practical value in the diagnosis of F toxicosis.

3.1.4 TOXICITY

Tolerance to dietary fluoride depends on the age and species of the animal, the chemical form of fluorine, the duration and continuity of intake and the nature and amount of the diet being consumed. At very high intakes, for example 100mg/l F, or more in cattle, these interacting factors are of minor importance because of immediate systemic toxic effects (Schlosberg, Bartana and Egyed, 1980). At lower intakes, typical of chronic fluorosis, tolerance depends markedly on the extent to which one or more of these influencing factors is operating.

Species differences in tolerance to dietary F are great, although the different forms of F used in some of the experiments make comparisons difficult. Thus maximum safe dietary levels of 300 - 400mg/l F as rock phosphate have been reported for growing chicks and 500 - 700mg/l F from this source for laying hens (Gerry, Carrick, Roberts and Hauge, 1949).

Tolerance levels similar to those given for chicks have been reported for growing female turkeys but 200mg/l F as the more soluble NaF decreased weight gains in young male turkeys (Anderson, Hurst, Strong, Nielsen, Greenwoord, Robinson, Shupe, Binns, Bagley and Draper, 1955).

In cattle, tolerance to the F in rock phosphate is approximately twice that to F in NaF. Calcium fluoride is less toxic than the sodium salt. In a direct comparison with dairy cows of the toxicity of NaF, CaF_2 and the F residue on the contaminated hay, in which the F in each source was 65mg/l of the ration, there was little difference in toxicity between the NaF and the contaminated hay, but the CaF_2 was only half as available as the other two sources, as judged by the F retention in the skeleton and by urinary F (Shupe, Miner, Harris and Greenwoord, 1962).

The margin of tolerance for dairy cattle is close to 40mg/l when ingested as NaF, while signs of fluorosis appear within 3 - 5 years at 50mg/l from this source (Suttie, Miller and Phillips, 1957). However, in a study beginning with young calves and lasting for 7 years it was concluded that the tolerance for soluble F is not more than 30mg/l of the dry diet. The dietary F tolerances of domestic livestock, as given in a review by Suttie and Kolstad, (1977b) are presented in Table 3.1.

Table 3.1 Dietary fluoride tolerance for domestic animals (Underwood, 1981)

Animal	mg/l F
Beef or dairy heifers	40
Mature beef or dairy cattle	50
Finishing cattle	100
Feeder lambs	150
Breeding ewes	60
Horses	60
Finishing pigs	150
Breeding sows	150
Growing or broiler chickens	300
Laying or breeding hens	400
Turkeys	400

The importance of continuity of F intake and the age of the animal is illustrated by experience with sheep consuming water-borne F. For example artesian bore water containing 5mg/l F induced severe dental abnormalities and other signs of fluorosis in sheep in the hot climatic conditions of Queensland (Harvey, 1952), whereas in the cooler conditions of south Australia, where very little water was drunk during the wet winter months, no ill effects were observed in mature sheep given 20mg/l F as NaF in the drinking water (Pierce, 1954).

When compared on the basis of total yearly intake skeletal F storage is similar for continuous and intermittent exposure. Alternating periods of high and low intakes can be more damaging than continuous intakes because of a more dynamic metabolism of F in skeletal tissue, with rapid increases in content during periods of high intake and rapid losses during periods of low ingestion (Suttie *et al.*, 1972). Furthermore, with short-term exposure to high intakes systemic reactions can arise, such as weight loss and unthriftiness due to decreased appetite.

General undernutrition tends to accentuate the toxic effects of F and increased proportions of dietary fat enhance the growth retarding effect of high intakes in rats and in chicks (Bixler and Muhler, 1960). This is believed to be due, at least in part, to the fact that F in the presence of fat causes delayed gastric emptying (McGown and Suttie, 1974).

Aluminium salts exert a protective effect against high intakes of fluoride by sheep, apparently through reducing absorption from the intestinal tract (Becker, Griffith, Hobbs and MacInitre, 1950). Calcium salts function similarly in rats (Weddle and Muhler, 1954).

Toxic amounts of F fed to an animal causes pitting, erosion and wearing down of the teeth, softening and overgrowth of the bones, loss of appetite, poor gains, harmful changes to the kidneys and finally death. Sometimes the pulp cavities may be exposed.

The actual limit in mg/l F which is in excess of the body's ability to cope with ingested F without accumulating it is difficult to determine. The 1974 National Research Council report

on "Effects of Fluorides in Animals" recommended the following levels of F as being safe for the pig:

Table 3.2 Recommended maximum levels of F for the pig.

Safe fluorine level in the total diet	Fluorine from NaF or other soluble fluorides		Fluorine from defluorinated natural rock phosphates	
	Sows	Finishing pigs	Sows	Finishing pigs
as mg/l in diet	100-150	150	150-225	225
as % in diet	0.01-0.015	0.015	0.015-0.0225	0.0225

Table 3.3 gives recommended safe levels of mineral elements in water for livestock, F being given as 2.0mg/l/ (Cunha, *et al.*, 1973).

Table 3.3 Recommended levels of mineral elements in water (Cunha, *et al.*, 1973).

Item	Safe upper limit of concentration in mg/l
Arsenic	0.2
Cadmium	0.05
Chromium	1.0
Cobalt	1.0
Copper	0.15
Fluoride	2.0
Lead	0.1
Mercury	0.001
Nickel	1.0
Nitrate-N	100.0
Nitrite	10.0
Vanadium	0.1
Zinc	25.0

Bowen (1980) gives the maximum permissible F level in human drinking water as 1.5mg/l. Diets containing different amounts of F (trace up to 970mg F/kg) were fed to a total of 230 pigs at all stages of growth and development over a 2 year period. The F was supplied as NaF or as a component of rock phosphate. It was concluded that up to 290mg F/kg diet had no adverse effect, but above that amount growth rate, bone strength and milk production of sows suffered. Osteomalacia occurred in pigs when phosphate contained more than 3g F/kg (Agricultural, 1981). It was recommended that the F should not exceed 220mg/kg diet (251mg/kg dietary DM).

There is no specified liveweight, estimated requirement or range of individual estimates as required due to insufficient evidence.

Suttie (1969) suggested that cattle exposed to the following fluorine concentrations (mg F/kg DM) for 2-3 years were likely to have the following results:

20 - 30	mild dental mottling
>40	dental enamel hypoplasia and periosteal hyperostoses of the long bones
>50	significant incidence of lameness with increasing risks of adverse effects on milk production, skeletal fluoride content rising to > 5000mg/kg in 5 year exposure.

Allcroft, Burns and Hebert, (1965) pointed out that dairy cattle are appreciably more susceptible to fluorosis than beef cattle.

Continuous exposure to dietary contents of F <35 or 40mg/kg diet DM would produce no adverse effects in lactating ruminants and that up to 60 to 100mg/kg was tolerated by non-lactating animals (Agricultural, 1984).

Most frequently F intoxication arises in small well defined areas, in the vicinity of industrial activity with emission of F-containing dusts. An similar example occurred at the Nelspruit Sappi pulp mills in the Transvaal, where irrigation of pastures containing F containing gypsum lead to fluorosis developing in the cattle grazing the pastures (Dr van Niekerk,

Voermol, personal communication). Less frequently it arises from inadequate monitoring of the diet and water source. Fluoridation of municipal water supplies adds another 20000 tons each year.

According to the US Environmental Protection Agency F concentrations in most fresh water streams are around 0.2 mg/l. Estimates of the minimum tolerate content of water borne F differ widely. In one group of studies the consumption of water containing 7 - 20mg F/l produced no adverse effects, whereas a second group found adverse effects from 2; 4; 5; 10 or 20mg F/l (Environmental Protection Agency).

Most plant species have a limited capacity to absorb F from the soil, and thus the F concentrations of pastures are usually low, unless contaminated otherwise. Uncontaminated pastures in England range from 2 - 16mg/l with a mean of 5.3mg/l on a dry basis (Allcroft *et al.*, 1965). Suttie (1969) found lucerne hay in the U.S.A. to have a mean of 3.6mg/l. Cereals and other grains and their by-products usually contain 1 - 3mg/l F (MacClure, 1949). It is therefore apparent that the feed, if uncontaminated or unsupplemented with materials high in F, can rarely be incriminated as a major factor in the incidence of chronic fluorosis in farm animals.

3.2 RESEARCH AREAS IDENTIFIED FOR INVESTIGATION

The review of literature on F revealed that much is already known about F. However, the following research needs have been identified concerning F in general and pertaining to the purposes of establishing criteria for water in particular.

3.2.1 ALLEVIATORS

There is a long list of possible alleviators of fluorosis:

CaCl Mg
Al (S + Cl)
Cu, Fe, Va, Se, Mo
diethylstibestrol
Vit C A D E
Iodenated Casein
Boron.

Of these only a few seem to be practical and/or effective, of which the most promising seems to be boron, as borate (Wheeler and Fell, 1983). Boron is listed as being a non-toxic determinant by Kempster *et al.* (1985), with a maximum permissable level of 5mg/l. It appears that boron works as an antidote by forming a BF_4 complex which is less toxic than F. Boron does not mobilise skeletal or visceral F, but the BF_4 complex appears to be formed at a metabolic or digestive level and it largely prevents the adverse effects of F on calcium and phosphate metabolism, and on clinical and nutritional status of the animals. Fluoride-induced skeletal changes are also largely prevented. Boron has been shown to increase urinary F excretion in rabbits (Elsair, Merad, Dnine, Azzouz, Khelfat, Hamrour, Alamir, Benal and Reggabi, 1981).

3.2.2 DIETARY MANIPULATIONS

3.2.2.1 DIETARY FAT AND PROTEIN

According to Buttner and Muhler, (1958) an increased fat percentage of the ration increases F retention via slower gastric emptying resulting in an increased F absorption since F spends more time in the acid environment of the stomach which promotes F absorption from the digestive tract. A decreased protein percentage seems to increase F retention. Parker, Sharma and Shupe, (1979) concluded that guinea pigs fed a F + low protein diet had elevated bone F deposition and Junkkarinen and Kreula (1976) confirmed that reduction of protein intake increased the amount of F retained in the body with considerably less F excreted in the urine. The possibility exists that an increase in dietary protein content of the diet via supplementation might alleviate high F levels exists.

3.2.2.2 FEEDING AND WATERING REGIME

Offering the animals water after feeding influences two factors:

(a) Fluoride is absorbed better on an empty stomach (due to the higher acid environment) and this would perhaps change by allowing water intake on a fuller stomach (Purdell-Lewis, Van Dijk, Heeres, Flissebaalje, Groenewald and Booij, 1985; Jeersak, Harold and Vaughan, 1989).

(b) The formation of HF in a relatively empty stomach results in severe gastric discomfort - sometimes lesions occur in the oesophagus causing further discomfort, with the resultant decrease in food intake. This applies mainly to monogastric animals but still needs to be quantified in ruminants.

Water temperatures of 0, 10, 20 and 30°C depressed rumen pH 2-4hrs post feeding (Brod, Bolsen and Brent, 1982).

3.2.2.3 Ca:P RATION RELATIVE TO F

The Ca:P ratio relative to F concentration plays an important dietary role. Increased Ca and P levels may alleviate high F levels and may even be beneficial to the growth of the animal, whereas high F levels without similar Ca and P increases eventually result in increased F retention and bone saturation of F after which F then invades the soft tissues (Phillips, 1976).

Suttie, Miller and Phillips, (1957) found that 200 g CaCarb/day decreased fluorotic symptoms in cattle. Calcium fluoride is less toxic than the sodium salt. Shupe *et al.*, (1962) found that CaF_2 was only half as available as NaF to dairy cows.

The synergistic effects observed when allowing animals on highly saline waters access to Ca:P licks are due to the protective action of Ca against Mg, S and F poisoning.

Parker, Sharma and Shupe (1979) judged that low protein and low calcium diets increased mortality of guinea pigs on a high F diet. According to Andersen (1986) an increased F intake results in the increase of the bodies requirement for Ca and vitamin D. The role played by ascorbic acid in Ca:P metabolism is well documented, as is the possible relationship between osteoporosis and vitamin C. Vitamin C deficiency may impede the transition of amorphic bone salts to the crystalline form. Dietary stress is known to effect skeletal fluorosis, with vitamin C, low protein and calcium the major factors involved.

3.2.3 MILK PRODUCTION AND REPRODUCTION RESPONSES TO INCREASING F

(a) MILK PRODUCTION

The decrease in milk production with increasing F is usually due to a decrease in food intake rather than a direct influence of F (Wheeler and Fell, 1983). Fluoride decreases Ca-mobilisation - the extent to which this affects the high demand for Ca during lactation is now known. It is known that the intermittently high intakes of F have a more deleterious effect than a constant high intake (Suttie *et al.*, 1972).

A dairy cow may drink twice as much in high production than in the dry period (Ensminger *et al.*, 1990). The possibility of alleviating fluorosis by supplementing F to the water supply in the dry period, and so maintaining a constant level of F ingestion, is one worth-while investigating.

(b) REPRODUCTION

The influence of excess F on reproduction was reported in 1966 by Van Rensburg and De Vos by the Veterinary Research Institute at Onderstepoort . Although the findings indicated that groups receiving 8 - 12mg/l F via the drinking water had significant decreases in calving percentage and number of oestrus/conception, their results were skewed due to the addition of defluorinated superphosphate that still contained F which added to the F load of these groups. In fact, they stated that the addition of defluorinated superphosphate seemed to aggravate the harmful effects of F on reproduction, thus rendering their comparisons between groups questionable.

Extrapolation of the levels used and their effect on reproduction to the same levels and other areas must be made with care as the groups received only grazing pasture and no supplements, resulting in their being on below borderline nutrition for 6 months of the year. Thus, the conclusion that for normal reproduction the F content of the drinking water should be below 5mg/l is questionable and this standard should be verified. Although there is controversy regarding placental transfer, it is generally agreed that it is limited and not of great concern (Clarke,*et al.*, 1981). The opposite is true of egg production.

3.2.4 THYROID AND PARATHYROID GLAND RESPONSES TO INCREASING F

(a) THYROID GLAND

Van Rensburg and De Vos (1966) postulated that the harmful effects on fertility may have resulted from a direct adverse influence of F on primarily the thyroid gland. Fluoride is antagonistic towards iodine, thyroxine and triiodothyronine. Wheeler and Fell (1983) proposed that F may compete with iodine when thyroxine is synthesized, leading to hypothyroidism.

Thyroxine and triiodothyronine are prerequisites of good reproduction. It is postulated that hypothyroidism results in prolonged di-oestrus and even anoestrus due to an influence in iodine uptake by F whereby thyroid excretion is decreased. This retards or inhibits gonadotrophins secretion by the anterior pituitary with subsequent functional disturbances in

the ovaries.

Serum thyroxine (T_4) and serum triiodothyronine (T_3) are depressed by F and directly correlated to urinary F concentration. The ability to alleviate this with iodinated casein is not known. Baer, Bech, Franke, Grunewlad, Kochmann, Melson, Runge & Wieder, (1977) studied the effects of boron with or without an iron supplement and varying F levels on rabbits. The F groups had reduced I in the blood, but in the F+ B groups the I in the blood was normal.

The question arises whether boron acts as an alleviator by maintaining blood iodine levels, thereby alleviating hypothyroidism with high F levels via the formation of the hypothetical BF_4 complex.

(b) PARATHYROID GLAND

Hyperparathyroidism is thought to occur with excess F intake (Wheeler and Fell, 1983). The parathyroid gland maintains blood Ca concentration via parathormone. The resultant change in blood and serum Ca with high F intakes has large implication in the development of fluorosis. Blood and serum Ca accelerate fluoroappatite crystal and ion exchange. Hypocalcaemia could develop due to ionised blood Ca decreasing with the formation of CaF in the blood. The monitoring of this mechanism to establish how it affects livestock production would be valuable.

3.3 FLUORIDE TRIAL : THE EFFECTS OF DIFFERENT LEVELS OF FLUORIDE IN THE DRINKING WATER ON SOUTH AFRICAN MUTTON MERINO WETHERS

3.3.1 MOTIVATION

In the water quality variables guidelines for livestock watering as published by the Department of Environment Affairs, the upper limit of acceptability for F is placed at 2 mg/l. Other countries also accept this level, such as Australia, Canada and the United States of America. South Africa has also accepted this level although in some cases 6 mg/l is quoted, notably by Adelaar (1974).

While it is regarded that 2 mg/l is the upper safe limit for F for livestock, it has been recorded on numerous occasions that livestock can withstand several fold increases without any effects other than mild dental mottling (section 3.1.4).

Visits were made to areas that had been identified in Chapter 2 as having potentially toxic amounts of F, TDS and Cl. Where subterranean waters had potentially toxic levels of F, the farmers had no recollection of fluorosis ever occurring nor was there any evidence of fluorosis. There are two main explanations for the apparent discrepancy. Firstly, synergistic and antagonistic effects occur with the various minerals present and the environment, and secondly, the standards used to classify the variables are conservative. Both seem to be relevant, although the latter point is more important when considered carefully. The fact that certain correlations in ground water constituents that occur naturally (discussed in Chapter 4 section 4.1), as were noted in the data received, that may be synergistic with F implies that the method of assessing the suitability of water is not accurate. These correlations and their effects are not accounted for in the guidelines, and the result is that the standards are too conservative.

A further possibility is that mild fluorosis may be prevalent and result in sub-optimal or decreased animal performance, but due to the extensive livestock production system and mild degree of fluorosis, symptoms are not observed by the livestock producer. Similar cases have been investigated, for example, in areas of Lebowa (Professor Boyazoglu, Medunsa, personal communication) and areas of Rust de Winter (Dr Schultheiss, Department of Etiology, Faculty of Veterinary Science, Onderstepoort, personal communication).

The environment has an effect too, for example, the desired level of a certain criterion as a maximum permissible level does not really apply to an arid region, such as investigated, as the naturally occurring levels often exceed the desired levels, but no alternative water source is available. In Australia it was recorded that 5 mg/l F were toxic to sheep in dry hot conditions of Queensland (Harvey, 1952), whereas 20 mg/l F not in wet conditions in South Australia (Pierce, 1954).

In the data received many samples were distributed just below the international level of 2mg/l F (1,8 mg/l - 2 mg/l F), between 2 - 6 mg/l F and as high as 15 mg/l F (Chapter 2). As these were borehole samples, the animals are realistically exposed to concentrations in excess of these levels. Harvey (1952) showed that a concentration of 5 - 15 mg/l F in the borehole can yield a concentration as high as 40 mg/l F in the drinking trough after evaporation (Harvey, 1952). This established the need to verify the two standards and do direct research towards finding solutions for these high levels.

Due to the distribution of the values and apparent discrepancies as mentioned above, it was decided to investigate the effects of F levels ranging from less than 1 mg/l to greater than 20 mg/l on the growth of sheep to market weight. The aim was two-fold. Firstly, it was to investigate the effect on animal performance in terms of growth rates, feed and water intakes. The second aspect involves the incidence and degree to which chronic fluorosis would develop during this period.

Although there are conflicting views it is generally accepted that placental transfer of F is limited (Clarke *et al.*, 1981) and that the F concentration of the ewe's milk is not significantly increased with increased F concentration in the drinking water. Hence the use

of wethers enabled extrapolation birth to market weight. The rationale behind this was that if growth was not significantly affected but clinical symptoms and histopathological lesions were observed, it would not influence the maximum permissible level of F over this growth phase.

Another important and critical point that makes the above rationale valid is the possibility of F invading the soft tissues on the levels used is extremely unlikely, and hence no health hazard is passed to the consumer. Had this not been so it would have been a limiting factor that must be included in the determination of a maximum permissible level.

3.3.2 MATERIALS AND METHODS

Fifty South African Mutton Merino wethers just weaned with an average live weight of 23 kg ranging from 18 kg to 26.3 kg were used as the experimental animals. They were assigned to five different groups and divided further to give 2 subgroups per group yielding 2 replicates using a complete randomized block design. The five groups each received different treatments via the drinking water as follows:

Group 1 = < 1mg/l F = Treatment 1
Group 2 = 6mg/l F = Treatment 2
Group 3 = 10mg/l F = Treatment 3
Group 4 = 14mg/l F = Treatment 4
Group 5 = 20mg/l F = Treatment 5

3.3.2.1 WATER TREATMENT

The water was offered *ad libitum* in plastic drinking troughs, and was reconstituted to the desired concentrations using a water source that was analysed prior to and during the duration of the trial. The F concentrations were achieved by adding measured amounts of sodium fluoride (NaF-AR grade) to each subgroup's drinking trough at each watering time. Watering times were 7h00 - 8h00 and 15h00 - 16h00 daily, with the drinking troughs being cleaned every morning.

3.3.2.2 RATION

A medium energy dry ration (10,58 MJ ME) was offered (Table 3.4 and Table 3.5) *ad libitum* after an initial two weeks adaptation period during which varying mixtures of lucerne hay and the ration were offered. Feeding times were the same as the watering times with the feed troughs being cleaned out in the afternoon. A medium energy dry ration was used for two reasons. Firstly, the dietary effects of a medium energy ration would be less than that of a high energy ration due to the decreased occurrence of dietary disturbances that might affect animal health and the uptake of fluoride from the digestive system. Secondly, the dry ration would result in a higher water intake via the drinking water and thus a higher F ingestion at a known concentration that was not diluted by water from the feed as opposed to a ration with a higher moisture content that would have had a dilution effect.

3.3.2.3 HOUSING AND HEALTH

The subgroups were housed in separate pens with a two week adaptation period during which they were subjected to standard dosing and innoculation programmes. The duration of the trial was three months (107 days with two weeks adaptation phase) by which time the average live weight ranged from 40 - 45 kg.

Table 3.4 Ration for 20 kg wethers.

Feedstuff	% ME (MJ/kg)	Prot (g/kg)	Ca (g/kg)	P (g/kg)	CF (g/kg)	Price R/ton
Maize meal gri	13.2	100	0.2	2	23	360
Kalori 3000	12	40	8.9	0.8	8	625
Tef	7.5	60	3.7	1.2	329	200
Cottonseed cake	12.1	437	2.5	10.7	168	720
Urea	-	2880	-	-	-	550
Feedlime	-	-	380	-	-	335
$NaHCO_3$	-	-	-	-	-	1874
DiCaP	-	-	250	210	-	885
Salt	-	-	-	-	-	80
Total	10.58	141.31	5.27	3.87	173.94	375.9

Table 3.5 Balanced Ration for 20 kg wethers.

Maize meal	31.8%
Kalori 3000	7.96%
Tef	42.78%
Cottonseed cake	15.02%
Urea	0.52%
Feedlime	0.34%
$NaHCO_3$	0.5%
DiCaP	0.5%
Salt	0.54%
Total	100.00

3.3.2.4 PARAMETERS MONITORED

(i) Daily water intakes (corrected for evaporation) and feed intakes were calculated for each subgroup.

(ii) Sixteen hour fasted live weight recordings were carried out weekly.

(iii) The sheep were observed throughout the duration of the trial with weekly inspections for clinical signs of fluorosis carried out on each sheep (Buck and Osweiler, (1976) and Shupe, (1980)). The diagnosis of chronic fluorosis was based primarily on dental lesions, skeletal fluorosis (external palpation of metacarpals, metatarsals and mandible) and signs of discomfort.

(iv) After the trial period of three months the sheep were slaughtered within 3 days at an average live weight of 42 kg with the heaviest in each subgroup slaughtered first.

Live weight was recorded prior to slaughter and hot carcass weight was recorded after slaughter.

Samples of the kidney from each sheep were collected for light microscopy and prepared as follows (Williams, 1990):

Slides less than 5 mm in thickness were fixed by immersion in 10% buffered formalin less than 20 min after slaughter. The fixed tissues were processed routinely, embedded in paraffin wax, sectioned at 3 - 4 μm and stained with haematoxylin and eosin (NE).

They were inspected by a pathologist at the Department of Pathology, Faculty of Veterinary Science, University of Pretoria (see Appendix Trial 1 for written report). The thyroid glands were excised from each sheep and excess adipose tissue removed before weighing. Liverweight from each sheep was also recorded.

The mandible, teeth, metacarpal and metatarsal bones were removed, cleaned and then macroscopically evaluated using colour, surface texture and appearance, presence of exostoses and thickening of periosteum as indicators of chronic fluorosis. The oesophagus was examined for ulcerations.

(v) Water samples were taken throughout the trial which monitored the initial reconstitution of the water and the evaporational effects on the concentration of F and several other variables (listed in Appendix for Trial 1). This was done as often reservoirs are used in reconstituting water but no monitoring of the drinking trough is carried out, so the level used may not be assumed to be the level exposed to the livestock.

3.3.2.5 STATISTICAL ANALYSIS

The SAS (statistical analyses system) software system (SAS INSTITUTE INC., 1985) was used under the guidance of the Department of Statistics (Faculty of Economics and Management Sciences, University of Pretoria) for data analysis. General linear model estimates and hypotheses tests were used for regression analysis.

Miscellaneous statistics :

F significance value - this is the ratio produced by dividing the Mean Squares (model) by the Mean Squares (error), and tests the ability of the model as a whole (after adjusting the mean)
to account for the dependent variable's behaviour.

R^2 - this measures how much variation in the dependent variable can be accounted for by the model, and is the ratio of the sum of squares for the model divided by the sum of squares of the corrected total.

Type III values - the type III sum of squares is a method of attaining values for partial analysis of variance by taking into account the full model with all the variables. The

resultant P values test the partial contribution of each predictor in the model over and above the other predictors in the model.

The F and P values for type III tests are equivalent to the results of a *t* test for testing the hypothesis that the regression parameter equals zero.

The Duncan Multiple Range Test is a procedure that best gives significant differences for multiple comparison *t* tests.

The correlation analysis used in attaining correlation coefficients were the Spearman and Pearson tests, depending on the nature of the data.

The P significance levels used were dependent on the trial design and the desired statistical data. Most regression analysis were tested at the 5% ($P < 0.05$) level, but in the case of the least square means analysis, stricter levels were used. These were calculated by statistical formulae dependent on the nature of the data and differ as a result of data differences.

3.3.3 RESULTS AND DISCUSSION

The results of 13 weekly live weight recordings are presented in Tables 3.6, 3.7 and 3.8. At no stage was there any significant treatment effect on liveweight (Table 3.6 and Figure 3.1). There were no significant differences between treatments in live weight (Table 3.7). The means and standard deviations are given in Table 3.8.

A number of factors must be made mention of. Firstly, the growth and weight gain on the medium energy ration is not as rapid as a high energy feedlot ration would have yielded. The dry ration also caused higher water intakes than would normally be encountered when grazing on succulent pastures. Fluoride ingestion can be assumed to have been higher under these trial conditions as opposed to natural grazing conditions.

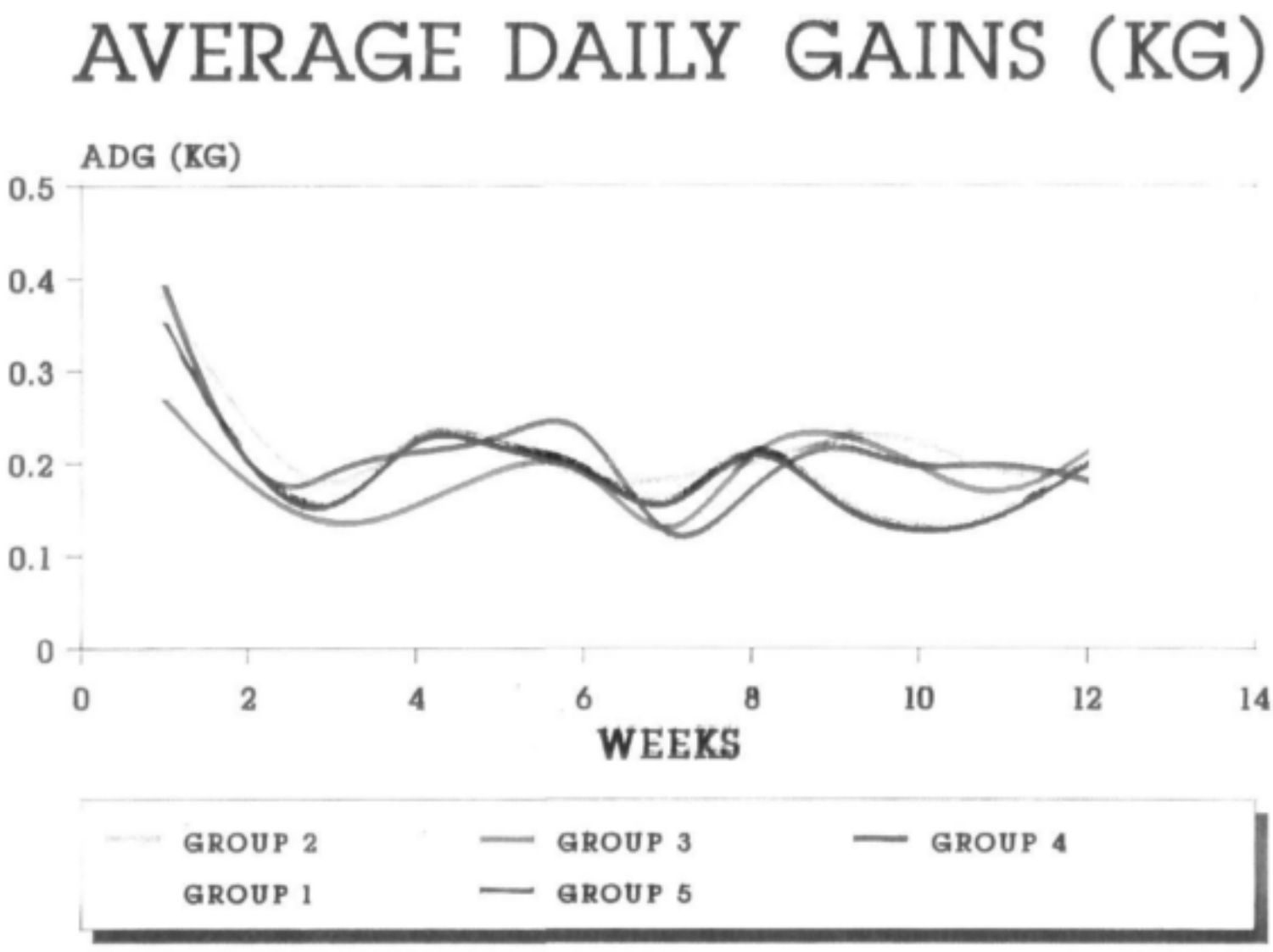


Figure 3.1 AVERAGE DAILY GAINS FOR SAMM WETHERS ON F TREATMENTS.

Table 3.6 Effect of fluoride in the drinking water at 5 levels on the live weight of SAMM wethers

Live weight	Significance values		
	P	R^2	F
1	0.976	0.01	0.12
2	0.859	0.03	0.33
3	0.742	0.04	0.49
4	0.527	0.06	0.81
5	0.223	0.12	1.48
6	0.349	0.09	1.14
7	0.291	0.10	1.28
8	0.283	0.10	1.30
9	0.372	0.08	1.09
10	0.384	0.08	1.07
11	0.209	0.12	1.53
12	0.135	0.14	1.85
13	0.108	0.15	2.02

Significance level ($P < 0.05$)

Thirdly, the water source used had a TDS concentration of < 1000 mg/l and a Ca concentration of < 50 mg/l which are both low enough to eliminate the synergistic effects that might occur were these higher (Florain, Ridlington and Bills, 1986). High levels of F are usually accompanied by high TDS levels in ground water (Hem, 1970), and Ca-P licks are often given under natural grazing conditions, both of which have synergistic effects. The absence of these factors under the trial conditions make the extrapolation of the above results to field conditions more possible. The effective F concentration that the sheep were exposed to was in fact higher than the levels administered due to evaporative effects (see Appendix for Trial 1 - water sample analyses).

Table 3.7 Least square means for 5 treatments for weekly live weight recordings over a 13 week period of SAMM wethers ($P < 0.005$)

Weeks	Least square means					
1	TRT	1	2	3	4	5
	1	.	0.7315	0.8345	0.6123	0.9405
	2		.	0.8931	0.8696	0.6763
	3			.	0.7654	0.7768
	4				.	0.5612
	5					.
2	TRT	1	2	3	4	5
	1	.	0.3151	0.9043	0.8600	0.7976
	2		.	0.3755	0.4061	0.4524
	3			.	0.9552	0.8916
	4				.	0.9361
	5					.
3	TRT	1	2	3	4	5
	1	.	0.3392	0.8152	0.8214	0.8214
	2		.	0.2364	0.4645	0.2396
	3			.	0.6462	0.9936
	4				.	0.6520
	5					.
4	TRT	1	2	3	4	5
	1	.	0.2433	0.9802	0.2998	0.9274
	2		.	0.2337	0.8946	0.2093
	3			.	0.2886	0.9472
	4				.	0.2601
	5			.		.

Table 3.7 (cont.)

Weeks	Least square means					
5	TRT	1	2	3	4	5
	1	.	0.0777	0.9282	0.1427	0.5723
	2		.	0.0644	0.7555	0.2227
	3			.	0.1205	0.5103
	4				.	0.3608
	5					.
6	TRT	1	2	3	4	5
	1	.	0.0897	0.8367	0.1536	0.3811
	2		.	0.1337	0.7782	0.3998
	3			.	0.2200	0.5017
	4				.	0.5737
	5					.
7	TRT	1	2	3	4	5
	1	.	0.1265	0.9226	0.1035	0.6571
	2		.	0.1514	0.9171	0.2728
	3			.	0.1248	0.7286
	4				.	0.2307
	5					.
8	TRT	1	2	3	4	5
	1	.	0.1039	0.8249	0.2209	0.6875
	2		.	0.0663	0.6777	0.2160
	3			.	0.1501	0.5335
	4				.	0.4072
	5					.

Table 3.7 (cont.)

Weeks	Least square means					
9	TRT	1	2	3	4	5
	1	.	0.0994	0.9948	0.2404	0.5312
	2		.	0.1006	0.6244	0.2986
	3			.	0.2430	0.5355
	4				.	0.5791
	5					.
10	TRT	1	2	3	4	5
	1	.	0.1243	0.9344	0.3020	0.8742
	2		.	0.1061	0.6042	0.1663
	3			.	0.2657	0.8099
	4				.	0.3809
	5					.
11	TRT	1	2	3	4	5
	1	.	0.0536	0.8968	0.2653	0.9638
	2		.	0.0706	0.3975	0.0591
	3			.	0.3238	0.9328
	4				.	0.2848
	5					.
12	TRT	1	2	3	4	5
	1	.	0.0290	0.7708	0.1270	0.6993
	2		.	0.0559	0.4869	0.0684
	3			.	0.2136	0.9243
	4				.	0.2497
	5					.
13	TRT	1	2	3	4	5
	1	.	0.0152	0.5112	0.0842	0.4914
	2		.	0.0693	0.4531	0.0740
	3			.	0.2756	0.9750
	4				.	0.2893
	5					.

Table 3.8 Weekly means and standard deviations of live weights of 5 levels of F in the drinking water over a 13 week growth period for SAMM wethers.

Week	TRT1 1mg/l		TRT2 6mg/l		TRT3 10mg/l		TRT4 14mg/l		TRT5 20mg/l	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	25.1	2.67	25.6	2.56	25.4	3.00	25.8	2.78	25.0	3.72
2	27.1	2.56	28.3	2.41	27.2	3.05	27.3	2.96	27.4	2.81
3	28.8	2.71	29.9	2.32	28.5	3.26	29.0	2.38	28.5	2.98
4	29.3	2.67	30.7	2.30	29.2	3.05	30.5	2.64	29.1	2.68
5	30.2	2.82	32.4	2.31	30.1	3.0	32.0	2.68	30.9	2.64
6	31.6	3.30	34.1	2.75	31.9	3.67	33.7	3.00	32.9	3.35
7	33.3	3.44	35.5	3.00	33.4	3.89	35.6	3.03	33.9	2.45
8	34.3	3.28	36.7	3.46	34.0	3.86	36.1	3.21	34.9	2.62
9	35.6	3.44	38.1	3.21	35.6	3.88	37.4	3.64	36.5	2.69
10	37.4	3.65	39.8	3.68	37.2	3.75	39.0	3.52	37.6	2.86
11	38.5	3.25	41.5	3.79	38.6	3.58	40.2	3.75	38.6	2.61
12	39.2	3.22	42.7	3.94	39.6	3.55	41.6	4.10	39.8	2.48
13	40.1	3.33	44.1	4.21	41.1	3.45	42.9	3.81	41.2	2.73

The results for the slaughter parameters are presented in Table 3.9. The type III sum of squares values (partial analysis of variance, significance level $P<0.05$) for the slaughter parameters hot carcass weight, and liver weight indicate that final weight (liveweight 13) had a highly significant effect on these parameters. The treatments and F intake (cumulative over trial period) had no significant effect on hot carcass weight and live weight.

Table 3.9 Significance levels of fluoride treatments on the hot carcass weight, liver weight and thyroid gland weight of SAMM wethers.

Dependant variable	P	F	R^2	Type III SS Groups = G Final weight = F Fluoride intake = I
Hot carcass weight	0.0001	36.00	0.833	G 0.156 F 0.0001 I 0.125
Liver weight	0.0001	7.57	0.513	G 0.665 F 0.0001 I 0.364
Thyroid gland weight	0.0095	3.29	0.314	G 0.0051 F 0.1996 I 0.0053

*significance level $p<0.05$.

Group and fluoride intake had significant effects on thyroid gland weight, while final weight did not. The least quare means are presented in Table 3.10. There were no significant differences between the treatments for hot carcass weight and liveweight. Four interactions differed significantly for thyroid gland weight, namely treatments 1 and 2, 1 and 3, 1 and 4 and 2 and 3.

Table 3.10 Least squares means for hot carcass weight, liver weight and thyroid gland weight between treatments of fluoride for SAMM wethers.

Treatment interactions	Hot carcass weight	Liverweight	Thyroid gland weight
1 & 2	0.1021	0.2623	0.0029
1 & 3	0.1574	0.3305	0.0026
1 & 4	0.1562	0.3509	0.0044
1 & 5	0.1270	0.3571	0.0055
2 & 3	0.3233	0.4916	0.0036
2 & 4	0.2172	0.4359	0.0069
2 & 5	0.1443	0.4122	0.0080
3 & 4	0.1689	0.4156	0.0172
3 & 5	0.1045	0.3912	0.0127
4 & 5	0.0754	0.3897	0.0127

Significance level ($P < 0.005$)

The thyroid gland weight increased in weight (corrected for live weight) as the F levels increased with significant differences between < 0.1 mg/l F and 6, 10 and 14 mg/l. Group 1 and group 5 had a least square means P-value of 0.0055, just above the significance level of 0.005. Group 2 had significantly heavier thyroid gland weight than group 1 and significantly lighter than group 3, with least square means P-value increasing to 0.0069 for group 4 and 0.0080 for group 5. The differences between groups 3, 4 and 5 were not significant. Van Rensburg and De Vos (1966) postulated that the harmful effects on fertility may have resulted from a direct adverse effect of F on primarily the thyroid gland. Fluoride is antagonistic towards iodine, thyroxine and triiodothyronine. Wheeler and Fell (1983) proposed that F may compete with iodide when thyroxine is synthesized, and hypothyroidism is thought to develop.

Although the results seem to suggest that increased F levels did lead to changes in the thyroid gland there were no clinical symptoms observed during the trial to suggest this, nor were there any histopathological abnormalities observed in the kidney sections examined

(Parenchymous nephritus can occur with chronic fluorosis - see Appendix Trial 1 for pathology report). There were no indications of fluorosis in the bones examined in the form of exostoses, colour and surface differences, and dental lesions. There were no oesophageal ulcerations observed.

Clearly, no clinical symptoms were observed as there were no dental or skeletal lesions that would have lead to the sheep exhibiting clinical signs of fluorosis.

Table 3.11 gives the correlations between water intake and feed intake for the various treatments on a daily basis (Spearman correlation coefficients) and weekly average basis (Pearson correlation coefficients). The correlation coefficient for the weekly average measurements are higher since there was more variation in daily intakes, but both show strong positive correlations of the same order for the treatments. This is significant as the economic losses from fluorosis occur primarily because of decreased feed and water intakes.

Table 3.11 Correlation coefficients for water and feed intakes of SAMM wethers over a 13 week period.

Group	Pearson correlation coefficients (weekly average)	Spearman correlation coefficients (daily average)
1	0.769	0.559
2	0.879	0.692
3	0.914	0.727
4	0.917	0.676
5	0.843	0.650

From the results of the trial, the low TDS and calcium levels and the medium energy dry ration used, the impression is gained that a level of 20 mg/l F in the drinking water could be safely recommended for the physiological growth stage used in the trial. More correctly this should be stated as an estimated intake of F per sheep for a suitable time period. This time period would differ from one environment to another.

The intake in mg/d that could be safely recommended is a more correct method of stating guideline levels. It enables the livestock producer to more accurately assess the suitability of a given water source as it takes into account the estimated intake of the variable in question. The concentration of the variable in the water source is not the relevant issue, but the ingestion of the variable from the water source is, and it is on this that calculations to assess the suitability of the water should be made as other relevant factors are also taken into account. This is discussed in more detail in Chapter 9. In this trial an ingestion of approximately 96 mgF/ d/ sheep at a live weight of 25 kg. and an ingestion of 122 mgF/ d/ sheep at a live weight of 42 kg is the level of consumption that was found to be safe.

The most important point that precipitates from the response of the animals in this trial, is that it casts doubt over the use of the present guidelines as a realistic means of assessing water quality. Research is currently directed to developing an index system with the main function to predict the impact of the water quality on water intake and animal health. Factors taken into consideration include among others the palatability of the water, toxicity of the respective variables, species differences, environment, production system and goal of the livestock in question.

This is perhaps best illustrated by an example. In many of the areas visited in the initial survey it was often the case that a particular borehole gave trouble to a farmer as opposed to all the water on the farm. In the case of F it would be able to limit the stock that are destined for slaughter to the borehole with F levels of 20 - 30 mg/l. The stock that will be kept for genetic material and so spend years in the production system in which they can accumulate F in the relevant tissues and ultimately develop fluorosis, can be kept off that water source. Intermittent high levels are more detrimental than continuous high levels

(Suttie *et al.*, 1972).

Although this example deals with F, the principle of the index system is similar for other variables as it is holistic in approach. Another way of explaining it would be that the definition of a maximum permissible level is that it is not constant, but changes depending on the species, environment, other mineral interactions with variables present and so on. Hence, the index system has a better chance of assessing the water quality.

While it is true that quantity is the problem rather than quality, the end goal being striven for is to be able (with the use of an index system) to increase the utilization of the water source by more accurately assessing its quality and effects on livestock.

3.3.4 CONCLUSION

It is thus concluded that to a level of 20 mg F/l, or an ingestion of 96 mg F/ d/ sheep (live weight of 25 kg) and 122 mg F/ d/ sheep (live weight 42 kg), the growth of SAMM wethers to an average slaughter weight of 42 kg live weight is not adversely affected.

Despite the possible indication of hypothyroidism, the absence of any other histopathological and clinicopathological abnormalities suggest that the manifestation of fluorosis and subsequent decline in performance and health, within the time frame, treatment levels administered and ingestion rates observed in this trial, are unlikely.

The results of this study offer an new approach to the evaluation of a water source and assessing its impact on livestock as it provides a basis of true variable exposure determination which allows for the incorporation of a number of crucial synergistic and antagonistic factors such as production system, time exposure, climatic, nutritional and physiological stage factors which singularly or cumulatively affect the water intake and metabolic effects the water quality variable exerts on the animal.

The results obtained suggest that water quality guidelines should therefore not contain merely a maximum permissable level as a constant value, but rather a maximum permissable ingestion rate calculated on a more holistic approach.

CHAPTER 4 EFFECTS OF VARIOUS SALTS ON THE ACCEPTABILITY OF WATER and the EFFECT OF SALINE WATER ON FLUORIDE CONCENTRATION IN BONE

4.1 INTRODUCTION AND MOTIVATION

Waters from natural subterranean and surface sources contain dissolved substances most of which are inorganic salts, with chlorides, sulphates and bicarbonates of sodium, calcium, magnesium and potassium predominating. The ions most commonly involved with salinity problems are Ca, Mg, Na, SO_4, Cl and bicarbonates (Environmental Protection Agency). High saline water may affect animal health and performance detrimentally by reducing water and feed intake. Furthermore unpalatable salts may become toxic when present in high concentrations or the water intake is high, depending on the animal's level of physiological stress and environmental factors. Animals' tolerance of saline water vary according to species, age, physiological condition and stressors, environment, water requirements and the type of salts present (for example, magnesium sulphate is more harmful than sodium chloride or sodium sulphate). Factors that are of major importance are those which affect the water intake, such as, age, body weight, production, heat and humidity, and the type of ration (Ensminger, *et al.*, 1990). Salts that have little effect on water palatability but are toxic, include nitrates, fluorine and the salts of various heavy metals. This chapter concerns itself with primarily those salts that have an effect on palatability, more specifically total dissolved salts (TDS), chloride and sulphate for reasons previously explained (Chapter 2).

4.1.1 CHLORIDE (Cl^-)

Chloride represents approximately 60% of the total anion in extracellular fluid, and approximately 0.15% of fresh body weight. The primary physiological roles include the chloride shift mechanism (respiration), HCl secretion for digestive purposes, and the activation of the enzyme amylase. Excessive chloride is excreted almost entirely via the urine. A dietary deficiency of Cl may lead to an abnormal increase of the alkali reserve of the blood (alkalosis), caused by an excess of bicarbonate due to the partial compensation by bicarbonate.

Chloride is by a wide margin the most important and widely distributed of the halogens in natural water (Hem, 1970), generally present in the anion form (Cl^-). The accumulation of airborne oceanic salt appears to be a likely source of salinity in arid interiors of continents. Calcium is usually the preferentially held ion, hence the mechanism for the origin of $CaCl_2$ brines. The most common type of water in which chloride is the predominant anion is the one in which sodium is the predominant cation.

4.1.2 SULPHATE (SO_4)

Sulphur is essential for life itself, mostly as a component of the amino acids cystine, cysteine, and methionine, the vitamins thiamine and biotin, and the hormone insulin. Approximately 0.15% of the body weight and 10% of the mineral content of the body are sulphur. As with Cl the small intestine is the major site of absorption. Excess sulphur is excreted in the urine and the faeces. The production of hydrogen sulphide, which is a highly toxic gas, by rumen microbes can lead to toxic effects when excessive amounts of sulphur are ingested, and is most common in cattle and sheep. A high sulphate level also interacts with molybdenum and copper absorption and influences the metabolism of these elements.

4.1.3 SALINITY AND ANIMAL PERFORMANCE

Due to the large differences in effects of salinity on different livestock species, guidelines have been developed for different species (Chapter 1), although these are still subject to individual differences and different environmental conditions which alter the levels of tolerance. A general guideline is that water with <3000mg/l NaCl can be used safely for all classes of livestock, but with increasing salinity existing guidelines must be used in conjunction with observation of the reactions of livestock to a saline water source (Ahmed, Farid, Safinaz, Shawket, and Hassan, 1989). Although most livestock can tolerate a TDS level of 15000 -17000mg/l, in all likelihood production and health will be affected adversely (Ensminger *et al.*, 1990). According to Ahmed, et al.,(1989) further experiments are needed to re-evaluate established tolerance levels (for salinity) which may differ according to types of salts in ground water.

Experiments by Peirce (1957,59, 60, 62, 63, 66) have indicated that wether sheep in pens on a roughage diet could tolerate drinking waters with 1.3% NaCl, 1.2% NaCl + 0.1% MgCl, 0.9% NaCl+ 0.5% Na_2SO_4, 1% NaCl + 0.3% $CaCl_2$, or 0.9% NaCl + 0.4% of equal proportions of sodium carbonate and sodium bicarbonate. They were however unable to tolerate 1.5% or 2% NaCl, 1.1% NaCl + 0.2% MgCl, or 0.7% NaCl + 0.5% MgCl. Further experiments were conducted (Peirce, 1968a) in pens to include ewes and lambs, and there was some indication in one experiment of a poorer reproductive performance by the ewes on chloride (1.3% TDS) or bicarbonate water compared with the control group. No adverse effects were observed on health, food consumption, or wool production of the ewes and lambs on any of the waters given (Table 4.1).

In one of the two experiments with chloride water containing 1.3% TDS, the growth rate of the treated lambs was significantly less than that of the control, however, in one experiment with chloride water with 1.3% TDS, one with 1% TDS, and all three with bicarbonate water (0.5% TDS) no reduction in growth rate was observed.

In a later experiment Peirce (1968b) administered the same saline treatments ewes grazing sown pastures, given in Table 4.1. It was reported that the chloride water with 1.3% TDS led to a reduction in the percentage ewes that lambed in one experiment, to decreased body weight gains in both experiments, and to increased diarrhoea and mortality in one experiment. Chloride water with 1% TDS was found to result in decreased body weight gains and reduced wool production without apparently adversely affecting the health of the lambs. The bicarbonate water led to a reduction in the percentage ewes that lambed in one of the experiments, but no adverse effects on the lambs in either experiments conducted.

Table 4.1 Concentration of salts in drinking waters of treatments 1, 2 and 3 (Peirce, 1968a+b). All values are percentages.

Group	NaCl Na_2SO_4 $NaHCO_3$ $CaCl_2$ $MgSO_4$	Total salts
1	0.9 0.05 0.05 0.18 0.15	1.3
2	0.68 0.03 0.03 0.12 0.14	1.00
3	0.21 0.015 0.25 0.02 0.005	0.5

These results are in contrast to those conducted with ewes and lambs in pens (Peirce, 1968a). Peirce (1968b) stated that under good nutritive conditions lambs could tolerate a higher concentration of chloride salts than under poor nutritive conditions, but that there was little difference in the ewe's tolerances. Bicarbonate water had no adverse effects on lambs in either conditions. Peirce (1968b) concludes that while under most natural conditions bicarbonate water could be used safely, the position with regard to chloride is less certain.

Eng (1989) reported that steers with a high energy diet adapted to high saline waters whereas those on a low energy diet did not. Wilson (1966) added NaCl to the diet of Merino sheep in individual cages at 0, 5, 10, 15 or 20 %, and 0, 0.5, 1, 1.5, and 20% in the drinking water. The addition of 1.5 or 2% NaCl to the drinking water did not yield the same detrimental effects on the sheep as Peirce (1957) had observed. Seddon (1927) and Heller (1933) also found that although sheep had reduced weight gains, they could tolerate 2% NaCl.

Contradictory findings as to TDS levels that various species can tolerate, and the different guidelines that exist for livestock with respect to TDS, indicate in part the changing influence of environment on the levels tolerated, and partly the inaccuracy or inability of a single value in the form of xmg/l TDS or xmg/l Cl to assess the suitability of water for livestock drinking purposes.

The effects of salinity seem to be more osmotic than related to a specific ion and it is suggested that livestock seem to refuse water with a high salinity at a level below that with which the kidneys cannot cope with (Environmental Protection Agency). This point is also illustrated by Peirce (1957) where some sheep showed a decline in food intake and body weight, but no adverse effects on health while receiving 1.5% NaCl with no effect on blood plasma concentrations of Na, K, Ca, Mg or Cl. Only in the group receiving 2% NaCl was the blood plasma chloride concentration significantly higher.

Similar occurrences were found by Peirce (1959) with regard to MgCl and NaCl in which sheep receiving 1.05 MgCl + 0.2 NaCl, 0.69% MgCl + 0.5% NaCl had a detrimental effect on some sheep, but only those receiving 0.5% MgCl had a significantly higher blood plasma concentration of Mg. In none of the NaCl + MgCl combinations was the blood plasma chloride concentration significantly higher. In 1960 Peirce reported similar trends with NaCl + Na_2SO_4 with significantly higher blood plasma concentrations of sulphate found with combinations of 1.05% + 0.3%, and 0.89% + 0.5% NaCl + Na_2SO_4 respectively, but no adverse effects on health, feed intake, weight gain or wool production.

Blood plasma concentrations of Na, K, Ca, Mg and Cl remained unchanged in sheep receiving the following mixtures of NaCl, carbonate and bicarbonate, given as percentages respectively : 1.26 + 0.015 + 0.25; 1.21 + 0.04 + 0.06; 1.12 + 0.08 + 0.13; 0.95 + 0.16 + 0.25 (Peirce, 1963). Again no adverse effects were observed on health, feed intake or wool production. Similar findings were reported by Peirce in later work (1966, 1968).

The adverse effects that are reported on principally growth and wool production are indirectly due to decreased water intakes and thus feed intakes as opposed to a direct adverse effect on the physiology of the animal. As mentioned the growth/wool production was detrimentally affected, but not the health.

Peirce (1959) suggested that the principle effect of the ingestion of water of high saline concentration was a depression of appetite. Wilson (1966) suggests that the acceptability or taste of food or water containing high levels of salt is a factor in determining the salt tolerance of sheep. Wilson (1966) reported that when NaCl was added to food or water, the

decline in feed intake was not solely related to the amount of salt ingested : higher concentrations of salt in the feed or water led to a decline in feed intake irrespective of the amount of salt ingested.

This implies that the acceptability or taste of high salt feed or water was partly attributable to the feed intake decline. At 0.5 or 1% NaCl no effect on intake was observed, but higher concentrations led to progressive declines in feed intake which was more variable when 2% NaCl was added to the water that when 20% was added to the feed. When offered 2% NaCl in the water, the sheep either increased their water (and salt) intake with little change in feed intake, or they did not increase the water intake and their feed intake fell. High water-low feed intake and low water-high feed intake combinations did not occur.

This suggests that it is choice (not kidney function) that gave rise to the variable response of the sheep to saline water at salt loads that need not have been detrimental.

Wilson (1966) did not observe the same detrimental effects with 1.5 or 2% NaCl in the drinking water as those observed by Peirce (1957), only observing a reduction in feed intake. These differences and others recorded (Seddon, 1927; Heller, 1933; Peirce, 1957,63) suggest that there is a variation in salt tolerance between sheep, strains of sheep and cattle.

Saul and Flinn (1985) reported that TSS (total soluble solids) concentration of 5000mg/l produced a large but non significant decrease in liveweight gain in Hereford heifers with no adverse affect on health. Climate may be partly responsible for the differences obtained, as mentioned previously, but there is no experimental evidence of a major temperature effect on salt tolerance. Peirce (1957, 59, 60, 62, and 63) did not obtain a seasonal variation in feed intake by sheep on various saline treatments.

The suggestion of taste as the determining factor leads to the proposal that salt tolerance in terms of drinking saline water can be expressed as the reduction in food intake compared with the fresh water treatment. Kidney function could be expressed as litres of increased water intake per 100g additional NaCl (Wilson, 1966). Potter (1963) could find no adaptive changes in kidney function after long periods of salt ingestion which also suggests that taste is an important factor in the adaptation that occurs.

As mentioned previously the farmers visited in the NW-Cape and the NW-Transvaal made mention of the fact that the livestock seemed to either accept a water source or not. If accepted there seemed to be no loss of condition, but if initially refused they would later drink reluctantly but their condition declined. This applied to both cattle and sheep.

Another observation was that some boreholes that were on record as being toxic to livestock were being drunk without any apparent adverse effects, while some that were listed as being acceptable were refused by the animals and if forced to drink from them they lost condition. The main criteria involved were TDS, Cl, F and SO_4.

This could be explained by synergistic and antagonistic effects between the various salts and ions present and the interaction with the environment that is not made allowance for by the guidelines given in the tables used to judge the suitability of the water for livestock. These effects and interactions are most probably due to a taste or palatability effect (as mentioned above).

Due to the variability in environment, species and physiology, and the fact that production decreases at a point before the health is adversely affected (as mentioned above), it is not feasible to try to establish a specific level at which TDS, Cl, SO_4 and F will result in an adverse effect on the health of the animal.

It is known that Cl imparts a negative influence on the palatability of water and so too SO_4, although the level at which this occurs is too varied to pinpoint.

4.1.4 OBJECTIVE

As a result of scientific evidence and derived theory of adaptation and physiological response of animals, it was thus decided that the objective should be rather to investigate if a ratio between these criteria could be established in which taste is the most important factor in terms of the animal's reluctance to accept water and/or decrease in feed intake. If such as ratio could be reached, the objective would then be to manipulate the ratios in an attempt to establish with more precision a relationship which could be used to predict the acceptability of the water.

This would be based on the ratios of the various salts and ions rather than on a number of specific levels or guideline values for TDS and the various ions above which is judged as unacceptable, as is the current practice.

Four treatments were decided upon. The first was to act as control with the TDS and various ions at levels which are judged as acceptable according to the standards set by Adelaar. The exception was the F level which was set at 15mg/l (5mg/l lower than the level found in the previous trial not to have a significant adverse effects - see Chapter 3). The second treatment was to consist of increasing levels of Cl, the third treatment of increasing levels of chiefly SO_4 and the last treatment of 15mg/l F with fresh water (TDS $<$ 1000mg/l). All groups received F at 15mg/l.

Once a point is reached where either the chloride or sulphate groups show a response to the water treatment in terms of a significant difference in water intake within treatments and across treatments with the same salinity, the following manipulations were decided upon. Firstly the level of salinity (and corresponding Cl or SO_4 values) will be dropped to a level dependant on the stage of the trial at which the response occurred and then increased at a faster rate to the same concentration at which the response occurred. The faster rate is to lessen the effects of adaptation and the increase to the same level is to verify the response as treatment related and not related to individual variation or non- treatment related effects.

Depending on the response shown and the treatment levels at which this occurs the levels will increase and follow the same procedure as mentioned, or dropped again to a slightly lower TDS level, but increased with different ratios, or the TDS level will be kept static and the ratios will be altered. The rationale behind this was to investigate the ability or inability of different ratios to alter water intake, and the use of these ratios to predict the response of the animal to various levels of water variables in terms of water intake, and are depicted by the Figures 4.1(a)-(d).

The first figure 4.1(a) illustrates the significant point of refusal **A** in terms of the TDS and Cl levels. Figure 4.1(b) illustrates this point in terms of the ratio of Cl to TDS and water intake **Z**. After manipulating the Cl level, for example, to a point **B** (Fig. 4.1c) where the water intake **K** differs significantly from water intake **Z**. By plotting these and other water intake points the curve **P** can be plotted (Fig.4.1d) and hence function **P** can be predicted. The final equation would therefore attempt to predict water intake with these ratios and other factors that have a major effect on water intake such as stage of production, production level and nutritional status. Equations would be species specific and ideally also breed specific.

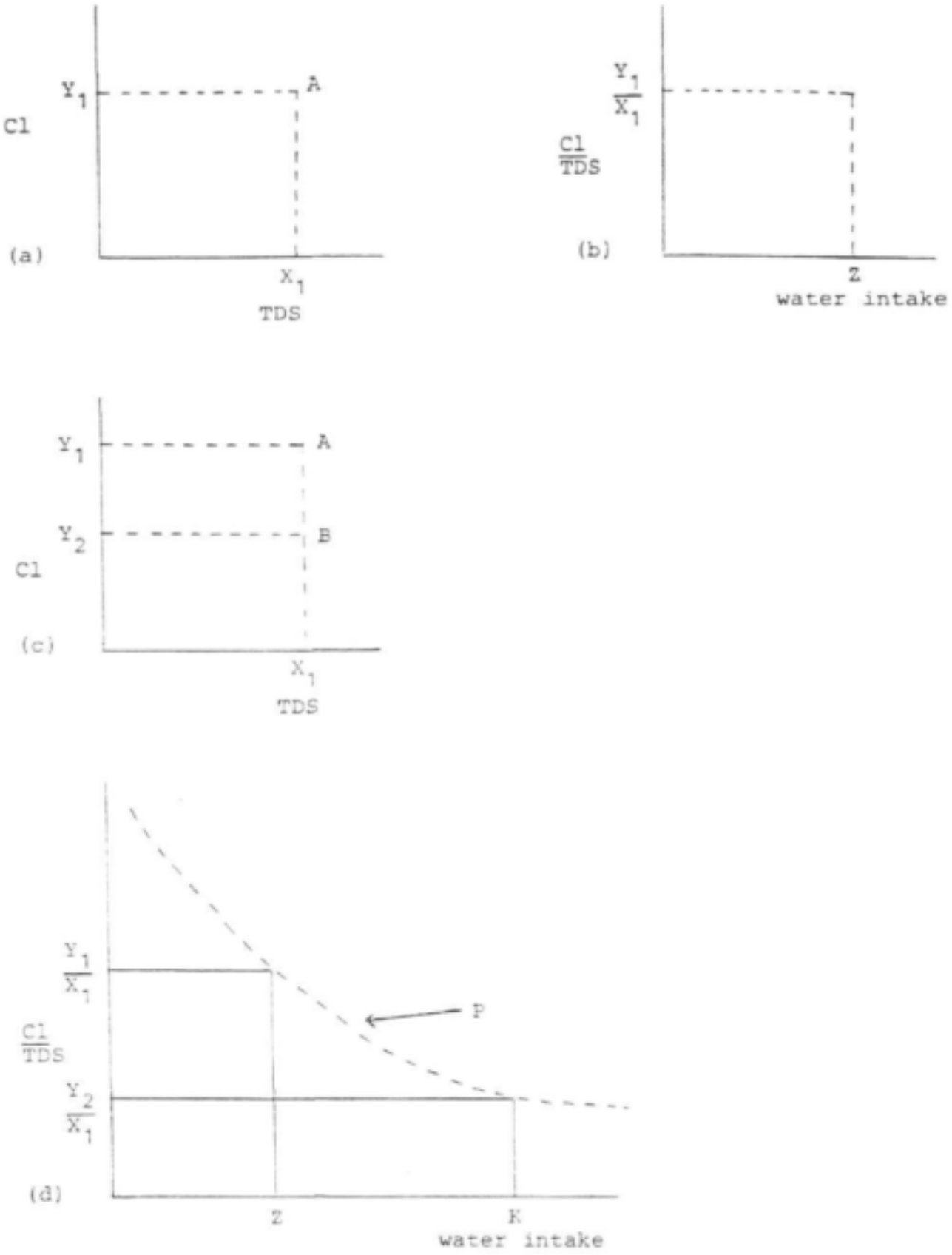


Figure 4.1 (a) (b) (c) & (d) Rationale behind the use of ratio manipulation to predict water intake.

The second factor that the trial incorporates involves salinity and fluoride, with F included at 15mg/l in all of the groups water treatments for reasons already mentioned. It has been recorded that a high level of NaCl reduced F retention in bone tissue (Florian *et al.*, (1986); Ruzika *et al.*, (1976)). It is not known why this occurs, but according to the former authors the possibility that a decreased dietary uptake of F from the digestive system is responsible can be excluded. It was mentioned in Chapter 2. that a similar occurrence was observed on the field trips undertaken to the NW-Cape and NW-Transvaal where the sheep did not show signs of fluorosis while drinking water with toxic amounts of fluoride (by international and local guidelines) and high levels of salinity. There is a correlation in ground water between F and TDS (Hem, 1970). When TDS values are below 1000mg/l, F values are seldom above 1mg/l. High TDS values had correspondingly high F values in the vast majority of the samples reviewed. This possibly explains why livestock in the areas visited did not show symptoms of fluorosis. The sheep in the NW-Cape seemed on appearance to be of a larger skeletal frame size than sheep in the Transvaal that are generally exposed to lower salinity levels. Although this is purely a subjective observation, it is well known that F is advantageous for bone formation. The incorporation of F in bone is advantageous in the sense that F lends itself to the formation of stable bone crystals (Spencer *et al.*, 1984), increases alkali-phosphatase levels which increase the mineralization of bone (Farley *et al.*, 1987) and inhibits osteoclast activity which results in a positive calcium balance (Spencer *et al.*, 1984).

Since most of the above mentioned work with respect to F refers to monogastric animals and has not been done on ruminants, the trial design allows for the effect of saline versus fresh water with both receiving high F levels on bone F concentration to be investigated.

4.2 MATERIALS AND METHODS

Twenty two South African Mutton Merino wethers, just weaned, were used as the experimental animals. They were from the same flock and had been on the same pasture since birth. The trial design consisted of four separate groups as mentioned above of 5 sheep/group with 2 sheep as extras. They were weighed the day after arrival and using a complete randomized block design allocated to their respective groups, using the lightest and heaviest sheep as extras.

4.2.1 ADAPTATION PHASE

On arrival the sheep were housed in a holding camp with water and chopped lucerne. On Day 1 the sheep were weighed and received 5ml/sheep oral dose of Seponver Plus, pulpy kidney inoculations and were tagged. Three sheep had their tails docked. They were then housed individually in crates after being randomly assigned to their groups.

For the first week they received fresh water (TDS <1000mg/l) and chopped lucerne hay *ad libitum*. On Day 8 they were removed from the crates and taken to the crush where they received 5ml/sheep Embemin (orally) and 1.5ml/sheep intramuscular injection of Vitamin A (Phoenix). They were treated in the crush as opposed to in the crates as it was also necessary for them to grow accustomed to being removed from the crates and to learn the route to the crush. This was accomplished by the third week. From Day 6 they received 0.5kg/sheep/day of the pelleted ration (described later) with the lucerne and increasing amounts of the ration were given daily. On Day 15 they received the second pulpy kidney inoculation, and were adapted to the ration and received only the ration. The sheep were weighed weekly except on Day 29 due to a fault in the scale. The water treatments are laid out in detail in the section on water treatments.

The reason for the lengthily adaptation to 3000mg/l TDS and then to 6000mg/l TDS is that it is necessary to allow several weeks for sheep to become accustomed to saline water. This was one of the most important findings according to Peirce (1959). During the adaptation phase the sheep grew accustomed to the removal of the water troughs and feed bins daily, and to the cleaning of the crates which was necessary every few days. The housing facility

was cleaned every morning.

4.2.2 TREATMENTS

4.2.2.1 RATION

The sheep all received the same medium energy (ME) ration *ad libitum* for the duration of the trial. A ME ration was given to lessen the possibility of a dietary effect playing a role. The same energy level was used in the fluoride trial without any dietary problems except for one sheep that had bloated on occasion (see Appendix trial 2 for composition). Measured amounts of the ration were given each morning with the feed bins cleaned of faeces each morning and weighed back each week. Some tended to contaminate the feed within the first half of the trial as they were of a small enough size to enable them to turn in the crates. The bins were emptied on such occasions into plastic troughs and weighed back each week with the feed bins.

4.2.2.2 WATER TREATMENTS

These commenced from Day 5 with the addition of chemicals to the water. The principal ions involved with salinity from underground waters are chlorides, sulphates, bicarbonates and carbonates of Na, Ca and Mg. Hence the compounds NaCl, Na_2SO_4, $CaCl_2$, $MgSO_4$ and $NaHCO_3$ were used. These chemicals were also used for the purpose of comparison with previous work done and to enable ratios of certain ions to salinity to be manipulated. Table 4.2 shows the salts and their ions that were used. Sodium fluoride (NaF) was used as described in the previous trial.

Table 4.2 Molar masses of chemicals used.

SALTS	Molar Masses	Percentages
NaCl	(23)(35) = 58	(39.65)/(60.3)
$NaHCO_3$	(23)[(1)(12)(48)] = 84	(27.38)/(72.6)
$CaCl_2$	(40)(70) = 110	(36.36)/(63.6)
$MgSO_4$	(24.3)[(32.06)(64)] = 120.36	(20.18)/(79.8)
Na_2SO_4	(46)(32.06)(64)] = 142.06	(32.4)/(67.6)

The calculated amounts of these salts (mg) were weighed and placed bags to yield specific concentrations of salts and ions for a set amount of water. A plastic container calibrated up to 20l was used to reconstitute the water each morning as required. Smaller plastic containers of up to 10l were used to supply each animal with water. Each group had their own such containers. Each sheep was supplied with a 10l plastic drinking trough which was cleaned each morning irrespective of the amount left. Larger drinking troughs were provided as the trial progressed (20l) where the consumption exceeded 10l.

The chemical treatments are dealt with in two sections. Firstly the adaptation phase and secondly the trial phase. In each instance the groups are dealt with separately.

4.2.2.2.1 ADAPTATION PHASE

Control group (C)

The purpose of the adaptation phase for the control group was to adapt the sheep to a TDS level of just less than 6000mg/l, as this level is generally recognized as a safe level for sheep and should not result in any adverse effects on health or growth once they were adapted to it. The sheep received the water source without any chemical addition for the first 4 days (see Appendix for chemical analyses of water source). The final concentration of the ions are below the recommended maximum limits according to Adelaar (except for F) and are shown in Table 4.3. The combinations of salts and the corresponding ion concentrations are shown in Table 4.4.

Table 4.3 Final concentrations for control group (mg/l).

IONS	Recommended max. limit (Adelaar, 1974)	Control group
Na	2000	1843.7
Cl	3000	2472.3
Mg	500	181.6
SO_4	1000	887.4
HCO_3	500	363
TDS	6000	5750

The C group also received NaF from Day 5 until the end of the trial at a concentration of 15mg/l.

Chloride and Sulphate groups

These two groups received the same water source from day 1 - 4 as the control group. The objective was to adapt these two groups to a TDS value of 6000mg/l from which the trial could commence, with this as the starting concentration. All the concentrations were within the recommended maximum limits (except for F) as shown in Table 4.5. The treatments from day 5 - 35 are presented in Table 4.6.

Table 4.5 Final concentrations of Cl + SO_4 groups for adaptation phase (concentrations in mg/l).

IONS	Recommended max. limit (Adelaar, 1974)	Cl + SO_4 groups
Na	2000	1706.8
Ca	1000	272.7
Cl	3000	1706.8
Mg	500	151.4
SO_4	1000	767.7
HCO_3	500	363
TDS	6000	6000

Table 4.4 Adaption phase water treatments for control group (concentrations in mg/l).

DAY	SALTS				IONS					TDS
	NaCl	$NaHCO_3$	$MgSO_4$	Na_2SO_4	Na	Cl	Mg	SO_4	HCO_3	
5	425	25	25	25	183.7	256.3	5	37	18	500
6	450	50	50	50	208.7	271.4	10	74	36	600
7	500	75	75	50	236.5	301.5	15	94	54	700
8	584	166	166	83	304.2	352.2	33.5	188.6	120.5	1000
9	840	180	180	100	415.2	506.5	36.3	211.3	130.7	1300
10	840	180	180	100	415.2	506.5	36.3	211.3	130.7	1300
11	1115	180	180	125	532.5	672.3	36.3	228.1	130.7	1600
12	1115	180	180	125	532.5	672.3	36.3	228.1	130.7	1600
13	1375	200	200	125	641.1	829.1	40.4	244.1	145.2	1900
14	1450	250	200	150	672.9	844.2	40.4	261	181.5	2000
15	1450	250	200	150	672.9	844.2	40.4	261	181.5	2000
16	1600	300	250	150	766	964.8	50.5	301	217.8	2300
17	1600	300	250	150	766	964.8	50.5	301	217.8	2300
18	1700	350	350	200	835.6	1025.1	70.6	414.6	254	2600
19	1700	350	350	200	835.6	1025.1	70.6	414.6	254	2600
20	1750	400	500	250	885.4	1055.3	100.9	568.1	290.4	2900
21	1750	500	500	250	911.9	1055.3	100.9	568.1	363	3000
22	1750	500	500	250	911.2	1055.3	100.9	568.1	363	3000
23	1900	500	550	250	977.3	1145.7	111	608	363	3200
24	1950	500	600	250	992.2	1175.9	121.1	648	363	3300
25	2000	500	650	250	1012	1206	131.2	687.8	363	3400
26	2100	500	650	250	1051.7	1266.3	131.2	687.8	363	3500
27	2313	500	675	250	1136.3	1394.7	136.2	707.8	363	3750
28	2625	500	688	250	1260	1582.9	138.8	718.2	363	4063
29	2975	500	775	250	1399	1793.9	156.4	787.6	363	4500
30	3250	500	813	250	1506.6	1959.8	164.1	817.9	363	4813
31	3625	500	875	250	1657.1	2185.9	176.6	867.4	363	5200
32	3875	500	875	250	1756.3	2336.6	176.6	867.4	363	5500
33	4100	500	900	250	1845.7	2473.3	181.6	887.4	363	5750
34	4100	500	900	250	1845.7	2472.3	181.6	887.4	363	5750
35	4100	500	900	250	1845.7	2472.3	181.6	887.4	363	5750

Table 4.6 Adaption phase water treatments for chloride and sulphate groups (concentrations in mg/l)

DAY	SALTS					IONS						TDS
	NaCl	$NaHCO_3$	$MgSO_4$	Na_2SO_4	$CaCl_2$	Na	Cl	Mg	SO_4	Ca	HCO_3	
5	400	25	25	25	25	173.8	257.1	5	37	9.1	18	500
6	400	75	75	75	75	203.7	288.9	15	110.6	27.3	54.5	700
7	500	167	167	83	83	271	354.6	33.7	189.4	30.2	121.2	1000
8	500	167	167	83	83	271	354.6	33.7	189.4	30.2	121.2	1000
9	600	251	221	124	104	346.9	428.3	44.6	260.2	37.8	182.2	1300
10	600	251	221	124	104	346.9	428.3	44.6	260.2	37.8	182.2	1300
11	800	300	250	124	126	439.6	562.9	50.5	283.4	37.8	217.8	1600
12	800	300	250	124	126	439.6	562.9	50.5	283.4	37.8	217.8	1600
13	994	300	300	166	140	530	705	60.5	351.7	60.4	217.8	1900
14	1000	334	334	166	166	541.8	730.3	67.4	378.8	60.4	242.5	2000
15	1000	334	334	166	166	541.8	730.3	67.4	378.8	60.4	242.5	2000
16	1100	400	400	200	200	610.6	791.2	80.7	454.5	72.7	290.4	2300
17	1100	400	400	200	200	610.6	791.2	80.7	454.5	72.7	290.4	2300
18	1200	450	450	250	250	680.1	883.3	90.8	528.2	91	326.7	2600
19	1200	450	450	250	250	680.1	883.3	90.8	528.2	91	326.7	2600
20	1400	500	500	250	250	773.1	1003.3	100.9	568.1	91	363	2900
21	1500	500	500	250	250	812.7	1064	100.9	568.1	91	363	3000
22	1500	500	500	250	250	812.7	1064	107	568.1	91	363	3000
23	1620	500	530	250	300	860.3	1168.6	107	592	109.1	363	3200
24	1720	500	530	250	300	900	1228.9	107	592	109.1	363	3300
25	1790	500	530	250	330	927.7	1290	107	592	120	363	3400
26	1840	500	550	250	360	944.6	1339.5	113.5	608	130.9	363	3500
27	2063	500	563	250	375	1076.4	1482.9	126.1	617.9	136.4	363	3750
28	2188	500	625	250	500	1085.5	1637.6	126.1	667.9	181.8	363	4063
29	2625	500	625	250	500	1258.8	1901	126.1	667.9	181.8	363	4500
30	2725	500	625	250	600	1298.5	2025	126.1	667.9	218.2	363	4700
31	2938	500	687	250	625	1382.9	2169.4	138.6	717.4	227.3	363	5000
32	3188	500	687	250	625	1482	2320.1	138.6	717.4	227.3	363	5250
33	3438	500	750	250	750	1581.2	2550.4	151.4	767.7	272.7	363	5600
34	3438	500	750	250	750	1581.2	2550.4	151.4	767.7	272.2	363	6000
35	3750	500	750	250	750	1706.8	1706.8	151.4	767.7	272.7	363	6000

Fluoride group

The F group received the water source from Day 1 of the adaptation phase till the end of the trial. Fluoride at a concentration of 15mg/l was administered from Day 5 as previously described until the end of the trial.

Extras

The two extras received the water source with no added chemicals for the first four days. They followed the control group treatment from Day 5 until Day 21 where they stabilized on 3000mg/l for the rest of the adaptation phase, and remained there until Day 41 of the trial phase.

4.2.2.2.2 WATER TREATMENTS FOR TRIAL PERIOD

Control group

The control group received as on Day 35 of the adaptation phase until Day 42 of the trial when they where incorporated into the chloride group. This will be discussed in the chloride section that follows.

Chloride group

Day 1 - 40

The chloride group increased in specifically Cl and TDS concentrations from Day 1 of the trial period. This increase was brought about by increasing NaCl and $CaCl_2$. The chemicals $NaHCO_3$, $MgSO_4$, Na_2SO_4 and NaF were retained at the same levels from Day 1 to limit the increase on ion concentrations above the recommended maximum limits to Na and Cl. The objective of this group was to increase the Cl concentration until an adverse effect in terms of water intake, feed intake or health was observed. The manipulations that would follow have already been dealt with. The changes in concentrations are shown in Table 4.7.

Day 41 - 70

On Day 41 sheep number 15 of the chloride group was observed at 7:00am to show signs of severe stress, nervous tremors, lack of coordination but not an elevated temperature, suggesting poisoning, not infection.

Table 4.7 Water Treatments for chloride group (concentrations in mg/l).

DAY	SALTS					IONS						TDS
	NaCl	$NaHCO_3$	$MgSO_4$	Na_2SO_4	$CaCl_2$	Na	Cl	Mg	SO_4	Ca	HCO_3	
1	4063	500	750	250	848	1557	2989	151.4	767.5	308	363	6411
2	4063	500	750	250	848	1557	2989	151.4	767.5	308	363	6411
3	4375	500	750	250	946	1955	3239.8	151.4	767.5	344.3	363	6821
4	4375	500	750	250	946	1955	3239.8	151.4	767.5	344.3	363	6821
5	4688	500	750	250	1044	2079.1	3490.9	151.4	767.5	379.6	363	7232
6	4688	500	750	250	1044	2079.1	3490.9	151.4	767.5	379.6	363	7232
7	5000	500	750	250	1143	2203	3741.9	151.4	767.5	415.6	363	7643
8	5000	500	750	250	1143	2203	3741.9	151.4	767.5	415.6	363	7643
9	5625	500	750	250	1339	2451.1	4243.5	151.4	767.5	486.9	363	8464
10	5625	500	750	250	1339	2451.1	4243.5	151.4	767.5	486.9	363	8464
11	5938	500	750	250	1437	2575.4	4494.5	151.4	767.5	522.5	363	8875
12	5938	500	750	250	1437	2575.4	4494.5	151.4	767.5	522.5	363	8875
13	6250	500	750	250	1535	2699.3	4745.1	151.4	767.5	558.1	363	9285
14	6250	500	750	250	1535	2699.3	4745.1	151.4	767.5	558.1	363	9285
15	6563	500	750	250	1633	2823.5	4996.1	151.4	767.5	543.8	363	9696
16	6563	500	750	250	1633	2823.5	4996.1	151.4	767.5	543.8	363	9696
17	7188	500	750	250	1829	3071.6	5497.6	151.4	767.5	665	363	10517
18	7188	500	750	250	1829	3071.6	5497.6	151.4	767.5	665	363	10517
19	7813	500	750	250	2026	3319.8	5999.1	151.4	767.5	736.3	363	11339
20	7813	500	750	250	2026	3319.8	5999.1	151.4	767.5	736.3	363	11339
21	8438	500	750	250	2222	3567.9	6501.3	151.4	767.5	807.9	363	12160
22	8438	500	750	250	2222	3567.9	6501.3	151.4	767.5	807.9	363	12160
23	8750	500	750	250	2320	3691.8	6751.8	151.4	767.5	843.6	363	12570
24	8750	500	750	250	2320	2691.8	6751.8	151.4	767.5	843.6	363	12570
25	9063	500	750	250	2516	3816	7065.2	151.4	767.5	914.8	363	13079
26	9688	500	750	250	2614	4064.1	7504.4	151.4	767.5	950.5	363	13802
27	9688	500	750	250	2614	4064.1	7504.4	151.4	767.5	950.5	363	13802
28	10000	500	750	250	2713	4188	7755.5	151.4	767.5	986.4	363	14213
29	10000	500	750	250	2713	4188	7755.5	151.4	767.5	986.4	363	14213
30	10625	500	750	250	2909	4436.1	8257	151.4	767.5	1057.7	363	15034

Table 4.7 (cont).

31	11250	500	750	250	3105	4684.3	8758.6	151.4	767.5	1239	363	15855
32	11875	500	750	250	3301	4932.4	9260	151.4	767.5	1200.2	363	16676
33	12500	500	750	250	3498	5180.5	9762.2	151.4	767.5	1271.9	363	17498
34	13125	500	750	250	3694	5428.6	10263.8	151.4	767.5	1343.1	363	18319
35	13750	500	750	250	3890	5676.9	10765.3	151.4	767.5	1414.4	363	19140
36	14375	500	750	250	4086	5924.9	11286.8	151.4	767.5	1485.7	363	19961
37	15000	500	750	250	4283	6173	11769	151.4	767.5	1557.3	363	20783
38	15625	500	750	250	4479	6421.1	12270.5	151.4	767.5	1628.6	363	21604
39	16250	500	750	250	4675	6669.3	12772.1	151.4	767.5	22425	363	22425
40	16250	500	750	250	4675	6669.3	12772.1	151.4	767.5	22425	363	22425
41	3750	500	750	250	750	1706.7	2738.3	151.4	767.5	273	363	6000

The group received the same treatment as the control group for that day, except for No 15 which received oral infusions of fresh water (3l morning and 3l afternoon). The reaction of No 15 was hyperacute, having shown no indication of an adverse effect previously.

The control group was incorporated into the chloride group with the two extra sheep and on Day 42 started increasing in Cl and TDS concentration to a level that was similar to Day 41. The reason for this was to increase the sensitivity of the response to the treatment. The control group had not adapted to the high $CaCl_2$, Cl, or TDS levels as the chloride group had. The extras were even more sensitive as they had been on 3000mg/l TDS.

The same concentration (22425mg/l TDS + 12772mg/l Cl) was reached on Day 50 and higher on Day 51. During this time no similar response to that of No 15 was observed. Both the control group and the extras appeared to have adapted without adverse effects. On Day 44 No 15 had been sent to the Department of Pathology at the Faculty of Veterinary Science, Onderstepoort, as the condition had remained unchanged.

Two aspects were apparent. Firstly, the response of No 15 was not exhibited by the chloride, control and extra sheep. Secondly, the sheep were adapted to such an extent that it was probable that only at a very high concentration of TDS and Cl would an adverse effect be noted, and that this could be toxicological rather than due to taste. The possibility existed therefore that the sheep had adapted to the taste to such an extent that they would not exhibit the expected response with a safety margin between that level and a toxic level. This was due to the slow rate at which Cl had increased and is further discussed in the results and conclusion. For the above two reasons, the control and chloride groups and the extras were lowered to 16500mg/l TDS and the ratio of Cl:TDS was manipulated from Day 52 to Day 60 to yield the ratios that would span a larger range than the ratio from Day 41 - 51 namely 0.456 - 0.571 versus 0.293 - 0.608.

The relatively fast increase to 0.608 from 0.578 and the relatively fast decrease from 0.608 to 0.292 was for the purpose to attain a clearer response should one occur, as opposed to a slower change that would probably not have as clear a response due to the adaptation that could occur. The control group and the extras were then stabilized at a 0.44 ratio till the end

of the trial whilst the chloride group were given a ratio of 0.196 from Day 61 - 67, and then a ratio of 0.608 from Day 68 - 70.

The control group and the extras were stabilized in order to enable them to be compared with the change in the chloride group, which were to left to stabilize at a similar ratio of 0.196, but which then received a rapid change to the highest ratio of 0.608 from Day 68 to the end. Sheep No 19 was brought into the chloride group from the control group on day 61 to bring the number of sheep in the chloride group to 5. A higher ratio could have been attained with a TDS ceiling of 16500mg/l with the use of only $CaCl_2$ (0.639), but this would have a resultant calcium concentration of 6000mg/l, six times the recommended maximum limit of 1000mg/l, which could have an unwanted interaction so this ratio was never used.

The changes in salts and ions for Day 41 - 70 are shown in Tables 4.8(a), (b)and (c), and the changes in ratios are shown in Table 4.9.

Sulphate group

The objective for this group was to increase the SO_4 concentration until an adverse effect was observed, whereafter the ratios of sulphate to TDS would be manipulated as previously described. The increases were brought about by increasing Na_2SO_4 and $MgSO_4$ in the chemical combinations. This group did not receive $CaCl_2$ as it was not desired to have the interaction with F referred to in the previous trial. The sulphate group increased steadily till Day 43 whereafter they were stabilized at 12000mg/l TDS for the remainder of the trial, but had the SO_4:TDS ratios manipulated after a period of Day 43 - 52 to establish a pattern for that ratio which could then be compared with possible changes till the end of the trial. Table 4.10 shows the ratios from Day 43 - 70. The reason for stabilizing the TDS level at 12000mg/l and not increasing it further was the same as the rationale for the chloride group, which was that the sheep had adapted to the sulphate water and may well reach a point where they would experience toxicological effects before a taste effect was observed. The Mg levels was above the 500mg/l recommended maximum limit hence predominantly Na_2SO_4 was increased. The ratio changes from 0.272 to 0.649 after having Day 43 - 70 to stabilize a pattern at 0.438. The ratio increased from 0.438 to 0.649 and then dropped sharply to 0.272 from Day 62 - 70, once again to obtain a clearer response should one occur.

Table 4.8(a) Water treatments for groups chloride, control and extras (concentrations in mg/l).

DAY	SALTS					IONS						TDS
	NaCl	$NaHCO_3$	$MgSO_4$	Na_2SO_4	$CaCl_2$	Na	Cl	Mg	SO_4	Ca	HCO_3	
42	5500	500	750	250	1000	2401.5	3952.9	151.4	767.5	363.6	363	8000
43	7750	500	750	250	1250	3294.8	5468.8	151.4	767.5	454.5	363	10500
44	9625	500	750	250	1375	4039.1	6679	151.4	767.5	500	363	12500
45	9625	500	750	250	1375	4039.1	6679	151.4	767.5	500	363	12500
46	11500	500	750	250	1500	4783.5	7889.1	151.4	767.5	545.4	363	14500
47	11500	500	750	250	1500	4783.5	7889.1	151.4	767.5	545.4	363	14500
48	13375	500	750	250	1625	5527.9	9099.3	151.4	767.5	590.9	363	16500
49	15000	500	750	250	4283	6173	11769	151.4	767.5	1557.3	363	20783
50	16250	500	750	250	4675	6669.3	12772.1	151.4	767.5	1699.8	363	22425
51	16700	500	750	250	5000	6847.8	13252.1	151.4	767.5	1818	363	23200
52	15000	250	500	0	781	6016	9542	100.9	399.1	284	181.5	16500
53	15000	250	500	0	781	6016	9542	100.9	399.1	284	181.5	16500
54	15000	0	0	0	1563	5947.5	10039.7	0	0	568.3	0	16500
55	15000	0	0	0	1563	5947.5	10039.7	0	0	568.3	0	16500
56	15000	0	0	0	1563	5947.5	10039.7	0	0	568.3	0	16500
57	9750	875	3188	2688	0	4976.4	5879.3	643.3	4361.8	0	635.3	16500
58	9750	875	3188	2688	0	4976.4	5879.3	643.3	4361.8	0	635.3	16500
59	8000	1000	3188	4313	0	4847.4	4824	643.3	5460.3	0	726	16500
60	8000	1000	3188	4313	0	4847.4	4824	643.3	5460.3	0	726	16500

Table 4.8(b) Water treatments for control group and extras (concentrations in mg/l).

DAY	SALTS					IONS						TDS
	NaCl	NaHCO3	$MgSO_4$	Na_2SO_4	$CaCl_2$	Na	Cl	Mg	SO_4	Ca	HCO_3	
61 - end	11250	1000	3563	0	750	4740.3	7269.7	719	2844	272.7	726	16500

Table 4.8(c) Water treatments for chloride group (concentrations in mg/l).

DAY	SALTS					IONS						TDS
	NaCl	$NaHCO_3$	$MgSO_4$	Na_2SO_4	$CaCl_2$	Na	Cl	Mg	SO_4	Ca	HCO_3	
61 - 67	5375	1000	3875	6250	0	4432.9	3241.1	782	7318	0	726	16500
68 - end	15000	0	0	0	1563	5955	10039.7	0	0	568.3	0	16500

The changes in salt and ion concentrations are shown in Table 4.11.

Fluoride group

No change.

Extras

Incorporated into the control group, but for statistical purposes were not included in analyses.

Table 4.9 Ratios of Cl:TDS for chloride + control groups and extras from Day 41 - end (concentrations in mg/l).

DAYS	RATIOS
	Cl:TDS
41	2738.3 : 6000 = 0.456
42	3952.9 : 8000 = 0.494
43	5468 : 10500 = 0.521
44-45	6679 : 12500 = 0.534
46-47	7889.1 : 14500 = 0.544
48	9099 : 16500 = 0.551
49	11769 : 20783 = 0.566
50	12772 : 22425 = 0.570
51	13252.1 : 23200 = 0.571
52-53	9452 : 16500 = 0.578
54-55-56	10039.7 : 16500 = 0.608
57-58	5879.3 : 16500 = 0.356
59-60	4824 : 16500 = 0.292
control + extras	
61-end	7269 : 16500 = 0.440
chloride	
61-67	3241.1 : 16500 = 0.196
67-end	10039.7 : 16500 = 0.608

Table 4.10 Ratios of SO4 : TDS for sulphate group from Day 39 - end (concentrations in mg/l).

DAYS	RATIOS
	SO_4 : TDS
39-42	5006.1 : 11694 = 0.428
43-51	5256.6 : 12008 = 0.438
52-53	5759.6 : 12000 = 0.480
54-55	6266.6 : 12000 = 0.522
56-57	6773.6 : 12000 = 0.564
58-59	7280.6 : 12000 = 0.607
60-61	7787.6 : 12000 = 0.649
62 - end	3266.5 : 12000 = 0.272

Table 4.11 Water treatments for sulphate group (concentrations in mg/l).

DAY	SALTS				IONS					TDS
	NaCl	$NaHCO_3$	$MgSO_4$	Na_2SO_4	Na	Cl	Mg	SO_4	HCO_3	
1	3750	500	813	324	1730.6	2261.3	164.1	867.9	363	5387
2	3750	500	813	324	1730.6	2261.3	164.1	867.9	363	5387
3	3750	500	875	398	1754.7	2261.3	176.6	967.3	363	5523
4	3750	500	875	398	1754.7	2261.3	176.6	967.3	363	5523
5	3750	500	938	472	1778.6	2261.3	189.3	1067.1	363	5660
6	3750	500	938	472	1778.6	2261.3	189.3	1067.1	363	5660
7	3750	500	1000	546	1802.6	2261.3	201.8	1167.1	363	5796
8	3750	500	1000	546	1802.6	2261.3	201.8	1167.1	363	5796
9	4375	500	1125	693	2098.3	2638.1	226.9	1366.3	363	6693
10	4375	500	1125	693	2098.3	2638.1	226.9	1366.3	363	6693
11	4375	500	1188	767	2120.1	2638.1	239.7	1466.5	363	6830
12	4375	500	1188	767	2120.1	2638.1	239.7	1466.5	363	6830
13	4375	500	1250	841	2144	2638.1	252.3	1566	363	6966
14	4375	500	1250	841	2144	2638.1	252.3	1566	363	6966
15	4375	500	1313	914	2167.8	2638.1	265	1665.7	363	7102
16	4375	500	1313	914	2167.8	2638.1	265	1665.7	363	7102
17	4375	500	1438	1062	2215.7	2638.1	290.2	1865.4	363	7375
18	4375	500	1438	1062	2215.7	2638.1	290.2	1865.4	363	7375
19	4375	500	1563	1209	2263.3	2638.1	315.4	2064.5	363	7647
20	4375	500	1563	1209	2263.3	2638.1	315.4	2064.5	363	7647
21	4375	500	1688	1357	2311.3	2638.1	340.6	2264.3	363	7920
22	4375	500	1688	1357	2311.3	2638.1	340.6	2264.3	363	7920
23	4375	500	1750	1431	2335.3	2638.1	353.2	2363.9	363	8056
24	4375	500	1750	1431	2335.3	2638.1	353.2	2363.9	363	8056
25	4375	500	1813	1503	2358.6	2638.1	365.9	2462.8	363	8191
26	4375	500	1938	1652	2406.8	2638.1	391.1	2663.3	363	8465
27	4375	500	1938	1652	2406.8	2638.1	391.1	2663.3	363	8465
28	4375	500	2000	1726	2430.8	2638.1	403.6	2762.8	363	8601
29	4375	500	2000	1726	2430.8	2638.1	403.6	2762.8	363	8601
30	4375	500	2125	1873	2478.5	2638.1	428.8	2961.9	363	8873
31	4375	500	2250	2021	2526.4	2638.1	454	3161.7	363	9146
32	4375	500	2375	2168	2574	2638.1	479.3	3360.9	363	9418
33	4375	500	2500	2316	2622.2	2638.1	504.5	3560.6	363	9691
34	4375	500	2625	2463	2669.6	2638.1	529.7	3759.8	363	9963
35	4375	500	2750	2611	2717.6	2638.1	555	3959.5	363	10236

Table 4.11 (cont)

DAY	NaCl	$NaHCO_3$	$MgSO_4$	Na_2SO_4	Na	Mg	SO_4	Cl	HCO_3	TDS
36	4375	500	2875	2758	2765.2	580.2	4158.7	2638.1	363	10508
37	4375	500	3000	2906	2813.1	605.4	4358.5	2638.1	363	10781
38	4375	500	3125	3053	2860.8	6630.6	4537.6	2638.1	363	11053
39	4375	500	3250	3569	3028	655.9	5006.1	2638.1	363	11694
40	4375	500	3250	3569	3028	655.9	5006.1	2638.1	363	11694
41	4375	500	3250	3569	3028	655.9	5006.1	2638.1	363	11694
42	4375	500	3250	3569	3028	655.9	5006.1	2638.1	363	11694
43	4375	500	3563	3569	3028	719	5256.6	2638.1	363	12008
44	4375	500	3563	3569	3028	719	5256.6	2638.1	363	12008
45	4375	500	3563	3569	3028	719	5256.6	2638.1	363	12008
46	4375	500	3563	3569	3028	719	5256.6	2638.1	363	12008
47	4375	500	3563	3569	3028	719	5256.6	2638.1	363	12008
48	4375	500	3563	3569	3028	719	5256.6	2638.1	363	12008
49	4375	500	3563	3569	3028	719	5256.6	2638.1	363	12008
50	4375	500	3563	3569	3028	719	5256.6	2638.1	363	12008
51	4375	500	3563	3569	3028	719	5256.6	2638.1	363	12008
52	3625	500	3563	4313	2836.5	719	5759.6	2185.9	363	12000
53	3625	500	3563	4313	2836.5	719	5759.6	2185.9	363	12000
54	2875	500	3563	5063	2917.4	719	6266.6	1733.6	363	12000
55	2875	500	3563	5063	2917.4	719	6266.6	1733.6	363	12000
56	2125	500	3563	5813	2863	719	6773.6	1281.4	363	12000
57	2125	500	3563	5813	2863	719	6773.6	1281.4	363	12000
58	1375	500	3563	6563	2808.6	719	7280.6	821.1	363	12000
59	1375	500	3563	6563	2808.6	719	7280.6	821.1	363	12000
60	625	500	3563	7313	2754.2	719	7787.6	376.9	363	12000
61	625	500	3563	7313	2754.2	719	7787.6	376.9	363	12000
62	7313	500	3563	625	3239.1	719	3266.5	4409.7	363	12000
"										
end										

4.2.3 PARAMETERS MONITORED

(a) **Water intake:**

Individual water intake was measured on a daily basis by using calibrated containers. Due to the season (March to July) and the housing environment the increase in concentration due to evaporation was not significant (<0.02ml/d) as it was in the first trial.

(b) **Feed intake:**

Average feed intake was measured by feeding pre-weighed amounts on a daily basis and weighing back.

(c) **Live weight:**

Weekly live weights were recorded, except on day 28 of the adaptation phase (faulty scale) and on Week 7 and Week 9 (Figure 4.2), due to the increased size of the sheep at that stage and the difficulty involved in removing the sheep from the crates and replacing them. The sheep were not fasted as in the first trial as it was found that it has a dramatic effect on the water intake for the following 2 days, and this would have resulted in an undesired effect on the water intake of the sheep.

(d) **Blood:**

Blood samples were taken from the jugular vein at intervals shown in Figure 4.2 (on the same days as weighing took place). The jugular vein was used as it is representative of the general circulation. The necks of the sheep were shorn to facilitate the drawing of blood. Samples of 10ml/sheep were drawn and centrifuged immediately afterwards (8000 rpm/ 10min) and the plasma removed with a syringe and placed in Ependorf tubes and frozen. The sampling time was from 8-9:00. The sheep were allowed to settle for an hour in the holding pens before samples were taken. The samples were analysed for chloride concentration by the Department of Chemical Pathology, Faculty of Medical Sciences, University of Pretoria. The samples were analysed using a silver detector electrode, platinum cathode electrode and a reference electrode, using an ASTRA Routine Analyzer system (Beckman, 1987).

(e) **Urine:**

Urine samples were obtained at the same frequency as shown in Figure 4.2. Urine collection funnels were harnessed to the sheep with a tube leading into a sample bottle after watering in the morning. Approximately 2-4 hours afterwards 20ml samples were removed and frozen. This method was used due to the structure of the crates in which the sheep were housed. The samples were analysed for chloride and creatinine concentrations (used as a constant reference) by the Department of Chemical Pathology, Faculty of Medical Sciences, University of Pretoria. The same method used for blood chloride was used for urinary chloride. Urinary creatinine was measured by calculating the change in absorptance in an alkaline picrate solution following sample addition, as described by Beckman (1987).

Figure 4.2 Schedule according to which live weight, urine and blood samples were recorded over the 71 day treatment period.

(f) **Slaughter measurements:**

(i) Hot carcass weight was recorded.

(ii) Thyroid glands were excised as described in the previous trial and weighed.

(iii) Kidney and liver sections were cut and dealt with as laid out in the previous trial.

(iv) Metacarpal and metatarsal bones were removed and 2-3mm thick cross sections cut at the same sampling point for all sheep and placed in distilled water in plastic sample bottles and later analysed for F potentiometrically (Van Staden and Janse van

Rensburg, 1991). The metacarpal and metatarsal bones were used as they are one of the most suitable sampling sites for post mortem diagnosis of fluorosis (Clarke *et al.*, 1981; Buck and Osweiler, 1976).

(v) Physiopathology of fluorosis - visual macroscopic evaluation for pathological lesions were conducted as in the first trial.

(g) **Health:**

Weekly inspections of teeth, palpation of limbs and clinical symptom observations for fluorosis as described in the first trial were carried out. Daily inspections of the faeces (for diarrhoea) and general health was done.

Statistical analyses used

The same methods described in Chapter 3 were employed in the statistical methods used in this trial (Chapter 3, section 3.2.2.5).

4.3 RESULTS

4.3.1 WATER INTAKE

1. Mean water intake (MWI) on a weekly basis between groups : Days 1 - 70

The MWI for each group is given in Table 4.12. The chloride and control groups did not differ significantly from each other, while the fluoride and sulphate groups differed significantly from each other, the chloride and control groups (P =0.0001; F = 57.5; R^2 = 0.38).

Table 4.12 Mean water intake (weekly basis) between groups for Days 1-70.

Duncans Multiple Range Test *	MWI (l/day)	TREATMENT
A	5.64	Chloride
A	5.48	Control
B	4.91	Sulphate
C	4.05	Fluoride

* Means with the same letter are not significantly different

(alpha = 0.05 d.f. = 276 MSE = 0.63).

2. MWI on a weekly basis within groups : Days 1-70

(i) **Control group**

The following differences were significant : Week 1 from Weeks 7, 8, 9 and 10; Week 2 from Weeks 7 and 8; Week 7 from Weeks 1, 2, 3, 4, 5, 6 and 8; Week 8 from Weeks 1, 2, 3, 4, 5, 6, 7, 9 and 10 (P = 0.0001, F = 7.31, R^2 = 0.52). The results are presented in the Appendix (Trial 2).

(ii) **Fluoride group**

The following differences were significant : Week 1 from Weeks 5, 8, 9 and 10; Week 2 from Weeks 5, 8, 9 and 10; Week 3 from Weeks 8 and 9 (P = 0.0067, F = 2.89, R^2 = 0.30). The results are presented in the Appendix (Trial 2).

(iii) **Chloride group**

The following differences were significant : Week 1 from Weeks 7 and 8; Week 7 from Weeks 1, 9 and 10; Week 8 from Weeks 1, 3, 4, 6, 9 and 10 ($P = 0.0009$, $F = 3.72$, $R^2 = 0.36$). The results are presented in the Appendix (Trial 2).

(iv) **Sulphate group**

Week 2 differed significantly from Weeks 5, 8, 9 and 10 ($P = 0.108$, $F = 1.7$, $R^2 = 0.20$). The results are presented in the Appendix (Trial 2).

3. MWI differences using treatments as narrower intervals

Using the results of the previous section as a guideline, further differences in MWI were investigated on narrower intervals as defined by the treatment changes (see Tables 4.7, 4.8 and 4.9 for chemical composition of treatments). The control group is compared with the chloride and sulphate groups for Days 1 - 40 for the purpose of investigating the salinity and palatability effects of the various chemicals on water intake. Between group analysis of the control group and the chloride group are used from Day 42 - 70 due to the group allocation and treatments received during this phase of the trial (calculations for Day 69 and 70 for the control group are made to enable comparison with the ratio change for the chloride group at this stage -see Table 4.9).

(a) **Control and Chloride groups**

(i) **Within group MWI differences : Days 1-40**

Weeks 3 (Days 15-22) and 6 (Days 35-38) were significantly different within the control group ($P = 0.0197$, $F = 1.71$, $R^2 = 0.37$), with Week 3 having the highest MWI of 5.5 l/d and Week 6 the lowest MWI of 4.27 l/d. There were no significant differences between weeks within the chloride group from Days 1 - 40 ($P = 0.26$, $F = 1.48$, $R^2 = 0.34$). The results for the Duncan Multiple Range Tests are given in the Appendix (Trial 2).

(ii) **Between group MWI differences : Days 1-40**

The results for between group analysis on a weekly basis for Days 1 - 40 are presented in Table 4.13. The MWI for Week 5 (Days 29-35) was significantly different.

The significant difference between the chloride and control groups for Week 5 (Table 4.13) led to the analysis of MWI between these two groups for the period Day 29 to 40 on a daily basis. The results are presented in Table 4.14. Days 31, 34, 36, 37, 38 and 39 had significant differences between the chloride and control groups for Duncans Multiple Range Test.

Table 4.13 Significance levels for between group MWI differences for Chloride and Control groups : Days 1-40.

Intervals		Significance values		
Days	Week	P	F	R^2
1 - 7	1	0.59	0.31	0.37
8 - 14	2	0.24	1.56	0.16
15 - 21	3	0.76	0.09	0.01
22 - 28	4	0.27	1.35	0.14
29 - 35	5	0.01	12.3	0.61
35 - 40	6	0.85	0.03	0.01

* significance level $P < 0.05$

(iii) **Within group differences for MWI by intervals: Days 42-70**

The intervals used in the following analysis are based on the treatment changes (Table 4.8) The intervals and means are presented in Table 4.15. The control group had the following significant differences between intervals : interval 1 from intervals 3, 4, 5 and 6; interval 2 from intervals 3, 4 and 5; interval 9 from intervals 3, 4, 5, 6, 7 and 8; interval 10 from interval 5; interval 11 from intervals 3, 4, 5, 6, 7 and 8.

Table 4.14 Significance levels for between group MWI differences for chloride and control groups for Days 29 to 40.

DAY	Significance values			Means(l/d)	
	P	F	R^2	Control	Chloride
29	0.48	0.54	0.06	*	*
30	0.09	3.62	0.31	4.53	5.4
31	0.02	7.27	0.48	4.01	6.08*
32	0.16	2.34	0.23	5.39	6.19
33	0.49	0.51	0.06	5.4	5.85
34	0.03	6.55	0.45	5.67	6.78*
35	0.005	14.44	0.64	4.98	7.14*
36	0.63	0.25	0.03	5.78	6.17
37	0.01	8.76	0.52	4.78	6.63*
38	0.0005	31.75	0.79	4.93	7.17*
39	0.08	3.98	0.33	4.65	2.7*
40	0.59	0.30	0.03	3.99	4.8

* significant difference ($P < 0.05$) for Duncans Multiple Range Test

The chloride had the following significant differences between intervals : interval 5 from intervals 1, 8, 9, 10 and 11; interval 6 from intervals 8, 9, 10 and 11; interval 11 from intervals 2, 3, 4, 5, 6 and 7. The results are presented in the Appendix (Trial 2).

(iv) **Between group differences for MWI by intervals : Days 42-70**

The comparisons between groups that were made are presented in Table 4.16. There was a significant difference between interval 10 of the control group and 11 of the chloride group.

Table 4.15 Intervals and MWI for control and chloride groups used for within and between group analysis.

Days	Interval	Mean Water Intake	
		Control	Chloride
42-43	1	5.29	5.57
44-45	2	5.45	6.10
46-47	3	7.02	6.31
48-49	4	7.03	6.54
50-51	5	7.43	7.44
52-53	6	6.57	6.96
54-56	7	6.43	6.45
57-58	8	6.26	5.31
59-60	9	4.83	5.24
61-68	10	5.79	5.14
69-70	11	4.86	4.44

Table 4.16 Between group significance levels for Days 42-70 for chloride and control groups for MWI ($P<0.05$).

Intervals		Significance values		
Chloride	Control	P	F	R^2
1	1	0.71	0.15	0.02
2	2	0.80	0.07	0.01
3	3	0.10	3.4	0.33
4	4	0.45	0.62	0.08
5	5	0.32	1.14	0.14
6	6	0.47	0.57	0.08
7	7	0.98	0	0.0009
8	8	0.27	1.39	0.16
9	9	0.60	0.29	0.04
10	10	0.16	2.37	0.25
10	9	0.45	0.61	0.07
11	11	0.11	3.38	0.36
11	10	0.04	5.95	0.43

(v) **Between and within group differences for MWI by days and intervals : Days 42-70**

Various comparisons were made between and within groups by days and intervals in order to compare immediate response to treatment changes and adaptation over intervals to treatments. Within the chloride group significant differences were found between the following comparisons : Day 68 to Day 69; Day 68 to Day 70; Day 68 to interval 11 (Day 69+70). Significant differences were found between the chloride and control groups for the following comparisons : interval 11 of chloride and Day 61 - Day 70 of control (intervals 10+11); Day 68 of control and interval 11 of chloride. The significance values are presented in Table 4.17.

Table 4.17 Significance levels for comparisons between chloride and control groups for Days 42-70.

Comparisons	Significance values		
within groups	P	F	R^2
chloride			
Day 68-69	0.005	14.47	0.64
Day 68-70	0.009	11.65	0.59
Day 68-days (69-70)	0.003	16.52	0.67
Interval 10-9	0.908	0.01	0.002
Interval 10-11	0.303	1.21	0.13
control			
Day (67+68)-Day (69-70)	0.22	1.70	0.175
between groups			
chloride - control			
Interval 11 - Days 61-70	0.11	3.22	0.29
Interval 11 - Day 68	0.003	18.75	0.70

* significance level $P < 0.05$

(b) **Sulphate group**

(i) **Within group differences for Days 1-42**

The days were analysed by weekly intervals and there were no significant differences within the group for the Days 1-42 ($P = 0.35$, $F = 1.21$, $R^2 = 0.30$). The Duncan groupings are presented in the Appendix (Trial 2).

(ii) **Within group differences for Days 42-70**

The only significant difference was found between the intervals 3 (Days 52-53) and 6 (Days 58-59), with 3 having the highest MWI (5.76) and 6 the lowest MWI (3.02). The results are presented in the Appendix (Trial 2).

(iii) **Between group differences for Days 1-45**

The control group was used in comparison with the sulphate group for this period in order to assess the effect of sulphate on the palatability of the water with increasing salinity. The interval Days 36-42 was not used in these comparisons as the control group treatment was changed on Day 42. There were no significantly different comparisons between the sulphate and control groups. The results are presented in Table 4.18.

Table 4.18 Significance levels for comparisons between control and sulphate groups for Days 1-35.

Days	Intervals	Significance values		
		P	F	R^2
1-7	1	0.44	0.65	0.07
8-14	2	0.69	0.17	0.02
15-21	3	0.91	0.01	0.001
22-28	4	0.59	0.31	0.04
29-35	5	0.83	0.05	0.01

* significance level $P < 0.05$

(c) **Fluoride group**

There were no significant differences found between selected intervals (on a weekly basis) for the fluoride group. The results and Duncan groupings are presented in Appendix (Trial 2).

4. Correlations between MWI and corresponding chemical levels

(i) Chloride group

The following correlations were found from Days 40-70 between MWI and TDS, Cl and SO_4 on a weekly basis : -0.47, 0.02 and 0.48. None were significant at the 0.05 level (see Appendix Trial2 for results). There was a significant negative correlation between MWI and the Cl concentration in the water between Days 68 and 69 on a daily basis (Table 4.19).

Table 4.19 Correlations between MWI and [Cl] for the chloride group.

Days	Pearson Correlation value	Significance	Coefficients
53-54	0.06	0.858	
56-57	-0.391	0.368	
58-59	0.20	0.568	
60-61	-0.61	0.056	
68-69	-0.82	0.003	

* significance level $P < 0.05$

(ii) **Control group**

There was a significant negative correlation between MWI and the chloride concentration in the water between Day 58 and Day 59. All other correlations were negative and non-significant. The results are presented in Table 4.20.

Table 4.20 Correlations between MWI and [Cl] for the control group.

Days	Pearson correlation coefficients	Significance values
53-54	-0.53	0.169
56-57	-0.09	0.818
58-59	-0.71	0.046
60-61	-0.11	0.782

* significance level $P < 0.05$.

(iii) **Sulphate group**

The following correlations were found between MWI and TDS, Cl and SO_4 on a weekly basis for Days 40-70 : -0.23, 0.43 and -0.45. None were significant at the 0.05 level (see Appendix Trial 2 for results). There were no significant correlations between sulphate concentration and MWI on a daily basis for Days 51-62 (Table 4.21).

Table 4.21 Correlations between MWI and [SO_4] for the sulphate group.

Days	Pearson Correlation coefficients	Significance values
51-52	0.29	0.409
53-54	0.14	0.681
55-56	0.25	0.477
57-58	-0.60	0.865
59-60	-0.18	0.600
60-61	0.27	0.444
61-62	0.27	0.445

* significance level $P < 0.05$

4.3.2 AVERAGE FEED INTAKE

Treatment only had a significant effect on average feed intake in Weeks 6 and 7. Table 4.22 gives the average feed intake recordings with the respective significance values. None of the least square means, besides some of those for Weeks 6 and 7, were significant. The least square means, means and standard deviations for Weeks 6 and 7 are given in Table 4.23. Treatments 2+1, 2+4 and 2+3 differ significantly from each other, while treatments 3+1, 4+1 and 4+3 do not (for both Weeks 6 and 7). Treatment 2 had a significantly lower mean intake (kg) than treatments 1,3 and 4 (Table 4.24).

Table 4.22 Significance levels for effects of treatment on average feed intake.

WEEKS	P	F	R^2
1	0.494	0.01	0.00
2	0.998	0.01	0.00
3	0.181	1.84	0.26
4	0.173	1.88	0.25
5	0.198	1.74	0.25
6	0.022	4.29	0.46
7	0.023	4.26	0.45
8	0.268	1.45	0.22
9	0.714	0.46	0.08
10	0.375	1.11	0.08

* significance level ($P < 0.05$)

Table 4.23 Least square means for average feed intake - Weeks 6 and 7.

TRT	Mean s.d.	Least Square Means
	week 6	
1	1.908 0.1780	TRT 1 2 3 4
2	1.447 0.1715	1 . 0.0068 0.7124 0.9320
3	1.856 0.2744	2 0.0068 . 0.0139 0.0080
4	1.896 0.2247	3 0.7124 0.0139 . 0.7765
		4 0.9320 0.0080 0.7765 .
	week 7	
1	1.934 0.102	TRT 1 2 3 4
2	1.554 0.156	1 . 0.0051 0.4864 0.7329
3	1.857 0.174	2 0.0051 . 0.0201 0.0100
4	1.893 0.232	3 0.4864 0.0201 . 0.7195
		4 0.7329 0.0100 0.7195 .

* significance level ($P < 0.05$)

Table 4.24 Effects of treatment on the mean average feed intake for Days 1 - 70.

Days	Significance values		
	P	F	R^2
1 - 70	0.4722	0.54	0.029

* significance level ($P<0.05$)

No significant correlations between treatment and AFI on a weekly basis were found (Table 4.25).

Table 4.25 Correlations between average feed intake (AFI) and treatment for Days 1 - 70.

AFI	Pearson correlation coefficient	P
1	0.162	0.494
2	-0.010	0.960
3	0.258	0.271
4	0.198	0.402
5	-0.079	0.738
6	0.132	0.590
7	0.073	0.764
8	0.288	0.231
9	0.214	0.378
10	0.156	0.522

* significance level ($P<0.05$)

4.3.3 LIVE WEIGHT

The P, R^2 and Type III sum of squares for the models Treatment = LW1 -LW9 are given in Table 4.26. At no live weight recording did treatment have a significant effect on liveweight (see Appendix Trial 2 for means and standard deviations) or were there any

significant treatment differences.

Table 4.26 Significance levels for effects of treatment on live weight.

Liveweight recordings	P	Type III LW1 = 1 TRT = 2	R^2	F
LW1	0.9565	- 0.9565	0.019	0.10
LW2	0.0001	0.0001 0.5555	0.91	38.36
LW3	0.0001	0.0001 0.9201	0.771	12.63
LW4	0.0015	0.0001 0.9534	0.668	7.56
LW5	0.0035	0.0002 0.9755	0.626	6.30
LW6	0.0076	0.0004 0.9907	0.582	5.24
LW7	0.0343	0.0048 0.8943	0.502	3.53
LW8	0.0587	0.0179 0.5491	0.456	2.94
LW9	0.0565	0.0068 0.9145	0.4599	2.98

* significance level $P < 0.05$

4.3.4 BLOOD

Treatment had a significant effect on blood for Days 43 ($P < 0.0001$) and 57 ($P < 0.006$). The results are presented in Table 4.27. The least square means (Table 4.28) showed significant differences between treatments 1+2, 1+3, 2+3 and 2+4 for Day 43, and 2+3,

2+4 and 1+4 for Day 57. For Day 43 the chloride group had the lowest mean blood [Cl], and for Day 57 the same pattern emerged (Table 4.29).

Table 4.27 The effects of treatment on blood serum chloride concentration in SAMM wethers.

Sampling day	Significance values		
	P	F	R^2
1	0.076	2.76	0.34
15	0.965	0.02	0.09
29	0.755	0.4	0.06
43	0.001	9.28	0.64
57	0.006	6.03	0.53
71	0.403	1.04	0.6

*significance level $P < 0.05$.

Table 4.28 Least square means for sampling Days 43 and 57 for blood serum chloride concentrations between treatments.

Sampling day	Least Square Means			
	TRT	1	2	3 4
43	1	.	0.0130	0.0303 0.2501
	2		.	0.0041 0.0013
	3			. 0.2504
	4			.
	TRT	1	2	3 4
57	1	.	0.3009	0.0622 0.0167
	2		.	0.0073 0.0018
	3			. 0.5136
	4			.

*significance level $P < 0.05$.

Table 4.29 Means and standard deviations for blood serum [Cl] for sampling Days 43 + 57.

Sampling day	Mean (mmol/l)	s.d.	[Cl] of drinking water (mg/l)
	TRT		TRT
43	1	105	3.08	1	5468
	2	100	1.91	2	5468
	3	108	2.60	3	719
	4	106	1.48	4	10
	TRT			TRT	
57	1	103	2.28	1	5879
	2	101	2,50	2	5879
	3	106	2.04	3	719
	4	107	2.58	4	10

A significant positive correlation was found between treatment and blood serum [Cl] for Day 1, and significant negative correlations for Days 43 and 57 for these two variables (Table 4.30).

Table 4.30 Pearson correlation coefficients for variables treatment ([Cl] of water treatments) and blood serum [Cl].

Sampling day	Pearson correlation coefficient	P
1	0.45	0.04
15	0.05	0.81
29	-0.11	0.62
43	-0.63	0.003
57	-0.70	0.0005
71	-0.38	0.095

*significance level $P < 0.05$.

4.3.5 URINE

Treatment had a significant effect on urinary [Cl] for Days 8+9, 36+37 and 65+66. Creatinine was significantly affected by treatment for Days 36+37 (Table 4.31). Table 4.32 presents the least square means for these significant treatment effects. Treatments 1+3, 1+4, 2+3 and 2+4 were significantly different for Days 8+9 (urinary [Cl]), treatments 1+4, 2+3, 2+4 (urinary [Cl])
and 1+3, 1+4, 2+3, 2+4 and 3+4 (urinary creatinine) for Days 36+37 and treatments 1+3, 1+4, 2+3, 2+4 and 3+4 (urinary [Cl]) for Days 65+66. The means and standard deviations are presented in Table 4.33, with treatment 4 (fluoride group) having the lowest mean for all the urinary [Cl] sampling days and treatment 2 (chloride group) for urinary [creatinine] for Days 36+37.

Table 4.31 The effect of treatment on urinary [Cl] and urinary [creatinine] (Days 36+37).

Sampling days	Significance values		
	P	F	R^2
8+9	0.0001	17.11	0.76
36+37	[Cl] 0.004	6.4	0.54
	[creat] 0.046	3.33	0.38
65+66	0.001	20.03	0.80

*significance level $P<0.05$.

Table 4.32 Least square means for sampling Days 8+9, 36+37 and 65+66 for urinary [Cl] and Days 36+37 for urinary [creatinine] between treatments.

Sampling Days	Least Square Means
	TRT 1 2 3 4
8+9	1 . 0.1710 0.0058 0.0001
	2 . 0.0003 0.0001
	3 . 0.0904
	4 .
	TRT 1 2 3 4
36+37	1 . 0.5060 0.0934 0.0047
[Cl]	2 . 0.0254 0.0011
	3 . 0.1540
	4 .
	TRT 1 2 3 4
36+37	1 . 0.0075 0.2157 0.0566
[creat]	2 . 0.0953 0.3289
	3 . 0.4552
	4 .
	TRT 1 2 3 4
65+66	1 . 0.5579 0.0027 0.0001
	2 . 0.0086 0.0001
	3 . 0.0112
	4 .

*significance level $P < 0.05$

Table 4.33 Means and standard deviations for urinary [Cl] for sampling Days 8+9, 36+37 and 65+66, and urinary [creatinine] for sampling Days 36+37 and corresponding [Cl] in the drinking water.

Sampling Days	Mean (mmol/l)	s.d	[Cl] in the drinking water (mg/l)
	TRT		TRT
8+9	1	283	44.72	1	2472
	2	339	61.18	2	3490
	3	159	96.75	3	2261
	4	89	12.45	4	10
	TRT			TRT
36+37	1	329	46.35	1	2472
[Cl]	2	365	82.66	2	12000
	3	234	134.78	3	2638
	4	155	29.24	4	10
	TRT			TRT
36+37	1	5.4	0.80	1	2472
[creat]	2	2.4	0.84	2	12000
	3	4.1	2.76	3	2638
	4	3.4	0.91	4	10
	TRT			TRT
65+66	1	462	104.33	1	7269
	2	437	51.34	2	3241
	3	302	44.16	3	4409
	4	173	39.85	4	10

Variables urinary [Cl] and urinary [creatinine] and urinary [Cl] and [Cl] of the treatments had significantly positive correlations for sampling Days 8+9 and 36+37. Variables urinary [Cl] and [Cl] of the water treatments had a significantly positive correlation for Days 65+66 (Table 4.34).

Table 4.34 Pearson correlation coefficients for variables treatment ([Cl] of the drinking water) and urinary [Cl] and urinary [creatinine].

Sampling Days	Pearson correlation	P coefficient
8+9	[Cl] 0.78	0.0001
	[creat] 0.72	0.0003
36+37	[Cl] 0.58	0.0076
	[creat] 0.45	0.0428
65+66	[Cl] 0.74	0.0003
	[creat] -0.20	0.41

*significance level $P < 0.05$.

4.3.6 SLAUGHTER PARAMETERS

4.3.6.1 HOT CARCASS WEIGHT

The effects of treatment on hot carcass weight (corrected for liveweight at the start of the trial - LW1) were non significant. The P, R^2 and Type III values are given in Table 4.35 together with the least square means (see Appendix Trial 2 for means and standard deviations).

Table 4.35 Significance levels for the effect of treatment on hot carcass weight.

Significance tests	Significance values	Least Square Means
P	0.0233	TRT
R^2	0.5319	1 2 3 4
Type III		1 . 0.2200 0.9728 0.6834
TRT	0.5597	2 0.2200 . 0.2094 0.3817
LM1	0.0070	3 0.9278 0.2094 . 0.6588
		4 0.6834 0.3817 0.6588 .
F	3.98	

* significance level $P < 0.05$

4.3.6.2 THYROID GLAND WEIGHT

Treatment did not have any significant effect on thyroid gland weight (corrected for hot carcass weight). There were no significant differences between treatments (see least square means). The significance values are given in Tables 4.36.

Table 4.36 Significance values for the effect of treatment on thyroid gland weight.

Significance tests *	Significance values *	Least Square Means **
P	0.0550	TRT 1 2 3 4
R^2	0.4624	1 . 0.4903 0.5917 0.0990
Type III		2 0.4903 . 0.2575 0.0370
TRT	0.1549	3 0.5917 0.2575 . 0.2428
HCM	0.0160	4 0.0990 0.0370 0.2428 .
F	3.01	

* significance level $P < 0.05$

** significance level $P < 0.0083$

4.3.6.3 KIDNEY AND LIVER HISTOPATHOLOGY

There were no histopathological lesions in the liver or kidney sections evaluated (see Appendix Trial 2 for pathology report).

4.3.6.4 FLUORIDE CONCENTRATION OF METACARPAL BONE

The metacarpal bone samples (right metacarpal) of the entire fluoride group and five selected from the other three treatments were analysed first (the metatarsal bone samples were not needed to obtain significant values). The treatments used were saline and fresh water treatments, with groups 1,2 and 3 comprising the saline treatment, and group 4 the fresh water treatment. Treatment was found to have a significant effect (corrected for F ion intake during the trial period) on the concentration of F in the right metacarpal bones analysed, with the saline water treatment yielding lower concentrations despite having a higher intake of the

ion. A positive correlation of 60% was found between the variables treatment and fluoride concentration, and a negative correlation of 40% was found between total fluoride intake and treatment. The results are given in Tables 4.37 and 4.38.

Table 4.37 Significance values for the effect of saline and fresh water treatments on metacarpal bone fluoride concentration of SAMM wethers.

Significance tests	Significance values	Means Standard deviation
P	0.0205	TRT F conc.
R^2	0.3669	1 660.940 94.8925
Type III		2 825.050 132.5307
TRT	0.0313	F intake
CFI	0.6787	1 5665.49 1376.9320
		2 4370.30 411.2982
F	4.93	

* significance level $P < 0.05$

CFI = corrected F intake

TRT 1 = Saline TRT 2 = Fresh

Table 4.38 Correlation coefficients and significance values between fluoride concentrations in metacarpal bone samples of SAMM wethers and (i) treatment (saline and fresh water) and (ii) corrected fluoride intake (CFI).

	F + TRT	F + CFI
Pearson corrl. coeff.	-0.40222	0.60022
P	0.0787	0.0051

* significance level $P < 0.05$

4.3.6.5 MACROSCOPIC PATHOLOGY

No pathological lesions were observed in the macroscopic evaluations conducted (Appendix, Trial 2 - pathology report).

4.3.7 HEALTH

No clinical symptoms of fluorosis or salt poisoning were observed, except for sheep No 15 (Appendix, Trial 2 - pathology report).

4.4 DISCUSSION

4.4.1 The effect of treatment on live weights for Weeks 1-9; hot carcass weight; average feed intake; health and histopathology of the kidney and liver; and blood and urinary [Cl + creatinine].

The mean water intake (MWI) on a weekly basis for Days 1-70 shows the significant effect of salinity on water intake, with the control and chloride groups having a significantly higher water intake than the sulphate and fluoride groups, with the fluoride group significantly lower than the sulphate group. Despite these differences there was no significant difference for live weight 1-9, hot carcass weight or mean average feed intake between groups for the trial duration.

The non significant effect of treatment on mean average feed intake (MAFI) and the absence of any significant correlation between treatment and average feed intake through Days 1-70 suggests that while there were significant differences in average feed intake (weeks 6 + 7), the overall feed intake was not adversely affected by changes in the palatability or salinity of the water. This is supported by the non significant differences between groups for liveweights 1-9 and hot carcass weight.

The histopathological results seem to indicate further that the sheep were able to tolerate the levels exposed to them without any adverse physiological effect. The adaptation phase and rate of salinity increase appears to have been of such a nature as to allow the sheep to cope with the treatments despite the significant effects of treatment on water intake at various

stages (discussed later). The response of sheep No 15 would seem to be an individual non-treatment related occurrence. This is supported by the ability of the chloride and control groups to reach the same level and higher without any similar response, and the absence of histopathological lesions in the organs investigated. A possible explanation is that sheep No 15 reacted to the pulpy kidney inoculation (pathologist Dr M. Williams, Onderstepoort, personal communication).

Blood plasma [Cl] seems to be negatively correlated with the [Cl] in the drinking water. Even though Day 71 did not have a significant correlation the trend was the same (R^2 = -0.38, P = 0.095). The chloride group had the lowest blood plasma [Cl], with the control group second. The chloride group was significantly lower than the control group, probably due to a carry-over effect from the previous treatment which was higher than the chloride group (Day 43) and lower than the control group (Day 43) with [Cl] of 7755mg/l and 2472mg/l respectively. This is supported by a similar pattern on Day 57 with the chloride and control groups significantly different from the sulphate and fluoride groups as for Day 43, but not significantly different from each other whilst being on the same [Cl] of 5879mg/l. The decreased blood plasma [Cl] with increased [Cl] in the drinking water observed was similar to the findings of Peirce (1966) for Na and Peirce (1968a) for Mg, and Little, Dansom, Manston and Allen (1984) for serum Na.

The least square means differences for Days 8+9 for urinary [Cl] are in line with the positive correlations found, i.e. increase in urinary [Cl] with increases in [Cl] of the drinking water. For Days 65+66 it appears that the chloride group had a carry-over effect as the group still had a high urinary [Cl] despite a lower [Cl] in the drinking water. The trend with urinary [creatinine] was less clear, with the chloride group having the lowest value despite the highest [Cl] in the drinking water for Days 36+37, yet for these days a significant positive correlation was found between urinary [creatinine] and [Cl] of the drinking water.

4.4.2 The effects of treatment on average water intake

Control group : Days 1-70 - weekly basis

The MWI on a weekly basis for Days 1-70 differed significantly from the sulphate and fluoride groups, with the control MWI higher than the sulphate and fluoride groups despite the higher salinity of the sulphate treatments for Days 1-40 (see Figure 4.3(a)). The non significant differences in weekly MWI for Weeks 1-6 show that the intake of <6000mg/l TDS was relatively constant, apart from an initial increase during Weeks 1 and 2. The significant difference in MWI for Weeks 7 + 8 from each other and all other weeks was due to the increase in salinity of the treatment. They reacted positively in the sense that their water intake increased with increase in salinity of the treatment. There was no adverse palatability effect with the increase in salinity from Days 42-51 (TDS concentrations of 6000mg/l - 22000 mg/l). This suggests that the previous 42 Days spent on <6000 mg/l enabled them to adapt readily to the increased salinity.

Chloride group : Days 1-70 - weekly basis

The lower MWI for Week 6 (5.22l/d) than Weeks 2, 3, 4 and 5, although non significant, would suggest that despite the higher salinity for Week 6 the water intake was lower due to a negative palatability effect. The response of sheep No 15 halted the increase in TDS and Cl, but for Weeks 7 and 8 the salinity increase to 22000mg/l TDS yielded an increase in water intake with salinity, with these two weeks having the highest MWI for the trial period, with Week 8 differing significantly from all weeks except Week 7.

Figure 4.3(a) Effect of treatment on weekly water intakes

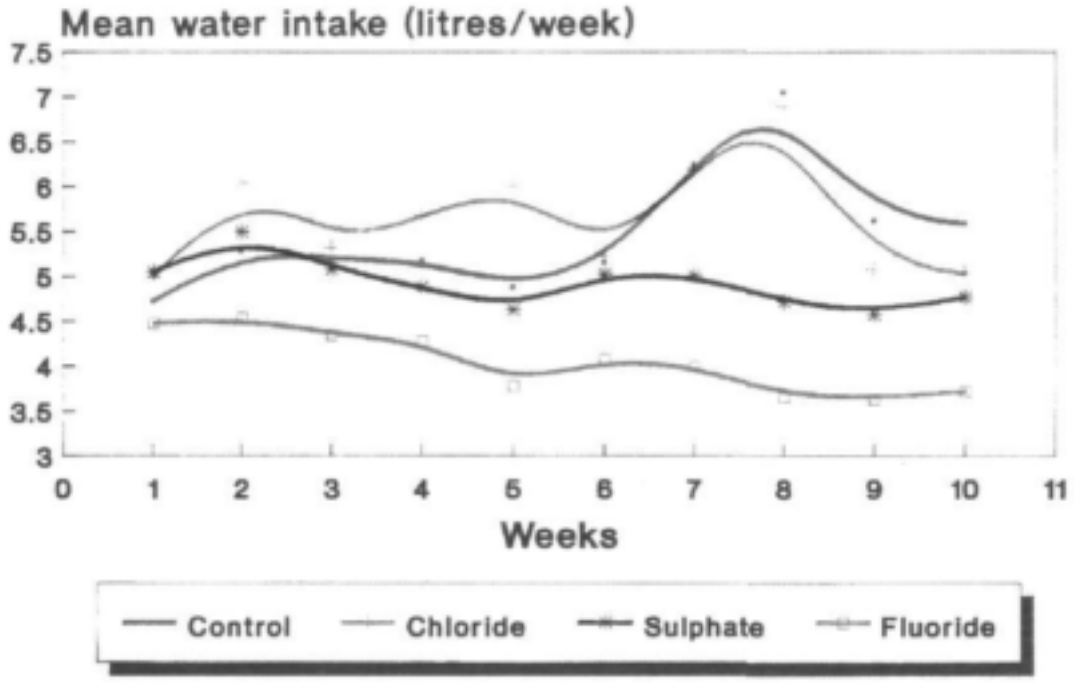
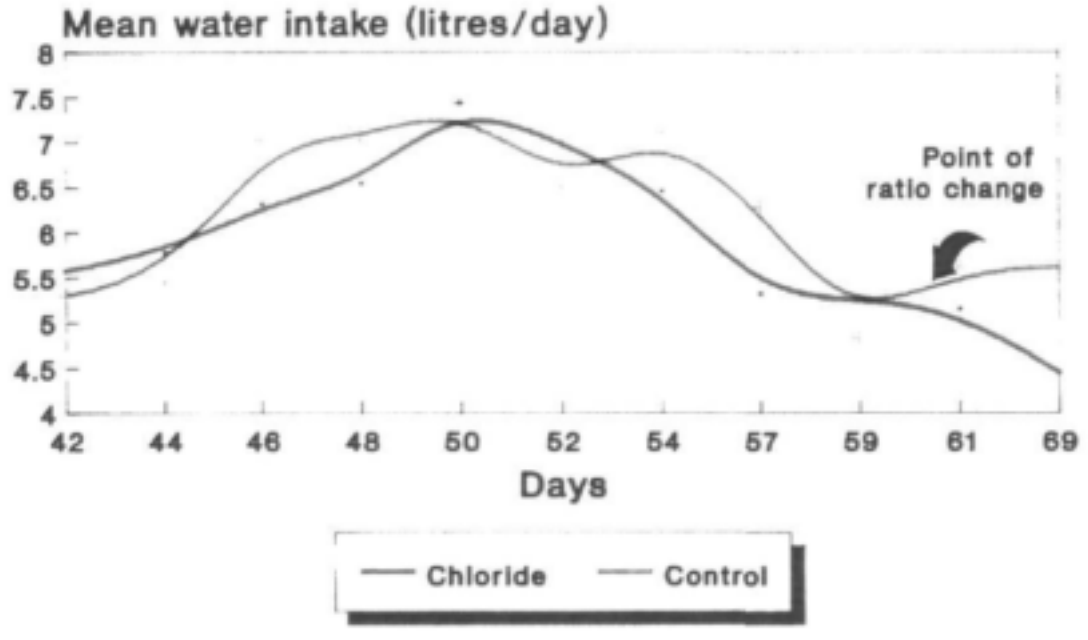


Figure 4.3(b) Effect of treatment on mean water intake from day 42 of Chloride and Control groups



Control and Chloride groups : Days 29-40 - daily comparisons

The significant increase in water intake of the chloride group at Days 29-35 is due to the higher salinity received at this stage. The non significant difference during Days 35-40 leads to speculation that palatability is beginning to have an effect, since despite the higher TDS level, the water intake was not significantly different. On a daily basis comparison a similar pattern emerges.

For Days 29-38 the chloride group has a higher water intake than the control group, with Days 31, 34, 35, 37 and 38 being significantly different. This was due to saline effects. On Days 39 and 40, however, the chloride group had significantly lower MWI than the control group. This was most probably due to a palatability effect. The increase in water intake from Day 39 to Day 40 shows a physiological need governing water intake. This response occurred with a Cl: TDS ratio of 0.57. The AFI for Week 6 (Days 36-42) was significantly lower for the chloride group compared with the other groups. This was most probably due to the decrease in water intake leading to the subsequent decrease in feed intake due to the strong positive correlation that exists between them.

Control and chloride groups : Days 42-70

Control group : Days 42-70

Intervals 1-5 (Days 42-51) increased in MWI with interval 5 having the highest for the Days 42-70, with a TDS concentration of >22000mg/l. The significant difference between intervals (1+2) and intervals (3, 4, 5, 6, 7 + 8) are due to the higher water intake with increase in salinity. Intervals 6, 7 and 8 (Days 52-58) did not differ significantly which suggests that there was no palatability effect, but the significant difference between interval 9 and intervals 6, 7 and 8 is contrary to the expected response with a lower water intake occurring with a lower Cl concentration. The explanation for this response is that the sudden increase in SO_4 (0mg/l - 5460mg/l) used to lower the Cl ratio to 0.292 overshadowed the expected increase in palatability.

The response to the Cl ratio of 0.44 was constant for the remainder of the trial. The lower intake of intervals 6, 7, 8, 9, 10 and 11 from intervals 3, 4 and 5 were probably due to the decrease in salinity from 2200mg/l TDS to 16500mg/l TDS. The non significant difference between intervals 1, 2, 9, 10 and 11 are possibly due to the lower salinity levels at this period as opposed to intervals 3, 4, 5 and 6. Intervals 6, 7 and 8 decreased in water intake in that order due to the lowered salinity, the high salinity in intervals 1-5 still affected the drinking pattern in these intervals.

Chloride group : Days 42-70

The water intake increase from interval 1-5 with each interval, and decrease again, was due to the increase in TDS to 22000mg/l at interval 5, and then drop to 16500mg/l TDS with interval 6. The significant difference between intervals 1 and 5 was probably due to this increasing salinity level (see Figure 4.3(b)).

The non significant difference between intervals 7, 8, 9 and 10 with the Cl:TDS ratio 0.5 - 0.196 would indicate that the changes in ratios were not of a large enough magnitude to result in a significant difference in water intake. The significantly lower water intake on interval 11 from intervals 2, 3, 4, 5, 6 and 7 seem to support the above finding as this was the most abrupt change possible between intervals 10 and 11 to the highest Cl concentration possible for 16500mg/l TDS (ratio 0.196 - 0.608) - see Figure 4.4(a).

The finding is that lowering the Cl concentration slowly does not increase the water intake, i.e. the water is not made more palatable and sheep drink according to physiologically dictated needs governed by salinity. But with a sharp enough change to a high Cl ratio from a low Cl ratio, the high ratio does result in a decrease in palatability that leads to a significant decrease in water intake. The following comparisons support this finding.

Comparisons between Chloride and Control groups : Days 42-70

The non significant difference between the control and chloride groups for intervals 1-9 shows a similar response of these two groups to the same treatment during this phase. The non significant difference between the groups for interval 10 support the previous finding that lowering the Cl concentration does not increase the water intake significantly (ratio 0.196 - 0.44).

The non significant difference between the groups for interval 11 is misleading as is seen when compared on a daily basis, and shows some of the sheep in the chloride group reacting more to the physiological need to increase their water intake than to the Cl ratio after initially responding to the ratio. Day 68 compared with Day 69 for the chloride group had a significantly lower water intake showing an immediate response to the increase in Cl concentration (ratio 0.196- 0.608). The water intake for Days 69 + 70 compared to Day 68 for the chloride group was also significantly lower. All sheep had a decrease in water intake from Day 68 to Day 69 in the chloride group, but 3 sheep increased their water intakes on the following day, and this overshadowed the further declines by the other two sheep on Day 70. Day 70 compared with Day 68 for the chloride group also yielded a significantly lower water intake, as did Days 69 + 70 compared with Day 68. Days 69 + 70 for the control group compared to Days 69 + 70 for the chloride group did differ significantly ($P \approx 0.11$). This was probably due to the 3 sheep in the chloride group that responded to the physiological need for water due to the salinity level. The non significant difference between Days 67 + 68 and 69 + 70 for the control group shows that the decrease in water intake in the chloride group was not due to the environment. The significant differences between these two groups for interval 11 of the chloride group and Days 61-70 and interval 10 for the control group further highlights the response of the chloride group to the higher Cl ratio during interval 11.

Figure 4.4(a) Effect of Cl:TDS ratio on mean water intake from day 42 for Chloride group

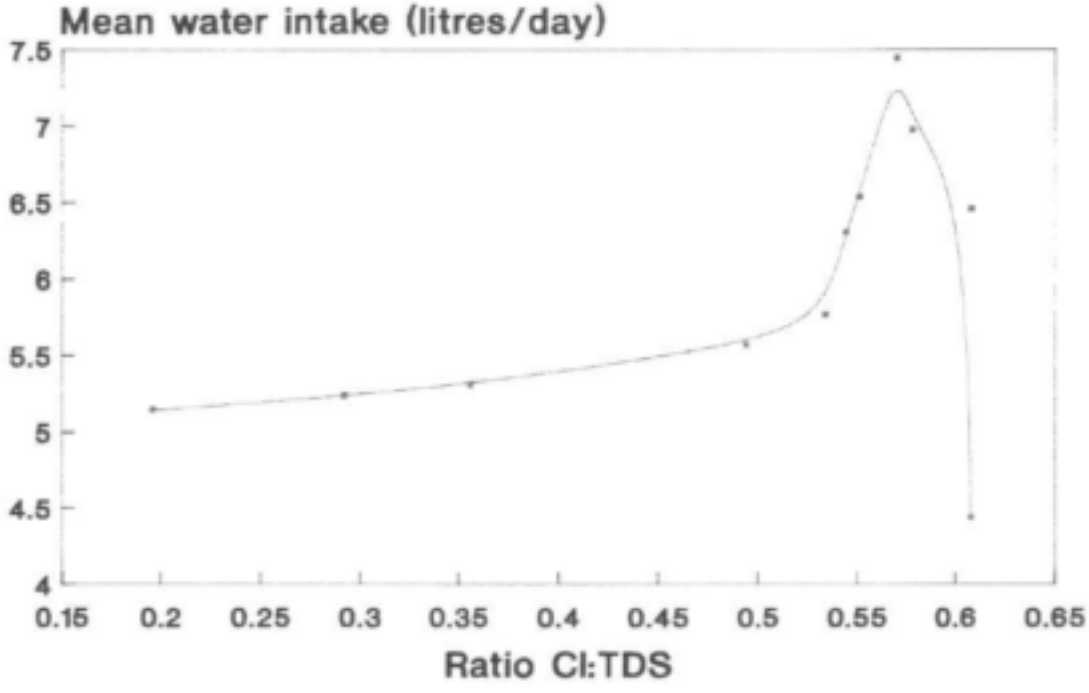


Figure 4.4(b) Effect of Sulphate:TDS ratio on mean water intake from day 39 for Sulphate group.

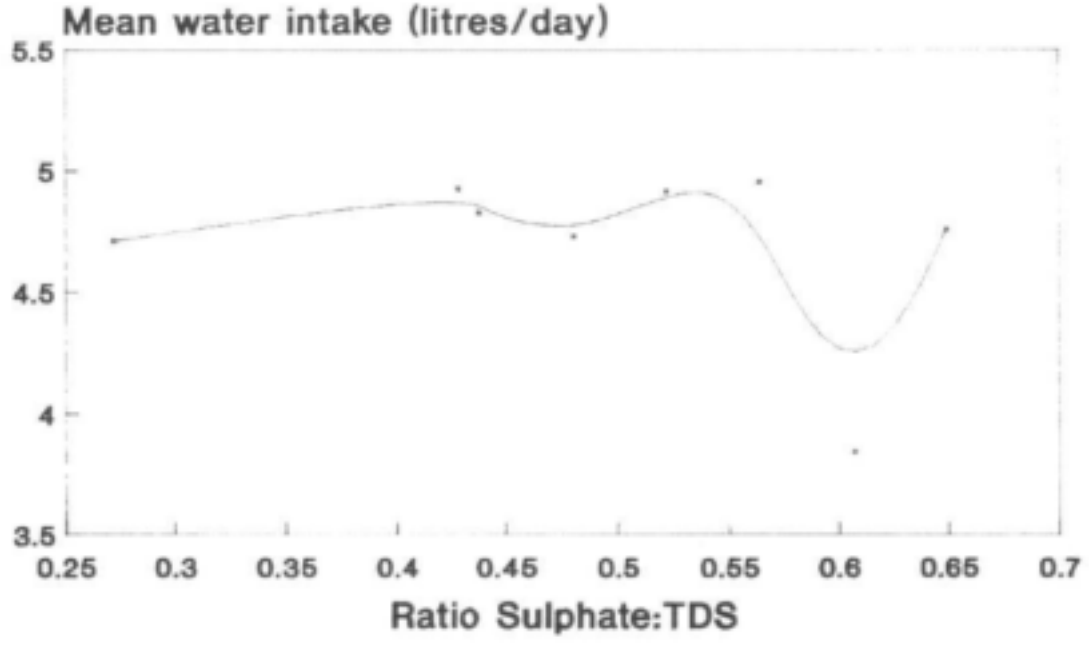


Figure 4.4(a) Effect of Cl:TDS ratio on mean water intake from day 42 for Chloride group

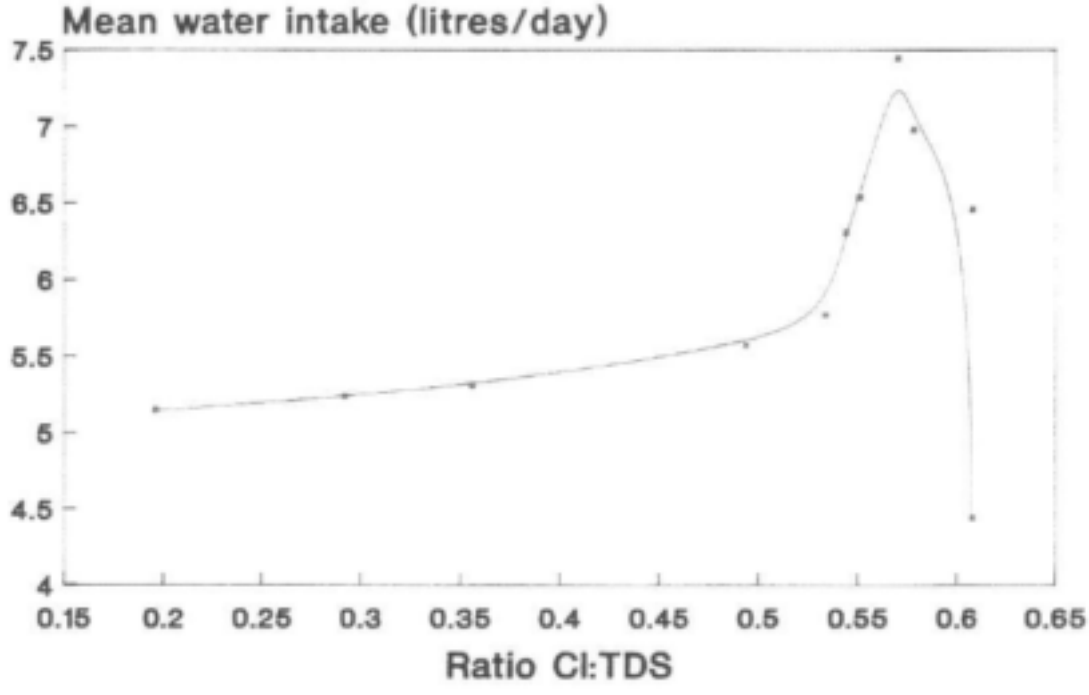
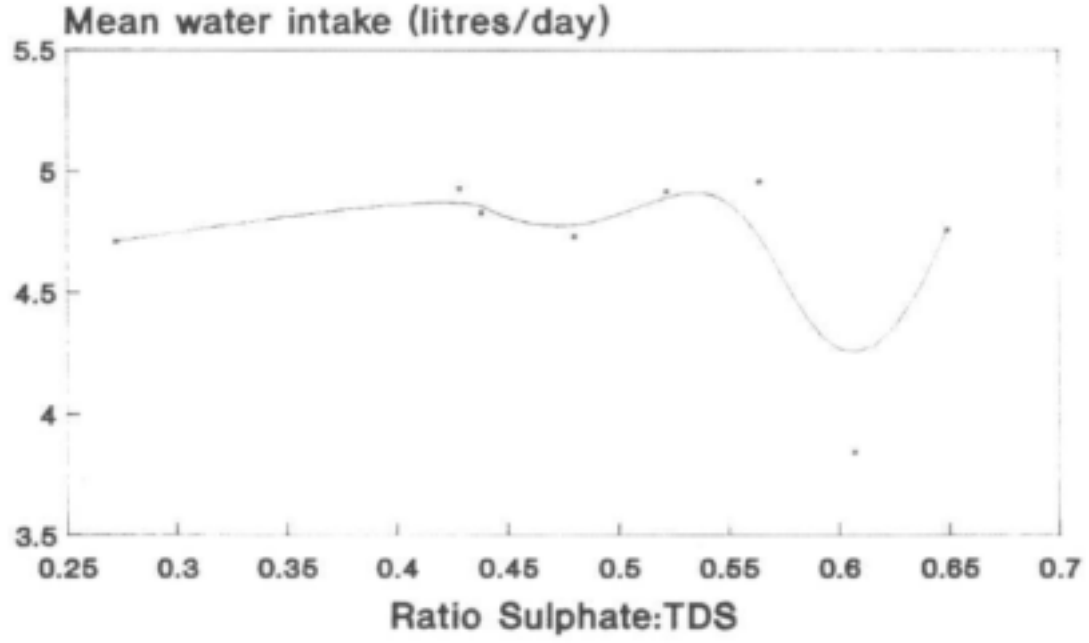


Figure 4.4(b) Effect of Sulphate:TDS ratio on mean water intake from day 39 for Sulphate group.



The significant negative correlations found between Cl and MWI (Days 58-59 and 68-69) seem to indicate further that Cl imparts a negative palatability effect on water.

Sulphate group : Days 1-70 - weekly basis

The significant difference between Week 2 and Weeks 5, 8, 9 and 10 with Week 2 having the highest MWI of 5.49 l/d would seem to be due to two factors. Firstly, the increase in salinity initially (Weeks 2+3 had the two highest MWI) , and secondly, the negative effect of SO_4 on the palatability of the water which led to low water intakes (Weeks 5, 8, 9 and 10) despite higher TDS levels.

The non significant difference between the control and sulphate groups for the period tested (Weeks 1-5) support this, with the poorest fit found at week 5 (R^2 = 0.001, P = 0.829) at the highest difference in TDS (5750mg/l for control group - 10000 mg/l for sulphate group). This shows that with the increase in TDS concentration MWI differed less between the control and sulphate groups. This culminated at Week 5 with the second lowest MWI for the sulphate group being recorded (comparisons stopped here as the control group changed treatment).

The negative correlations found during Days 40 - 70 between MWI, SO_4 (-0.45) and TDS (-0.23), although non significant, show a palatability effect overriding the normal response to increased salinity (usually strongly positive). The higher intake in week 6 with increases in the SO_4 levels seems to be similar to the findings with the chloride and control groups that the physiological need dominates the palatability effect. It has been found that with highly saline waters rats tended to refuse the water until they were eventually driven to it by thirst where they consumed large amounts and then died (Environmental Protection Agency). The effect of the environment in which the sheep were, may have significantly altered their response to the unpalatable treatments in the sense that they were confined to crates and had little else to do but feed and drink. It seems plausible that under natural conditions the initial response shown to unpalatable water would have continued for longer.

A similar pattern was found with Week 9 recording the lowest MWI and having a high intake the following week. This pattern is clearer when viewed on a narrower interval based on treatment changes where interval 6 (ratio SO_4 = 0.607) had the lowest MWI, significantly different from interval 3. The following interval 7, despite a higher ratio of 0.649, had the second highest water intake for the period Days 39-70. This is similar to the response mentioned above with the physiological need dominating the palatability effect, but with a different time scale. The MWI for intervals 5-6 had a negative correlation of -0.60 (P = 0.06) with SO_4 (see Figure 4.4(b)).

The non significant difference between interval 8 (ratio = 0.201) and intervals 1, 2, 3, 4, 5 and 7 (all high ratios) seems to be in line with the findings of the chloride and control groups that lowering the ratio does not necessarily increase water intake.

The negative effect of SO_4 on palatability seems to be greater or more pronounced than that of Cl. An example of this is the lower water intake in interval 9 for the control group which had a low Cl ratio of 0.292, but a low MWI was recorded, possibly due to the increase in SO_4.

Fluoride group

The lowest MWI (Days 1-70) on a weekly basis was recorded for the fluoride group. This is directly due to the lower salinity (< 1000 mg/l TDS) received by this group. The reasons for the higher MWI during Weeks 1, 2 and 3 are not clear, but the Weeks 4, 5, 6, 7, 8, 9 and 10 did not differ significantly from each other and this group showed a stable MWI.

4.4.3 The effects of saline water treatment compared with fresh water treatment on bone F concentration

The strong negative correlation that was found indicated that further investigation as to the mechanism responsible is needed. The effects of 15mg/l F on the growth and health of SAMM wethers to market weight were the same as observed in the fluoride trial (Chapter 3). The [F] of >600mg/l for the saline group and >800mg/l for the fresh water group are above the concentrations of around 370-500mg/lm F for "normal" bone (Phillips and Suttie, 1960) and 300-360mg/l (Underwood, 1981), although the techniques used in estimation of F previously are questionable.

4.4.4 SUMMARY OF MAIN POINTS

1. Sheep tolerated the concentrations of salts and ions administered for the duration of the trial without any adverse effects on health, live weight, hot carcass weight, feed or water intake.

2. Blood [Cl] was significantly affected by treatment and showed a significant negative correlation existed between blood [Cl] and [Cl] of the drinking water.

3. Urinary [Cl] was significantly affected by treatment and showed a significant positive correlation between urinary [Cl] and [Cl] of the drinking water.

4. Certain levels of Cl and SO_4 were found to have a significant adverse effect on palatability , expressed by a significant decrease in water intake for the sulphate and chloride groups, and a significant decrease in feed intake for the chloride group.
This palatability effect occurred at Cl:TDS ratios of 0.608 compared to 0.196 and 0.44 for Cl, and 0.607 and 0.400 for SO_4:TDS ratios.

5. The adverse effects occurred when drastic changes in ratios were made from low to high.

6. The lowering of ratios with constant TDS levels did not increase the water intake.

7. For the sulphate and chloride groups adverse effects on water intake were noted with the gradual increase in Cl and SO_4 concentrations. For the sulphate group this appeared to occur earlier, with the water intake not rising with the increase in TDS as long as it did for the chloride group (22000mg/l TDS).
This occurred for the chloride group at a ratio of 0.57 compared to the control and a ratio of 0.4 for the sulphate group, and suggests that at these ratios water intake was adversely affected significantly in both groups.

8. The significantly lower feed intake for the chloride group at the 0.57 ratio shows the resultant affect of decreased water intake.

9. The adverse effects on water intake were accentuated when drastic changes were made to the ratios.

10. The lowering of ratios did not increase the water intake significantly.

11. Saline water significantly lowered the metacarpal bone [F], and no adverse effects were noted on the growth or health of the sheep on 15mg/l F in the drinking water.

4.5 CONCLUSION

The adaption phase and slow increase in the Cl, TDS and SO_4 concentrations appear to have enabled the sheep to adapt completely to the treatments. Were changes made more drastic, similar adverse effects in water intake may have occurred at lower concentrations. Due to this fact it is not possible to identify specific levels at which adverse effects might occur, but as mentioned in the introduction to this chapter, it is not practical to attempt this, although in this case adaptation altered response that may have occurred in practice, there are too many other variables that occur under natural conditions to be able to do this anyway.

The objective was to identify the degree to which the palatability of water was affected by Cl and SO_4 and the ratios at which this occurred. Based on the results obtained it would seem that SO_4 may have a stronger effect on palatability than Cl, but that both affect palatability negatively, at ratios of 0.4 -0.6 for SO_4 and 0.57-0.6 for Cl.

The recovery of the sheep with high water intakes following low water intakes that occurred may be due to the housing of the animals and may not occur under normal conditions.

It is realistic to postulate that the sheep may have had longer periods of low water intakes, lower feed intakes and consequently lower production under normal conditions. This is more true for the NW-Cape region where the moisture obtained from the pasture in less. Under normal conditions the sheep would also be unlikely to have been adapted at the slow rate of this trial. The impression gained from the visits mentioned was that sheep would be moved from one borehole which is of low salinity too another borehole which is often drastically different. This would lead to sudden changes in ratios and thus water intakes which would probably be longer lasting.

Despite this occurring, it appears that the effects, although more accentuated, would not result in direct adverse effects on health, i.e. the sheep could cope physiologically with the levels used in this trial under normal conditions (provided the sheep had been introduced to some extent previously to saline waters). This is based on the total absence of histopathological lesions in the organs investigated, and again emphasizes the inadequacy of

a single guideline for water quality variables to estimate the suitability of the water and the response of livestock to the variable.

The effect of salinity on bone F concentration appears to be a strongly negative correlation, i.e. despite the higher ingestion of F there is a significantly lower bone concentration. The mechanism for this is not known, but possibilities are discussed in the final chapter.

The above mentioned points seem to explain to a certain degree the observations and discrepancies that occurred in the assessment of the suitability of water for livestock production and the reaction of livestock to various waters. The findings of this trial lead to recommendations of how the index system should be developed and the ions and ratios that seem to play a major role in the equation used to predict water intake (Chapter 9).

CHAPTER 5. FLUORIDE TOLERANCE IN BROILERS

5.1. INTRODUCTION

Fluoride is a potentially hazardous substance for livestock where it occurs in surface and subterranean water sources. In South Africa, fluoritic water is associated with the fluorspar deposits of which 99% is in the Bushveld complex and the Zeerust Fluorite Field (Crocker *et al.*, 1988). The drainage regions from these fluorspar deposits are into the Limpopo valley and the subterranean water sources have varying levels of fluoride. The region has a 90% annual rainfall probability of 400 mm with a 50% variation in the summer (wet season) to 150% in the winter (Tyson, 1986). Farmers in the region are by necessity dependent on ground water. The livestock systems include intensive broiler production.

The review of international published guideline levels and criteria for water quality in Chapter 2, noted that the target guideline range for fluoride in drinking water for monogastric animals is 2 mg/l. This guideline value may be too restrictive since it does not take the production system and exposure time into account. Fluoride has a cumulative effect and chronic fluorosis occurs in livestock and man after medium to long term exposure in terms of concentration and time. The potential toxicity of fluoride depends on these factors and the type of animal, its physiological condition and environmental conditions which may determine the animal's water intake requirements.

Fluoride in the form of NaF through the drinking water resulted in increased bone ash content and strength (Merkely, 1976). However, previous research found no effect on bone ash (Raica *et al.*, 1957) or bone morphology or strength (Suttie *et al.*, 1984). Hauck *et al.*, (1933) reported no unfavourable effect on the appearance or weight gain of chicks fed up to 0.15% NaF (679 ppm F). At a level of 0.3%, appetite was depressed, and weight reduced, but the treatment had little effect on chicks two and three months old. Phillips *et al.*, (1935) corroborated the negative impact of NaF on growth rate.

NaF is ideal for testing the effect of fluoride exposure since it is highly soluble in water, is absorbed passively and has a 95% absorption rate (Janse van Rensburg and Pitout 1991). The objective of this experiment was to quantify the response of broilers to fluoride in the levels administered, and their ingestion rates over a 1 to 49 day exposure frame. This information is intended to refine current water quality guideline values.

5.2. MATERIALS AND METHODS

Sodium fluoride was provided through the drinking water to 700 Ross broiler hens over a 49-day growth period. The trial design was five treatment levels of NaF (6, 10, 14 and 20 mg/l and a negative control) with four replicates and 35 birds per replicate. The water was from the Pretoria municipal source and the negative control contained 0.21 mg/l F. This was taken into account in formulating the 6 to 20 mg/l treatments.

The chicks were vaccinated for Mareks and Newcastle diseases and randomly allocated to 20 pens (2 x 3 m) with 35 chicks in each pen. The chicks were housed in a temperature controlled broiler house with a starting temperature of 32 °C to Day 7, 30 °C to Day 14 and 28 °C to Day 49.

All groups received the same standard commercial starter diet (ME = 3030 kcal/kg, Protein content = 22%, Days 1 - 21), a grower diet (ME = 3100 kcal/kg, Protein content = 20%, Days 22 - 42) and a finisher diet (ME = 3150 kcal/kg, Protein content = 18%, Days 43 - 49), *ad libitum*. Water intake was measured using standard calibrated water troughs. Water and feed intake were measured daily and the birds were weighed weekly. Ingestion of F (mg/bird/day) was calculated. Mortalities were recorded. The birds were slaughtered and a mid - shaft sample of the femur was excised for analysis of fluoride content. Analyses were done on dried bone, (24h at 100°C) using the method of Van Staden and Janse van Rensburg (1991) which entails isolating and concentrating the fluoride in the bone and then determining it potentiometrically. Livers and carcasses were weighed.

Statistical analyses were conducted with a PC - SAS Version 6.08 commercial statistical software, analysis of variance and Tukey's multiple range test were used to determine the significance and difference between treatment means at a $P<0.05$ significance level.

5.3. RESULTS

It was found that an average F intake of 4 mg F/chick was obtained over the first three weeks, for the highest treatment group and 18 mg F/chick for the remainder of the trail. The average fluoride doses given over both periods were, 22.440 mg/l (Table 5.3.1.).

Table 5.3.1. Fluoride ingestion rate (mg) per chicken.

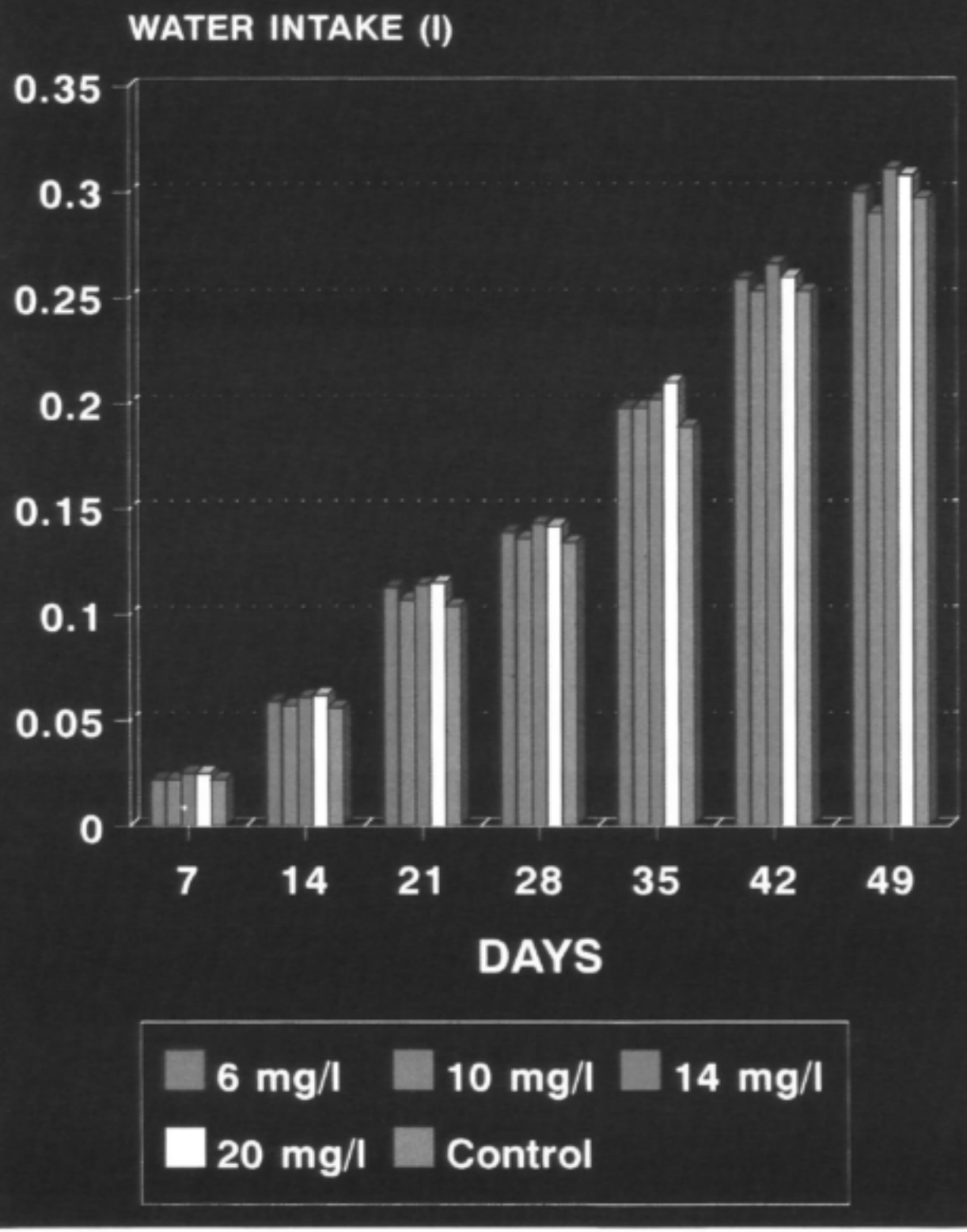
DAY 1 - 21				
0.21 mg/l	**6 mg/l**	**10 mg/l**	**14 mg/l**	**20 mg/l**
0.038	1.164	1.860	2.800	4.040
DAY 22 - 49				
0.21 mg/l	**6 mg/l**	**10 mg/l**	**14 mg/l**	**20 mg/l**
0.663	5.376	8.770	12.908	18.400

No differences between treatments and water intake as a percentage of body weight ($P = 0.999$) occurred. The overall mean for each treatment coincides with the control, which is a 35% water intake over a 7 week period (Table 5.3.2. and Figure 5.3.2.).

Table 5.3.2. Average water intake (% body weight) per chicken.

Weeks	Treatment mg/l					MEAN	SD
	0.21	6	10	14	20		
Week 1	58.0%	56.4%	57.8%	65.7%	65.7%	60.70	4.60
Week 2	60.2%	62.8%	60.6%	61.6%	61.4%	61.30	1.00
Week 3	41.6%	43.1%	41.8%	41.8%	41.5%	41.96	0.65
Week 4	25.9%	25.7%	25.7%	25.7%	23.7%	25.30	0.92
Week 5	23.5%	23.9%	25.0%	24.0%	24.3%	24.10	0.56
Week 6	20.1%	20.2%	20.0%	20.0%	14.4%	18.90	2.50
Week 7	15.9%0	15.8%	15.3%	16.1%	15.8%	15.80	0.29
OVERALL MEAN	35.0%	35.4%	35.2%	36.4%	35.3%	35.4%	1.503

Figure 5.3.2.
Water intake (l)



Body weight showed a tendency to increase in the highest treatments, in the 6th week this was significant (P = 0.0001). These tendencies were however not supported for the model over the entire treatment period (Table 5.3.4.). Body weight was not influenced by fluoride treatment (P = 1.000).

Table 5.3.3. Least Square Means for body weight (kg) over production period.

Treatment mg/l	LS Means
0.21	0.3509
6	0.3589
10	0.3563
14	0.3665
20	0.3783

Table 5.3.4. Mean body weight per chicken (kg).

Weeks	Treatment mg/l					P value
	0.21	6	10	14	20	
Week 1	0.038[a]	0.039[a]	0.038[a]	0.038[a]	0.038[a]	0.8593
Week 2	0.093[a]	0.094[a]	0.094[a]	0.099[a]	0.101[a]	0.0314
Week 3	0.250[b]	0.262[ab]	0.256[ab]	0.273[a]	0.277[b]	0.0116
Week 4	0.517[a]	0.541[b]	0.530[b]	0.557[b]	0.599[b]	0.0016
Week 5	0.804[b]	0.828[ab]	0.791[b]	0.841[a]	0.864[a]	0.0143
Week 6	1.258[b]	1.280[b]	1.266[b]	1.325[b]	1.805[a]	0.0001
Week 7	1.866[a]	1.901[a]	1.893[a]	1.934[a]	1.951[a]	0.0679

* Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Feed intake was not influenced by the fluoride treatments. (Table 5.3.6.) but the 5th week was the exception with the highest treatment having a significantly higher intake than the control ($P = 0.0205$). The P value for the model was $P = 0.9992$ (Table 5.3.5). Feed intake was therefore not influenced by fluoride treatment.

Table 5.3.5. Least Square Means for feed intake (kg) over production period.

Treatment	LS Means
0.21	0.0803
6	0.0812
10	0.0857
14	0.0835
20	0.0839

Table 5.3.6. Mean feed intake (kg) per chicken.

Week	Treatment mg/l					P value
	0.21	6	10	14	20	
Week 1	0.016[a]	0.016[a]	0.017[a]	0.017[a]	0.017[a]	0.9858
Week 2	0.045[a]	0.047[a]	0.047[a]	0.048[a]	0.050[a]	0.1404
Week 3	0.077[a]	0.079[a]	0.083[a]	0.080[a]	0.083[a]	0.2904
Week 4	0.075[a]	0.075[a]	0.072[a]	0.076[a]	0.074[a]	0.1181
Week 5	0.106[b]	0.113[ab]	0.112[ab]	0.112[ab]	0.155[a]	0.0205
Week 6	0.117[a]	0.115[a]	0.142[a]	0.122[a]	0.118[a]	0.0549
Week 7	0.126[a]	0.123[a]	0.128[a]	0.128[a]	0.130[a]	0.3071

[*] Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

There were no significant differences between treatments as far as feed conversion was concerned. Feed conversion was significantly lower ($P < 0.0013$) in the fourth week for the 20 mg/l treatment compared to the other treatments, but not significant for the model. (Table 5.3.7.).

Table 5.3.7. Feed conversion ratio per chicken

Week	Treatment mg/l					P
	0.21	6	10	14	20	
Week 2	4.058[a]	4.290[a]	4.230[a]	3.911[a]	3.908[a]	0.1330
Week 3	2.360[a]	2.366[a]	2.525[a]	2.268[a]	2.270[a]	0.1943
Week 4	1.328[a]	1.343[a]	1.285[a]	1.326[a]	1.090[b]	0.0013
Week 5	1.733[a]	1.974[a]	2.088[a]	1.947[a]	2.057[a]	0.2086
Week 6	1.365[a]	1.439[a]	1.530[a]	1.390[a]	1.532[a]	0.1608

[*] Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Carcass weights (Table 5.3.8.) showed a tendency to increase with increased fluoride, but this was not significant ($P = 0.1508$).

Table 5.3.8. Carcass weights (kg).

Treatment mg/l	Mean Weight	SD
0	1.489[a]	0.1083
6	1.426[a]	0.1011
10	1.454[a]	0.0507
14	1.557[a]	0.0292
20	1.536[a]	0.0707

[a] Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Liver weights (Table 5.3.9. and Figure 5.3.9.) increased in the higher fluoride treatments ($P = 0.0144$). The mean liver weight for the control was 41.345 g and 52.775 g for the 20 mg/l treatment.

Table 5.3.9. Liver weights (g).

Treatment mg/l	Mean weight	SD
0.21	41.345[b]	5.9647
6	41.250[b]	5.3533
10	50.385[ab]	4.0303
14	56.365[a]	9.3570
20	52.775[a]	6.5412

[a] Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

A significant increase in fluoride content of the femur occurred (P = 0.0001), most probably due to fluoride absorbtion being almost completely in calciferous tissue. The normal level of fluoride in whole bone on a fat free basis is 200 - 500 mg/kg. In this trial the control had a fluoride concentration of 273.432 mg/kg and the 20 mg/l a fluoride concentration of 863.741 mg/kg (Table 5.3.10 and Figure 5.3.10.).

Table 5.3.10. Fluoride content of the femur (mg/kg).

Treatment mg/l	Mean [Fluoride]	SD
0.21	273.432[d]	26.2258
6	468.223[c]	29.1799
10	588.762[bc]	20.5822
14	740.697[ab]	44.9503
20	863.741[a]	153.5804

* Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Figure 5.3.9.
Liver weights (g)

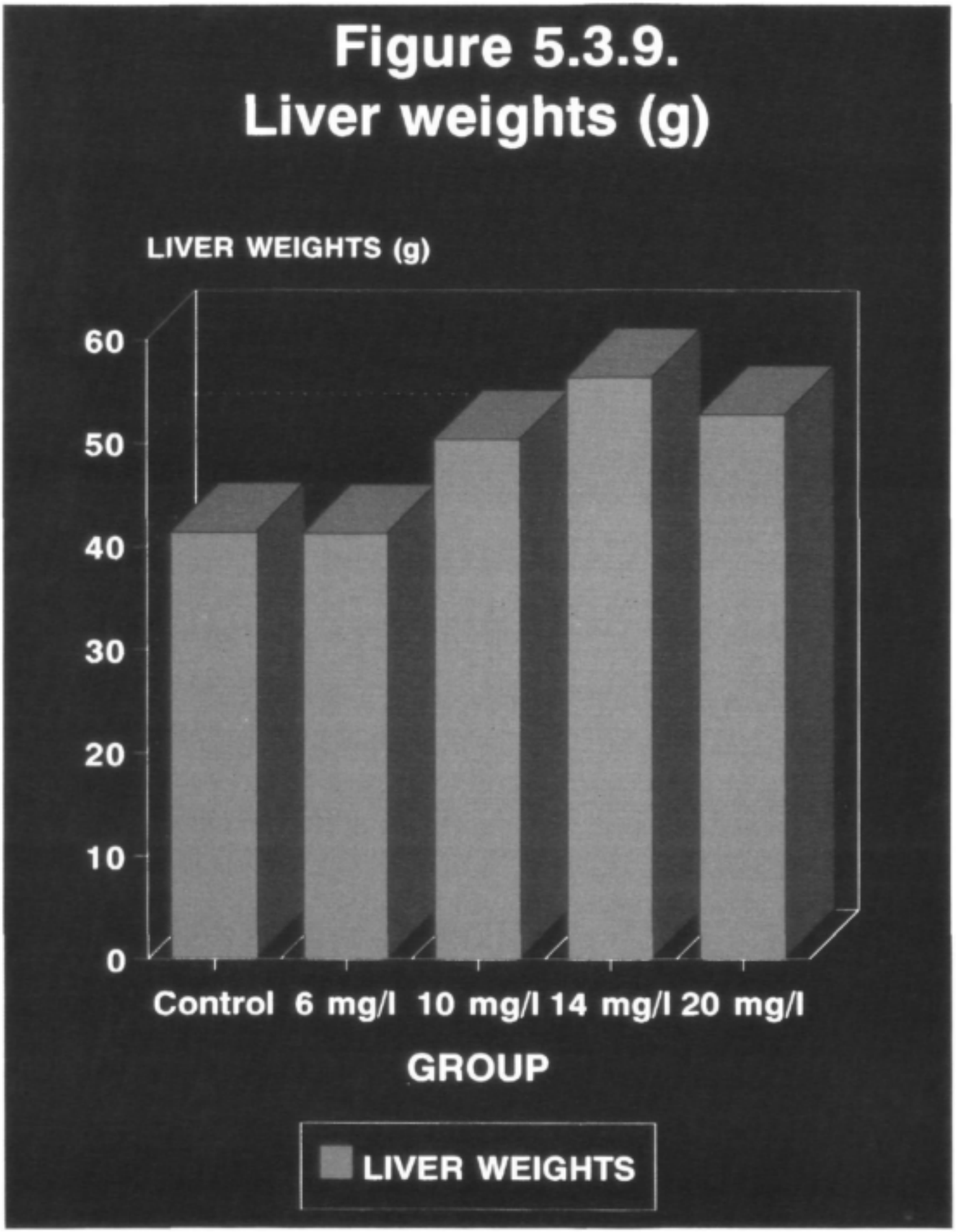
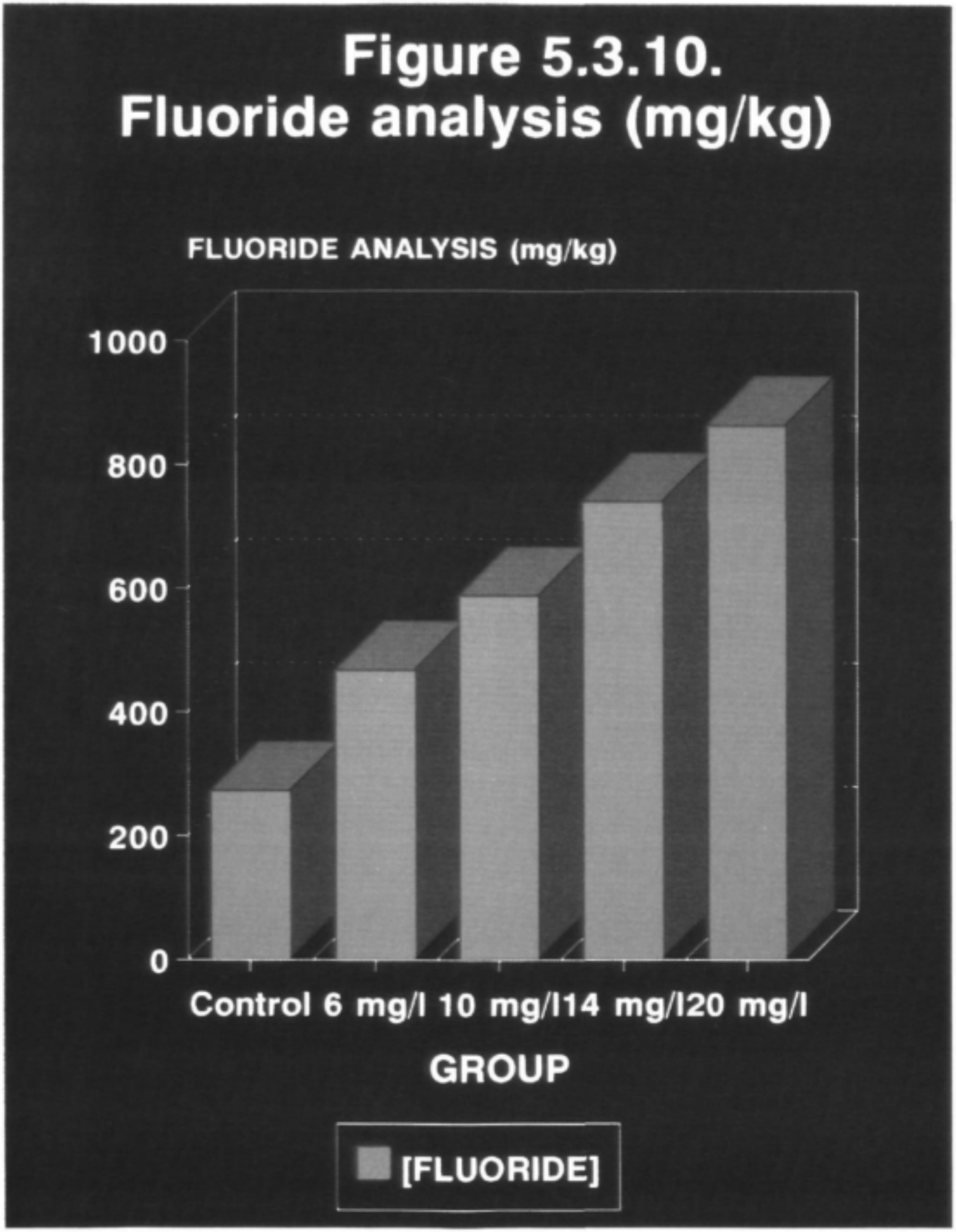


Figure 5.3.10.
Fluoride analysis (mg/kg)



5.4. CONCLUSION

The results suggest that fluoride at the dosage levels used in this trial, over the 49 day growth period, did not adversely affect performance. Furthermore, very high levels of fluoride are required before the soft tissue is affected as fluoride has a high affinity for calcified tissues and its first form of absorption is in bone (Janse van Rensburg and Pitout, 1991). In this investigation the levels of fluoride administered and the exposure time would not result in fluoride deposition in the soft tissue and therefore no consumer health hazard arises.

The implications of the results obtained for water quality guideline formulation are that firstly, ingestion rates need to be calculated and secondly, the production system must be taken into account in order to fully utilise the water source. This is important as it is the actual ingestion of the water quality variable that is relevant and not only the concentration of the variable in the water source. Generally speaking, similar environmental controls for intensive broiler production units are prescribed. This enables the practical application of the results obtained in this investigation to the commercial industry and hence for guideline formulation.

There were no significant differences between treatments regarding feed and water intake, weight gain or feed conversion. Liver weight was significantly influenced by treatment. The fluoride contents of the femurs ($P < 0.0001$) were: Control - 273.4 mg/kg; 6 mg/l - 468.2 mg/kg; 10 mg/l - 588.7 mg/kg; 14 mg/l - 740.7 mg/kg; 20 mg/l - 863.7 mg/kg. Carcass weights showed a tendency to increase with increased fluoride dosage, this was however not significant.

These results indicate that fluoride up to levels of 20 mg/l in the drinking water, or an ingestion rate of up to 3.206 mg/bird/day had no negative effect on production characteristics over a 49 day growth period. These results contribute to refining current water quality guidelines.

CHAPTER 6. FLUORIDE TOLERANCE IN LAYERS

6.1. INTRODUCTION

The development of bone fragility in caged layers is a major problem in the poultry industry. The incidence of bone breakage during the processing of spent hens substantially reduces the economic returns from these birds. Merkley (1981) found that F treatment increased the breaking strength of humeri from 6.86 kg to 13.35 kg and that of tibiae from 6.61 kg to 13.10 kg. The fluoride treatment also significantly ($P < 0.01$) increased the percentage of bone ash. Egg quality and rate of production were not reduced by the F treatment.

Previous work done on the effect of NaF on egg production and bone strength on caged layers were done via the diet. Much higher levels of NaF were used (0; 300; 600; 900 and 1200 mg/kg - Van Toledo *et al.*, 1983). In this chapter treatments much closer to the natural F content of RSA ground water sources were used.

Halpin and Lamb (1932) reported that egg production was not affected by 1 and 2% levels of rock phosphate but was decreased by a 3% level in two five month experiments. At these levels the rock phosphate supplied the ration with approximately 0.035, 0.070 and 0.105% of F respectively.

Gerry *et al.*, (1947) reported that although as little as 1% of rock phosphate (0.038% of F in ration) depressed the growth of young chicks, the chicks overcame the detrimental effect as they grow older.

Thus the toxic effects of F and its cumulative manifestations depend upon the susceptibility of the species, the amount and period of F ingestion and the carrier of F, i.e. the toxicity of certain forms of fluorine has been shown to be of the following order: fluosilicates, NaF, rock phosphate and CaF_2. It is known that various physiological manifestations occur following the ingestion of fluorides over an extended period of time. It is the object of this study to present the analytical results of an investigation to determine NaF long term effect on growth and production of layers.

6.2. MATERIALS AND METHODS

Sodium fluoride was provided through the drinking water to 1000 Hy - line silver grey layers over a 74 week period. The trial design included five treatment levels of NaF (6, 10, 14 and 20 mg/l and a negative control) with four replicates and 50 birds per replicate. The water was from the Pretoria municipal source and the negative control contained 0.21 mg/l F in the first 17 weeks and 0.29 mg/l F for the rest of the trial. This was taken into account in formulating the 6 to 20 mg/l treatments.

The chicks were randomly allocated to 20 pens (2 x 3 m) with 50 chicks per pen in a temperature controlled broiler house using a gas fired hover. The starting temperature was 35 °C and was reduced by 3 °C per week until 21 °C was reached.

An specifically designed vaccination programme was followed taking, maternal immunities, disease exposure and expected and vaccines available into consideration.

Chickens were not debeaked as the space requirements for birds were greatly exceeded and the hens were caged individually in the layer house.

Table 6.2.1. Vaccination Programme

Age	Vaccine	Method
Day old	Mareks rispins	Under skin
8 days	Gumboro inactivated	In neck
10 days	Clone 30 (New Castle)	Eye drop
12 days	Gumboro mild/live	Water
16 days	Gumboro mild/live	Water
5 weeks	First I.L.T.	Eye drop
7 weeks	La Sota	Water
8 weeks	I.B.	Water
10 weeks	Pox	Wing stab
11 weeks	La Sota	Water
14 weeks	First Coryza	Under skin
14 weeks	2nd I.L.T.	Eye drop
14 weeks	E.D.S.	Under skin
17 weeks	2nd Coryza	Under skin
17 weeks	I.B. oil base	Under skin
17 weeks	N.C.D. oil base	Under skin
40 weeks	La Sota	Water
42 weeks	I.B.	Water

All groups received the same standard commercial diets *ad libitum*. Feed intake was measured daily for the first 17 weeks. The feeding regime followed was:

STARTER DIET: 0 - 6 weeks

Protein: 20%

ME: 2970 kcal/kg

GROWER 1: 7 - 8 weeks

Protein: 19%

ME: 3080 kcal/kg

GROWER 2: 9 - 14 weeks

Protein: 17%

ME: 3190 kcal/kg

DEVELOPER: 15 - 16 weeks

Protein: 15%

ME: 2930 kcal/kg

PRE - LAYER: 17 - 18 weeks

Protein: 17%

ME: 3190 kcal/kg

Mortalities were recorded with accompanying post mortem reports. Ingestion of F (mg/bird/day) was calculated. Body weights were monitored weekly over the first 17 weeks. Due to technical and labour problems it was not possible to monitor body weights in weeks, 12, 14 and 15.

The following lighting programme was followed up to 17 weeks:

Day 1 - 2 : 24 hours at 10 lux intensity.

Day 3 - 21: 15 hours per day at 5 lux intensity.

Three weeks - 17 weeks: 12 hours per day at 5 lux intensity.

At 17 weeks the flock was moved to a convection layer house.

The following lighting programme was then used.

Table 6.2.2. Lighting Programme

Age	Total daylight	Lights on	Lights of
19 weeks	13 hours	05:30am	18:30pm
20 weeks	13.5 hours	05:00am	18:30pm
21 weeks	14 hours	05:00am	19:00pm
22 weeks	14.5 hours	04:30am	19:00pm
23 weeks	15 hours	04:30am	19:30pm
24 weeks	15 hours	04:30am	19:30pm
25 weeks	15.5 hours	04:00am	19:30pm
26 weeks	16 hours	03:30am	19:30pm
27 weeks	16 hours	03:30am	19:30pm
28 weeks	16.5 hours	03:30am	20:00pm
Keet at	16.5 hours	03:30am	20:00pm

Nutrition during the laying period was based on adequate daily levels of crude protein using the following percentages:

18 - 40 weeks: 18% protein

40 - 60 weeks: 16% protein

60 + weeks: 15.5% protein

Layers were caged individually in the layer house. A controlled water dosing system was installed enabling each group's intake to be measured daily.

Egg production was determined daily for each hen individually. Later this was recalculated into eggs/group/week. From peak production, the breaking strength of a randomly selected representative sample of the egg population were determined over a 20 week period.

At 74 weeks the trial was terminated. The birds were slaughtered, carcasses and livers were weighed. Livers and kidneys were histo - pathologically examined for fatty changes and protein "lakes" in the liver and kidney round cell infiltrations. A mid - shaft sample of the femur was excised for analysis of fluoride content. Analyses were done on dried bone, (24h at 100°C) using the method of Van Staden and Janse van Rensburg (1991) which entails isolating and concentrating the fluoride in the bone and then determining it potentiometrically.

The breaking strength of a representative sample of femurs were established, Rowland *et al.*, (1967) method with the Allo - Kreamer shear press was used. The attachment for the shear press was designed whereby pressure could be placed on the midpoint of the femur which was supported near the end. The bottom area of the attachment was 3 mm X 21 mm and had been machined from a 21 mm diameter steel rod. An adjustable support (vice) was designed to fit the Instron platform and permitted an unlimited selection of support widths. In this study each femur was supported near the ends approximately 20 mm from the midpoint at which the force was applied. The press descended at the rate of 120 mm per minute. The maximum force required to break each femur was read from the chart of the shear press recorder.

Statistical analyses were conducted with PC - SAS Version 6.08 commercial software analysis of variance and Tukey's multiple range test, and the Kruskall Wallis test using chi - square distribution with 4 degrees of freedom, were used to determine the significance between treatment means at a $P < 0.05$ level.

6.3. RESULTS

6.3.1. Growth results (week 1 - 17).

In the seventh and eighth weeks the controls had higher body weights than the 10 and 14 mg/l treatments, these differences were significant (P = 0.0116 and P= 0.0029). Fluoride had no influence on body weights (P = 0.9989).(Table 6.3.1.2.)

Table 6.3.1.1. Average body weights per chicken per week.

Week	Treatment mg/l					P value
	0.21	6	10	14	20	
Week 1	0.032[a]	0.033[a]	0.033[a]	0.032[a]	0.033[a]	0.2986
Week 2	0.059[a]	0.059[a]	0.060[a]	0.062[a]	0.060[a]	0.1728
Week 3	0.112[a]	0.113[a]	0.113[a]	0.117[a]	0.112[a]	0.4646
Week 4	0.187[a]	0.190[a]	0.185[a]	0.189[a]	0.185[a]	0.6065
Week 5	0.283[a]	0.283[a]	0.271[a]	0.277[a]	0.276[a]	0.1235
Week 6	0.369[a]	0.368[a]	0.357[a]	0.360[a]	0.364[a]	0.0765
Week 7	0.482[a]	0.477[ab]	0.461[b]	0.465[b]	0.474[ab]	0.0116
Week 8	0.602[a]	0.602[a]	0.576[b]	0.579[b]	0.594[ab]	0.0029
Week 9	0.739[a]	0.732[a]	0.700[a]	0.728[a]	0.750[a]	0.2739
Week 10	0.825[a]	0.617[a]	0.779[a]	0.776[a]	0.804[a]	0.5437
Week 11	0.953[a]	0.938[ab]	0.893[c]	0.898[c]	0.924[b]	0.0001
Week 13	1.139[a]	1.139[a]	1.108[a]	1.108[a]	1.135[a]	0.0828
Week 16	1.375[a]	1.401[a]	1.356[a]	1.361[a]	1.384[a]	0.6576
Week 17	1.414[a]	1.445[a]	1.372[a]	1.386[a]	1.387[a]	0.0775

* Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Table 6.3.1.2. Least square means for body weights over growth period.

Treatment mg/l	LS Means for body weight over production period.
0.21	0.612[a]
6	0.610[a]
10	0.590[a]
14	0.596[a]
20	0.606[a]

[a] Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Table 6.3.1.3. Least Square Means for feed intake over growth period.

Treatment mg/l	LS Means for feed intake
0.21	0.066[a]
6	0.067[a]
10	0.065[a]
14	0.062[a]
20	0.063[a]

[a] Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Fluoride treatment had no significant effect on feed intake ($P = 0.8136$). Although there were differences between treatments within certain weeks this was not significant for the model.

In the ninth week the 20 mg/l treatment had a significantly ($P = 0.0106$) higher feed intake than the rest of the treatments (Table 6.3.1.4.).

Table 6.3.1.4. Mean feed intake (kg) per chicken.

Week	Treatment mg/l					P value
	0.21	6	10	14	20	
Week 1	0.018[a]	0.020[a]	0.020[a]	0.020[a]	0.018[a]	0.6343
Week 2	0.029[a]	0.029[a]	0.031[a]	0.029[a]	0.026[a]	0.0621
Week 3	0.027[a]	0.029[a]	0.026[a]	0.024[a]	0.023[a]	0.1882
Week 4	0.034[a]	0.035[a]	0.035[a]	0.035[a]	0.033[a]	0.9553
Week 5	0.042[a]	0.045[a]	0.042[a]	0.041[a]	0.040[a]	0.5575
Week 6	0.052[a]	0.058[a]	0.048[a]	0.048[a]	0.047[a]	0.1926
Week 7	0.064[a]	0.064[a]	0.048[a]	0.054[a]	0.055[a]	0.0796
Week 8	0.062[a]	0.067[a]	0.048[a]	0.052[a]	0.056[a]	0.0838
Week 9	0.071[b]	0.070[b]	0.074[ab]	0.075[ab]	0.087[a]	0.0106
Week 10	0.082[a]	0.094[a]	0.081[a]	0.089[a]	0.089[a]	0.4419
Week 11	0.088[a]	0.072[b]	0.067[b]	0.067[b]	0.072[b]	0.0013
Week 12	0.071[a]	0.075[a]	0.073[a]	0.067[a]	0.073[a]	0.3577
Week 13	0.110[a]	0.106[a]	0.110[a]	0.097[a]	0.097[a]	0.8618
Week 14	0.117[a]	0.109[a]	0.118[a]	0.102[a]	0.098[a]	0.6559
Week 15	0.086[a]	0.084[a]	0.094[a]	0.085[a]	0.080[a]	0.7795
Week 16	0.061[a]	0.089[a]	0.086[a]	0.079[a]	0.085[a]	0.2640
Week 17	0.114[a]	0.094[a]	0.101[a]	0.084[a]	0.087[a]	0.2360

[a] Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test

Fluoride treatment did not influence feed conversion (Table 6.3.1.5.).

Table 6.3.1.5. Average feed conversion ratio per chicken.

Week	Treatment mg/l					P value
	0.21	6	10	14	20	
Week 2	4.741[a]	4.697[a]	4.556[a]	4.544[a]	4.754[a]	0.9637
Week 3	5.628[a]	5.616[a]	6.178[a]	5.748[a]	5.428[a]	0.6102
Week 4	2.441[a]	2.355[a]	2.585[a]	2.338[a]	2.450[a]	0.1198
Week 5	1.339[a]	1.362[a]	1.322[a]	1.347[a]	1.342[a]	0.3443
Week 6	3.531[a]	3.462[a]	3.263[a]	3.440[a]	3.221[a]	0.1848
Week 7	3.455[a]	3.352[a]	3.242[a]	3.245[a]	3.160[a]	0.8103
Week 8	3.572[a]	3.143[a]	3.525[a]	3.323[a]	2.965[a]	0.4087
Week 9	3.661[a]	3.665[a]	3.900[a]	3.466[a]	2.991[a]	0.8121
Week 10	6.983[a]	6.545[a]	6.719[a]	2.466[a]	11.784[a]	0.7409
Week 11	4.152[a]	4.413[a]	4.572[a]	4.407[a]	4.492[a]	0.6093

[a] Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Table 6.3.1.6. Mortalities over 17 week growth period.

Treatment	Total no of Mortalities
0.21 mg/l	37
6 mg/l	29
10 mg/l	36
14 mg/l	29
20 mg/l	23

None of the mortalities were caused by fluoride linked illness. See Table 6.3.2.13 for causes of mortalities.

6.3.2. Production results (week 18 - 74).

Carcass weights were significantly higher (P = 0.0332) in the high fluoride treatment groups. The 20 mg/l group had a mean carcass weight of 2.5523 kg and the 0.29 mg/l group a mean of 2.1529 kg (Table 6.3.2.1. and Figure 6.3.2.1.).

Table 6.3.2.1. Carcass weights (kg).

Treatment	Mean weight
0.29 mg/l	2.1529[c]
6 mg/l	2.2329[bc]
10 mg/l	2.3831[abc]
14 mg/l	2.4693[ab]
20 mg/l	2.5523[a]

* Means with different superscripts are significantly different (P < 0.05) according to Tukey's Multiple Range Test.

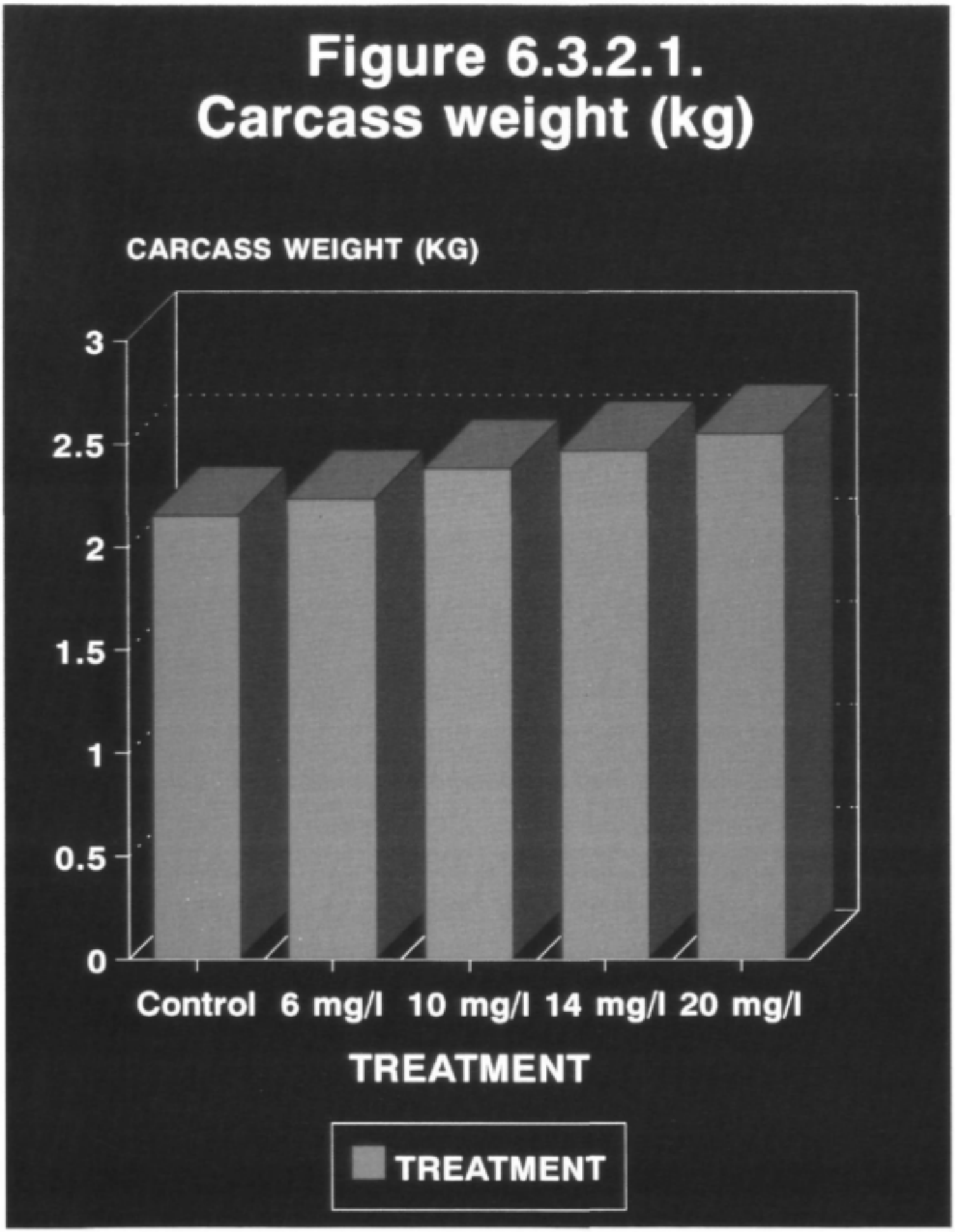
Fluoride treatments had no significant influence (P = 0.0902) on liver weights (Table 6.3.2.2.).

Table 6.3.2.2. Liver weights (g).

Treatment	Mean weight
0.29 mg/l	56.226[a]
6 mg/l	51.682[a]
10 mg/l	48.760[a]
14 mg/l	52.407[a]
20 mg/l	56.226[a]

* Means with different superscripts are significantly different (P < 0.05) according to Tukey's Multiple Range Test.

Figure 6.3.2.1. Carcass weight (kg)



A significant increase (P = 0.0001) in fluoride content of the femur occurred, due to fluoride absorbtion being almost completely in calciferous tissue. The normal level of fluoride in whole bone on a fat free basis is 200 - 500 mg/kg. In this trial the control had a fluoride concentration of 573.69 mg/kg and the 20 mg/l a fluoride concentration of 1671.39 mg/kg (Table 6.3.2.3. and Figure 6.3.2.3.).

Table 6.3.2.3. Fluoride content of the femur (mg/kg).

Treatment	Mean [Fluoride]
0.29 mg/l	573.69[d]
6 mg/l	947.28[c]
10 mg/l	1139.91[b]
14 mg/l	1329.00[b]
20 mg/l	1671.39[a]

Means with different superscripts are significantly different (P < 0.05) according to Tukey's Multiple Range Test.

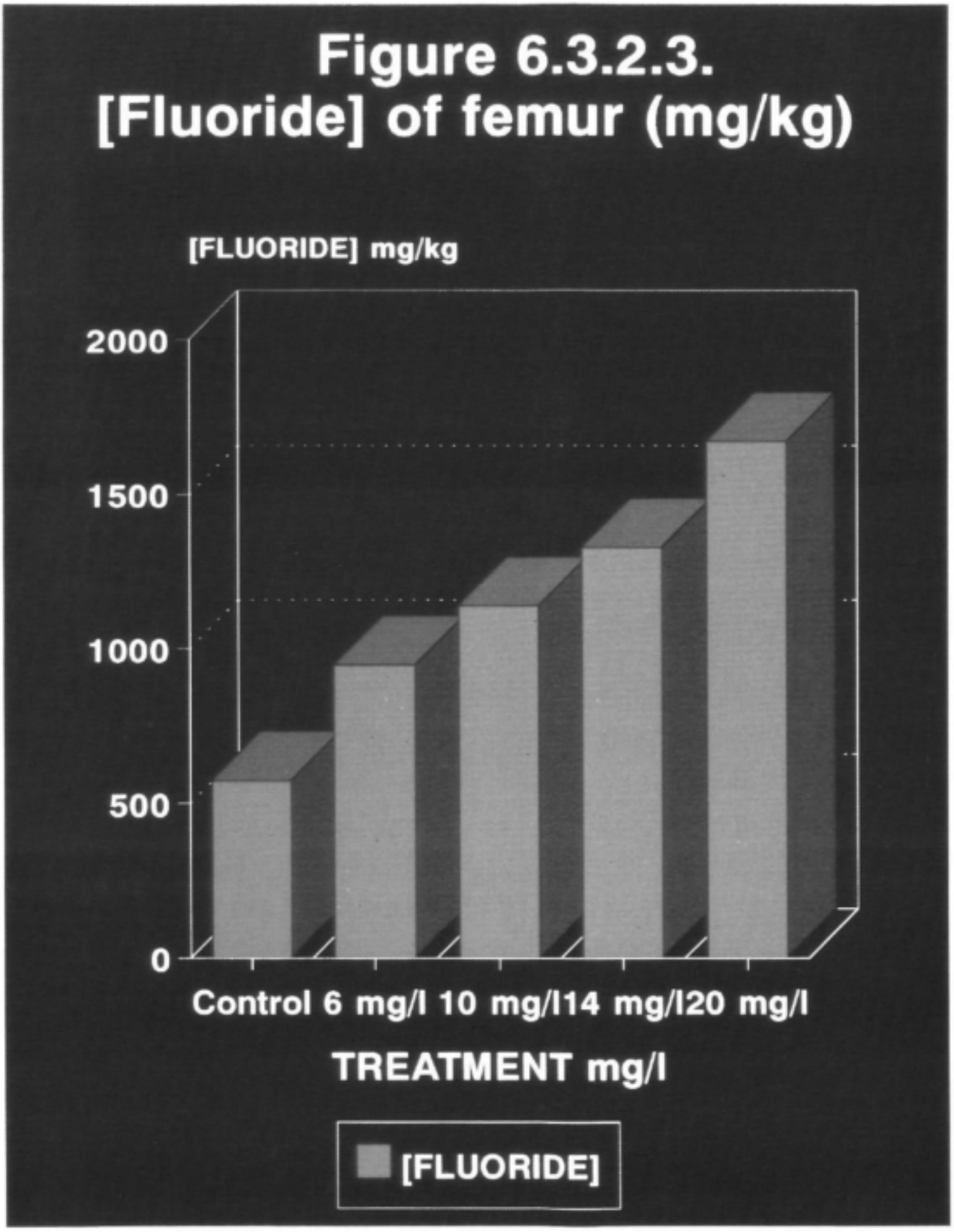
The breaking strength of the femurs was not significantly influenced (P = 0.5005) by fluoride treatment (Table 6.3.2.4.). The mean breaking strength for all groups was 24.041 kg.

Table 6.3.2.4. Breaking strength of femurs (kg).

Treatment	Mean breaking strength
0.29 mg/l	24.794[a]
6 mg/l	24.693[a]
10 mg/l	24.574[a]
14 mg/l	22.776[a]
20 mg/l	23.370[a]

Means with different superscripts are significantly different (P < 0.05) according to Tukey's Multiple Range Test.

Figure 6.3.2.3. [Fluoride] of femur (mg/kg)



In the fifth week there was a significant (P = 0.04) increase in the breaking strength of the 20 mg/l treatment group (4.4450 Nm), compared to the 3.99225 Nm for the control group (Table 6.3.2.5.). This trend continued for the duration of the trial (breaking strength over total period). The P value was 0.0681 for the model (Table 6.3.2.6).

Table 6.3.2.5. Breaking strength (Nm) of eggs over 20 weeks.

Week	Treatment mg/l					P value
	0.29	6	10	14	20	
1.	4.0550[a]	4.2900[a]	4.1550[a]	4.0300[a]	3.7450[a]	0.9767
2.	3.7800[a]	3.8925[a]	3.8625[a]	3.9350[a]	4.3425[a]	0.9121
3.	3.1300[a]	3.8025[a]	3.9750[a]	3.8275[a]	3.8250[a]	0.8358
4.	2.5825[a]	3.4700[a]	3.9850[a]	3.6200[a]	4.1225[a]	0.1932
5.	3.9225[a]	4.4300[a]	3.2975[a]	4.3375[a]	4.4450[a]	0.0400
6.	3.7425[a]	4.0675[a]	4.0425[a]	3.3925[a]	4.0125[a]	0.6200
7.	3.2400[a]	4.8275[a]	3.9750[a]	4.0100[a]	4.2775[a]	0.1200
8.	3.6500[a]	3.9175[a]	3.7600[a]	4.2000[a]	4.1950[a]	0.8005
9.	3.5750[a]	3.5500[a]	4.0200[a]	3.8050[a]	3.7300[a]	0.9357
10.	3.4175[a]	4.2750[a]	3.6575[a]	4.2700[a]	3.2575[a]	0.2455
11.	3.1375[a]	3.1925[a]	3.0750[a]	3.8750[a]	3.0675[a]	0.5633
12.	4.5350[a]	3.5750[a]	3.4175[a]	3.4675[a]	3.7925[a]	0.1733
13.	3.9175[a]	2.6625[a]	3.6900[a]	3.4475[a]	3.8750[a]	0.2796
14.	3.3275[a]	3.2875[a]	3.8650[a]	3.3825[a]	3.7050[a]	0.6413
15.	3.5500[a]	3.7625[a]	4.0325[a]	3.7200[a]	4.8125[a]	0.5451
16.	3.3625[a]	3.2150[a]	3.0425[a]	3.6050[a]	4.0150[a]	0.5891
17.	3.6925[a]	4.3067[a]	3.3375[a]	3.6375[a]	3.3950[a]	0.3545
18.	3.1875[a]	3.4850[a]	3.2125[a]	3.0750[a]	3.3175[a]	0.9042
19.	3.0400[a]	4.0500[a]	3.1600[a]	2.8500[a]	4.1950[a]	0.1689
20.	3.1675[a]	3.2175[a]	3.6000[a]	4.1400[a]	3.2450[a]	0.0605

Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Although no significant differences were found between treatments on a weekly basis, fluoride treatment had a significant influence (P = 0.0681) on the breaking strength of eggs. Breaking strength tended to increase in the higher treatments (Table 6.3.2.6.), the 20 mg/l treatment had a mean breaking strength of 3.879 Nm and the control a breaking strength of 3.501 Nm. This shows that fluoride strengthens egg shells at a 20 mg/l dosage level.

Table 6.3.2.6. Least Square Means for breaking strength of eggs over 20 weeks.

Treatment mg/l	LS Means for breaking strength over 20 weeks
0.29	3.501^{b}
6	3.761^{ab}
10	3.658^{ab}
14	3.731^{ab}
20	3.879^{a}

* Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Histopathology of livers and kidneys.

Fatty changes in the liver (FC).

Fatty change was present in most of the livers examined. The degree of fatty change varied from case to case and was scored on a scale of 0 to 4, according to a subjective appraisal.

Protein "lakes" (PL).

A minority of livers contained variable numbers and sizes of irregular, homogeneous, proteinaceous accumulations in the parenchyma. These were scored subjectively on a scale of 0 to 3. Morphologically and tinctorially these accumulations most closely resemble the beta - pleated protein group known as amyloid. This could be verified histochemically by the demonstration of fluorescence following staining with thiovlavin - T and examination by UV - microscopy. After examination it proved not to be amyloid.

Kidney round cell infiltrations (KRC).

The only significant lesion in the kidneys was the presence of variable numbers of interstitial lympho - plasmacytic foci. These were assessed semi-quantitatively by counting the number

of foci per section and grading them as follows: no foci = 0; 1 - 4 foci = 1; 5 - 8 foci = 2; >9 foci = 3.

Note: lympho - plasmacytic foci were also encountered in the livers but were not specifically evaluated as nearly every liver was affected and such foci are very common in "normal" chicken livers.

Subcapsular haemorrhages/haematomas were observed macro- and microscopically in the livers of some birds.

Subcapsular haematomas (livers):

No haematomas occurred in the control group.

In the 6 mg/l F group, several, chronic subcapsular haematomas and multiple fresh subcapsular and intra hepatic haematomas occurred.

In the 10 mg/l F group, small and large, chronic subcapsular haematomas occurred as well as organising subcapsular haematomas.

In the 14 mg/l F group, subacute and chronic subcapsular haematomas occurred.

In the 20 mg/l F group, large acute and subacute, subcapsular haematomas associated with protein accumulations occurred. Acute, intra-hepatic haemorrhages were also observed.

No significant differences were found between treatments for the three variables accounted above. The presence of these histopathological phenomena can be directly linked to the time the hens were fed. Normally layers are slaughtered at ± 65 weeks. In this trial the exposure time to fluoride was extended as long as possible, the hens were on a high level of feeding and therefore the fatty livers and protein lakes can be attributed to the high level of nutrition.

Figure 6.3.2.7a. Normal chicken liver (FC 0).

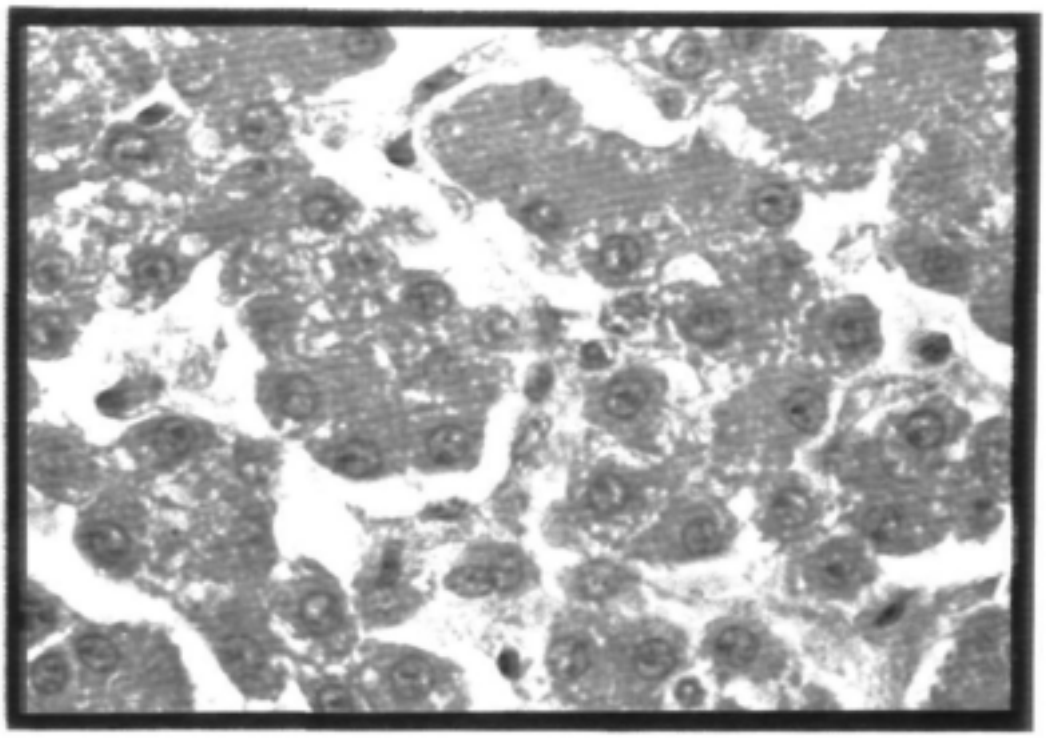


Figure 6.3.2.7b. Fatty change in the liver (FC 4).

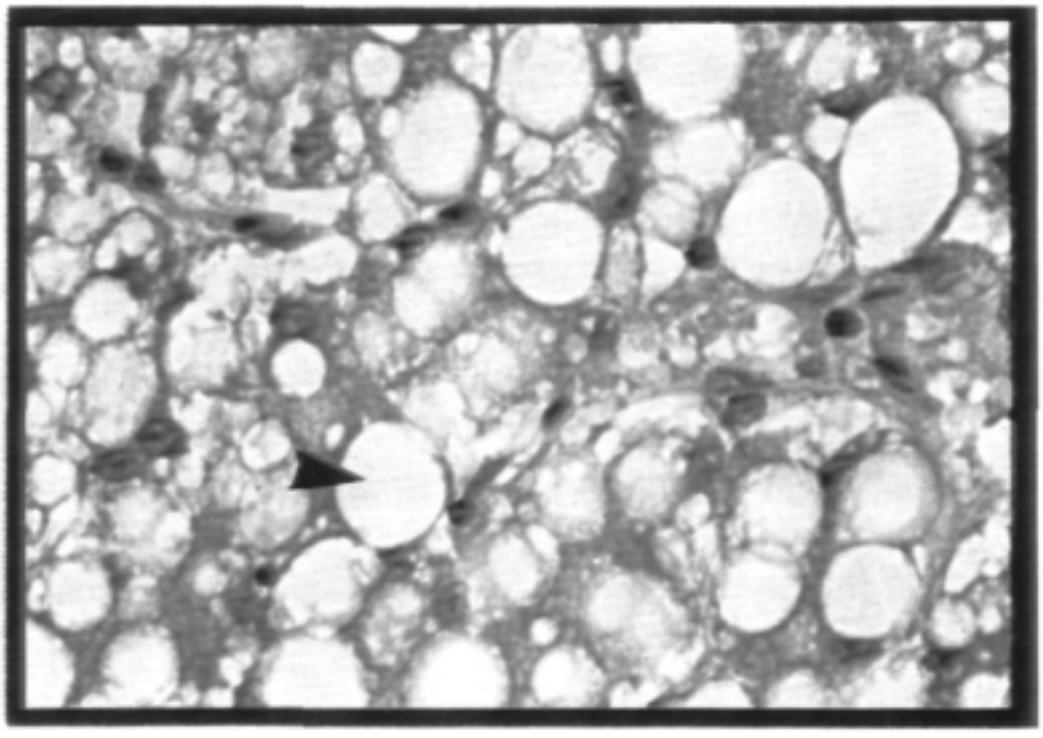


Figure 6.3.2.7c. **Protein "lakes" in the liver.**

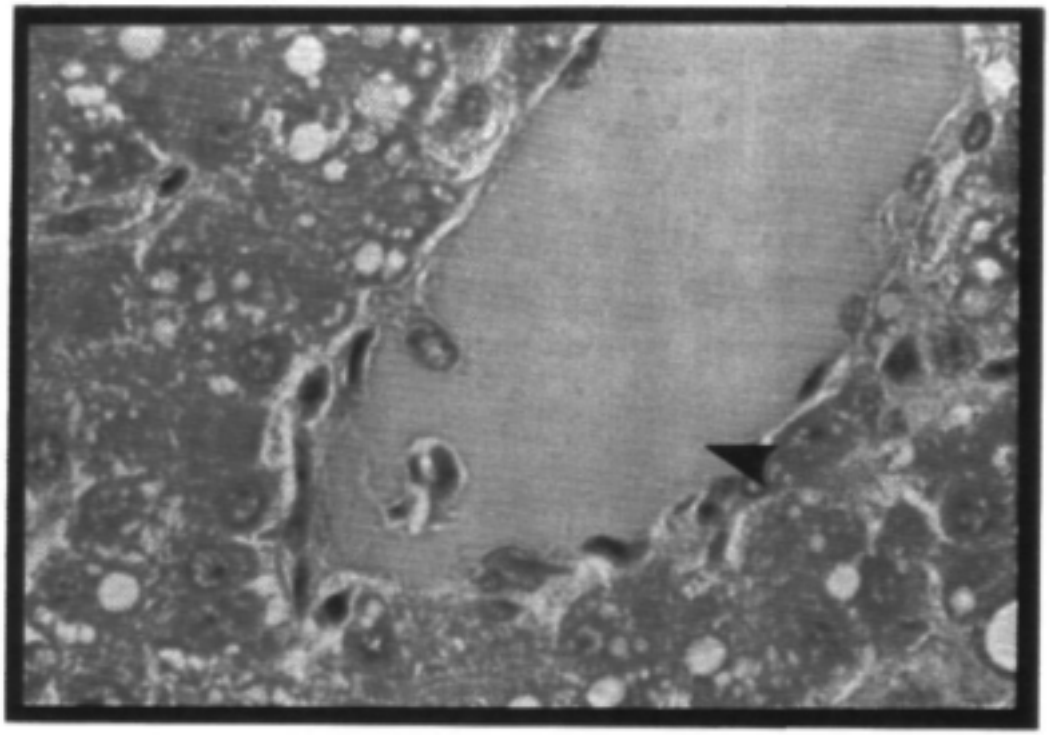


Figure 6.3.2.7d. **Kidney round cell infiltration.**

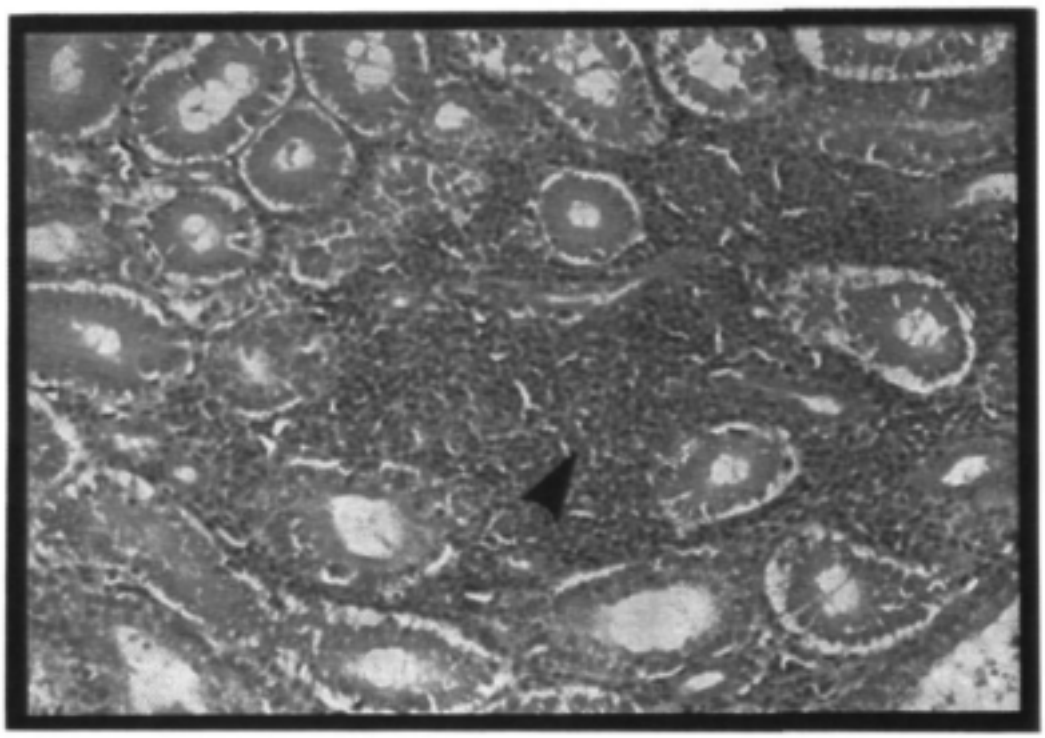


Table 6.3.2.7. Histopathology of livers and kidneys.

Variable	Treatment mg/l					P value
	0.29	6	10	14	20	
Variable in livers						
FC 0	4	7	3	5	9	0.3092
FC 1	16	14	20	14	11	0.3229
FC 2	10	11	10	13	16	0.5446
FC 3	6	8	7	6	4	0.7959
FC 4	4	0	0	2	0	0.0272
Variable in livers						
PL 0	36	30	32	27	32	0.1734
PL 1	3	6	3	4	5	0.7725
PL 2	1	4	1	7	2	0.0535
PL 3	0	0	4	2	1	0.0829
Variable in kidneys						
KRC 0	8	7	9	1	3	0.0448
KRC 1	21	17	21	20	17	0.7954
KRC 2	11	13	10	14	15	0.7389
KRC 3	0	3	0	5	5	0.0355

Key: **FC** = fatty changes in the livers (Figure 6.3.2.7a and Figure 6.3.2.7b).

PL = protein "lakes" in the livers (Figure 6.3.2.7c).

KRC = Kidney round cell infiltration (Figure 6.3.2.7d).

Although some significant differences occurred between groups (weeks 19, 20 and 40), this was not constant throughout the laying period (Table 6.3.2.9. and Figure 6.3.2.9). Fluoride had no significant effect (P = 0.8224) on egg production when looking at the means for the whole model (Table 6.3.2.8.).

Table 6.3.2.8. Least square means for egg production.

Treatment mg/l	LS Means
0.29	6.134
6	6.035
10	6.133
14	6.077
20	6.059

Table 6.3.2.9. Egg production per hen per week.

Week	Treatment mg/l					P value
	0.29	6	10	14	20	
19	0.224^{ab}	0.435^{a}	0.257^{ab}	0.124^{b}	0.187^{b}	0.0157
20	2.792^{ab}	3.328^{ab}	2.833^{ab}	2.387^{b}	3.482^{a}	0.0077
21	5.917^{a}	6.287^{a}	6.253^{a}	6.041^{a}	6.277^{a}	0.4298
22	6.954^{a}	6.951^{a}	6.874^{a}	6.889^{a}	6.926^{a}	0.9111
23	6.847^{a}	6.916^{a}	6.808^{a}	6.824^{a}	6.887^{a}	0.6708
24	6.826^{a}	6.904^{a}	6.886^{a}	6.877^{a}	6.864^{a}	0.8170
25	6.829^{a}	6.873^{a}	6.858^{a}	6.763^{a}	6.889^{a}	0.2082
26	6.779^{a}	6.728^{a}	6.812^{a}	6.722^{a}	6.863^{a}	0.2857
27	6.752^{a}	6.829^{a}	6.764^{a}	6.858^{a}	6.695^{a}	0.1877
28	6.745^{a}	6.811^{a}	6.732^{a}	6.825^{a}	6.855^{a}	0.4326
29	6.722^{a}	6.856^{a}	6.517^{a}	6.757^{a}	6.811^{a}	0.3785
30	6.796^{a}	6.750^{a}	6.675^{a}	6.860^{a}	6.747^{a}	0.2207
31	6.788^{a}	6.654^{a}	6.661^{a}	6.833^{a}	6.697^{a}	0.4605
32	6.798^{a}	6.652^{a}	6.618^{a}	6.660^{a}	6.656^{a}	0.6049
33	6.648^{a}	6.561^{a}	6.703^{a}	6.745^{a}	7.138^{a}	0.5954
34	6.707^{a}	6.727^{a}	6.800^{a}	6.911^{a}	6.673^{a}	0.4244
35	6.754^{a}	6.674^{a}	6.786^{a}	6.877^{a}	6.683^{a}	0.1645
36	6.579^{ab}	6.503^{ab}	6.692^{a}	6.615^{ab}	6.468^{b}	0.0395
37	6.554^{a}	6.422^{a}	6.477^{a}	6.538^{a}	6.443^{a}	0.6215
38	6.591^{a}	6.459^{a}	6.583^{a}	6.602^{a}	6.475^{a}	0.3406
39	6.477^{a}	6.311^{a}	6.440^{a}	6.547^{a}	6.512^{a}	0.3770

Table 6.3.2.9. (continued)

Week	0.29 mg/l	6 mg/l	10 mg/l	14 mg/l	20 mg/l	P value
40	6.607[a]	6.357[b]	6.554[ab]	6.676[a]	6.513[ab]	0.0046
41	6.506[a]	3.221[b]	6.489[a]	6.403[ab]	6.318[ab]	0.0101
42	6.271[a]	6.196[a]	6.371[a]	6.161[a]	6.266[a]	0.2444
43	6.846[a]	6.614[a]	6.853[a]	6.677[a]	6.743[a]	0.1224
44	6.664[a]	6.483[a]	6.796[a]	6.640[a]	6.579[a]	0.1846
45	6.411[a]	6.326[a]	6.604[a]	6.502[a]	6.472[a]	0.2190
46	6.396[a]	6.312[a]	6.562[a]	6.290[a]	6.380[a]	0.2177
47	6.522[ab]	6.293[ab]	6.564[a]	6.234[b]	6.382[ab]	0.0308
48	6.587[a]	6.342[a]	6.640[a]	6.488[a]	6.533[a]	0.1900
49	6.339[a]	6.182[a]	6.395[a]	6.192[a]	6.234[a]	0.1610
50	6.538[a]	6.413[a]	6.458[a]	6.410[a]	6.270[a]	0.4609
51	6.431[a]	6.345[a]	6.373[a]	6.412[a]	6.379[a]	0.09776
52	6.426[a]	6.252[a]	6.452[a]	6.343[a]	6.275[a]	0.2666
53	6.282[a]	6.283[a]	6.482[a]	6.411[a]	6.123[a]	0.0719
54	6.139[a]	5.795[a]	5.774[a]	6.160[a]	5.823[a]	0.0154
55	6.186[b]	6.362[ab]	6.724[a]	6.220[b]	6.274[b]	0.0112
56	6.257[a]	6.144[a]	6.221[a]	6.270[a]	6.076[a]	0.6710
57	6.124[a]	6.143[a]	6.265[a]	5.968[a]	5.905[a]	0.2289
58	6.089[a]	6.098[a]	6.212[a]	5.954[a]	5.959[a]	0.0966
59	6.157[a]	6.184[a]	6.172[a]	5.965[a]	5.975[a]	0.5535
60	6.158[a]	6.010[a]	6.119[a]	5.922[a]	5.942[a]	0.3342

Table 6.3.2.9. (continued)

Week	0.29 mg/l	6 mg/l	10 mg/l	14 mg/l	20 mg/l	P value
61	6.068[a]	5.973[a]	5.806[a]	5.776[a]	5.717[a]	0.0793
62	5.827[a]	5.828[a]	5.760[a]	5.759[a]	5.636[a]	0.7097
63	6.107[a]	5.995[a]	5.955[a]	5.995[a]	5.772[a]	0.4203
64	6.084[a]	5.988[a]	5.969[a]	5.713[a]	5.825[a]	0.2614
65	5.484[a]	5.285[a]	5.324[a]	5.417[a]	5.224[a]	0.5284
66	5.828[a]	5.116[a]	5.861[a]	5.863[a]	5.530[a]	0.6271
67	5.596[a]	5.401[a]	5.659[a]	5.615[a]	5.632[a]	0.4577
68	5.795[a]	5.092[a]	5.806[a]	5.695[a]	5.326[a]	0.0303
69	5.287[a]	5.062[a]	5.276[a]	5.210[a]	5.394[a]	0.2795
70	5.401[a]	5.138[a]	5.294[a]	5.340[a]	5.134[a]	0.7808
71	5.357[a]	5.202[a]	5.378[a]	5.086[a]	5.216[a]	0.6968
72	5.394[a]	5.088[a]	4.993[a]	5.177[a]	5.114[a]	0.5595
73	5.116[a]	4.985[a]	5.113[a]	5.036[a]	5.077[a]	0.9790

[a] Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Table 6.3.2.10. Total amount of eggs produced per group over laying period (corrected for mortalities).

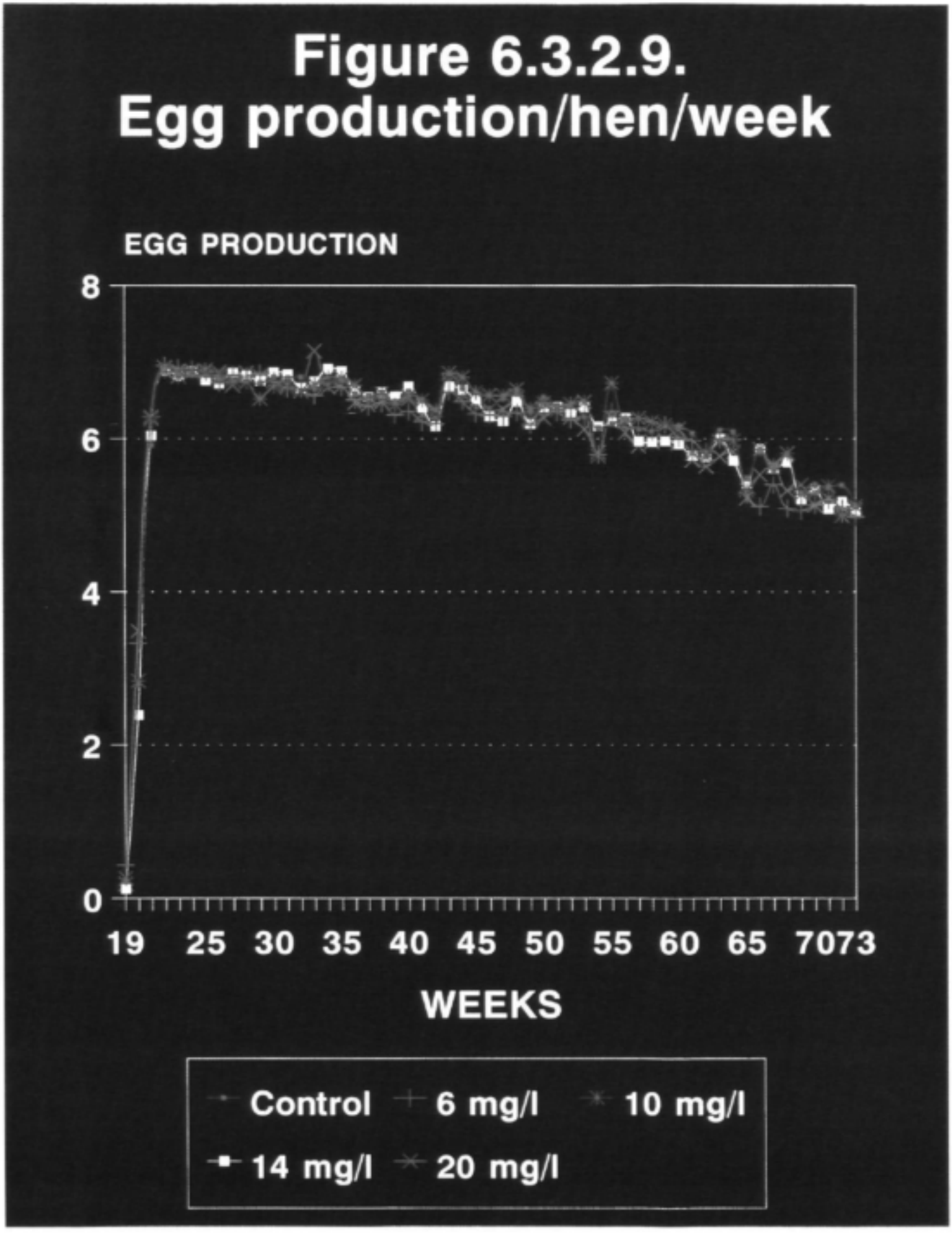
Treatment	0.29 mg/l	6 mg/l	10 mg/l	14 mg/l	20 mg/l	P value
Amount	1349.37	1333.69	1349.19	1336.94	1332.98	0.4060

The 20 mg/l group produced 203 soft eggs over the laying period and the control only 133. The control on the other hand produced more (133) double yolked eggs than the 20 mg/l group (95). These two indices of egg quality were subjectively appraised.

Table 6.3.2.11. Amount of soft and double yolked eggs over laying period.

Treatment mg/l	0.29	6	10	14	20
Amount of soft eggs	133	153	134	113	203
Amount of double yolked eggs	133	83	70	104	95

Figure 6.3.2.9.
Egg production/hen/week



These mortalities (Table 6.3.2.12.) were not linked to fluoride treatments. See Table 6.3.2.13. for causes of mortalities.

Table 6.3.2.12. Mortalities over production period (week 19 - 74).

Treatment	Mortalities
0.29 mg/l	45
6 mg/l	38
10 mg/l	50
14 mg/l	44
20 mg/l	34

Table 6.3.2.13. Causes of mortalities.

Cause	Total amount of chickens that died from specific cause per treatment (mg/l).				
	0.29	**6**	**10**	**14**	**20**
Dehydration	12	10	7	4	6
"Flip over"	2	2	3	1	-
Liver rupture	4	5	5	6	6
Yolk sac infection	3	1	-	2	2
Pericarditis	2	4	2	1	1
Septicemia	3	2	1	2	2
Necrotic Enteritis	1	-	-	-	-
Anaemia	-	1	-	-	-
Visceral Gout	-	1	1	-	1
Egg retention peritonitis	-	-	3	1	2
Aspergilloses	-	-	2	2	1
Mite infection	-	-	1	-	-
Hydro pericardium	-	-	1	-	-
Too decomposed	9	8	14	11	6
No visible symptoms	10	5	10	13	3
Torsion of small intestines	-	-	-	1	-
Peritonitis	-	-	-	2	1
Splenomegaly	-	-	-	-	1
Prolapse	-	-	-	-	2

Although the fluoride treatments were 6, 10, 14 and 20 mg/l the effective amount of fluoride ingested per hen per day over the laying period was 1.361, 1.979, 2.661 and 4.453 mg/week respectively.

Table 6.3.2.14. Fluoride ingestion rates from week 27 - 74.

	Treatment mg/l				
	0.29	**6**	**10**	**14**	**20**
Total amount of water ingested (l) over laying period per hen.	11.365	12.701	11.082	10.642	12.469
F ingested over laying period (mg) per hen.	3.300	76.206	110.820	148.988	249.380
Average amount of F ingested/hen/day (mg)	0.059	1.361	1.979	2.661	4.453

Water intake was significantly ($P < 0.0001$) influenced by fluoride treatment. The 6 mg/l and 20 mg/l treatments drank more water than the control group (Table 6.3.2.15.).

Table 6.3.2.15. Least square means of water intake for weeks 27 - 74.

Treatment mg/l	LS Means
0.29	0.237^{b}
6	0.264^{a}
10	0.231^{c}
14	0.222^{d}
20	0.260^{a}

* Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

Table 6.3.2.16. Water intake per hen per week (l).

Weeks	Treatment mg/l					P value
	0.29	6	10	14	20	
27	0.263[a]	0.290[a]	0.259[a]	0.227[a]	0.298[a]	0.0686
28	0.247[b]	0.319[a]	0.241[b]	0.239[b]	0.309[a]	0.0001
29	0.266[b]	0.337[a]	0.258[b]	0.246[b]	0.340[a]	0.0001
30	0.258[b]	0.315[a]	0.247[b]	0.240[b]	0.300[a]	0.0001
31	0.300[b]	0.357[a]	0.293[b]	0.291[b]	0.343[a]	0.0001
32	0.243[b]	0.34[a]	0.237[b]	0.235[b]	0.305[a]	0.0001
33	0.284[b]	0.352[a]	0.287[b]	0.270[b]	0.348[a]	0.0001
34	0.249[b]	0.324[a]	0.248[b]	0.235[b]	0.338[a]	0.0001
35	0.272[b]	0.345[a]	0.279[b]	0.260[b]	0.335[a]	0.0001
36	0.274[b]	0.342[a]	0.279[b]	0.257[b]	0.327[a]	0.0001
37	0.277[b]	0.361[a]	0.298[b]	0.265[b]	0.359[a]	0.0001
38	0.262[ab]	0.310[a]	0.263[ab]	0.249[b]	0.302[ab]	0.0070
39	0.231[b]	0.291[a]	0.226[b]	0.217[b]	0.279[a]	0.0001
40	0.251[b]	0.318[a]	0.247[b]	0.237[b]	0.311[a]	0.0001
41	0.243[b]	0.295[a]	0.244[b]	0.234[b]	0.283[a]	0.0001
42	0.247[b]	0.285[a]	0.241[b]	0.228[b]	0.279[a]	0.0001
43	0.232[bc]	0.275[a]	0.231[bc]	0.218[c]	0.263[ab]	0.0001
44	0.224[b]	0.259[a]	0.215[b]	0.206[b]	0.242[a]	0.0001
45	0.255[b]	0.302[a]	0.253[b]	0.246[b]	0.274[a]	0.0001
46	0.227[b]	0.274[a]	0.226[b]	0.216[b]	0.273[a]	0.0001
47	0.234[b]	0.267[a]	0.224[b]	0.218[b]	0.277[a]	0.0001

Table 6.3.2.16. (continued)

Weeks	0.29 mg/l	6 mg/l	10 mg/l	14 mg/l	20 mg/l	P value
48	0.229^{b}	0.266^{a}	0.222^{b}	0.218^{b}	0.271^{a}	0.0001
49	0.237^{b}	0.273^{a}	0.229^{b}	0.225^{b}	0.278^{a}	0.0001
50	0.236^{a}	0.237^{a}	0.214^{b}	0.208^{b}	0.245^{a}	0.0001
51	0.243^{a}	0.255^{a}	0.221^{b}	0.211^{b}	0.264^{a}	0.0001
52	0.232^{b}	0.241^{a}	0.216^{b}	0.204^{c}	0.252^{a}	0.0001
53	0.239^{bc}	0.254^{ab}	0.229^{cd}	0.216^{d}	0.264^{a}	0.0001
54	0.232^{a}	0.239^{a}	0.212^{b}	0.210^{b}	0.245^{a}	0.0001
55	0.203^{b}	0.223^{a}	0.210^{ab}	0.193^{b}	0.227^{a}	0.0001
56	0.208^{a}	0.211^{a}	0.198^{ab}	0.189^{b}	0.204^{ab}	0.0107
57	0.194^{ab}	0.202^{a}	0.194^{ab}	0.185^{b}	0.194^{ab}	0.0649
58	0.207^{a}	0.205^{a}	0.208^{a}	0.199^{a}	0.205^{a}	0.5777
59	0.217^{a}	0.211^{ab}	0.2091^{ab}	0.195^{b}	0.206^{ab}	0.0298
60	0.222^{a}	0.210^{ab}	0.204^{ab}	0.198^{b}	0.203^{ab}	0.0102
61	0.219^{a}	0.207^{ab}	0.209^{ab}	0.202^{b}	0.202^{b}	0.0279
62	0.226^{a}	0.219^{a}	0.213^{a}	0.209^{a}	0.216^{a}	0.2117
63	0.229^{a}	0.216^{ab}	0.209^{ab}	0.203^{b}	0.213^{ab}	0.0096
64	0.200^{a}	0.193^{a}	0.183^{a}	0.176^{a}	0.192^{a}	0.1541
65	0.235^{a}	0.220^{ab}	0.211^{ab}	0.207^{b}	0.212^{ab}	0.0136
66	0.229^{a}	0.247^{a}	0.206^{a}	0.203^{a}	0.213^{a}	0.1778
67	0.205^{a}	0.195^{a}	0.192^{a}	0.192^{a}	0.192^{a}	0.2194
68	0.216^{a}	0.212^{a}	0.217^{a}	0.209^{a}	0.206^{a}	0.2753

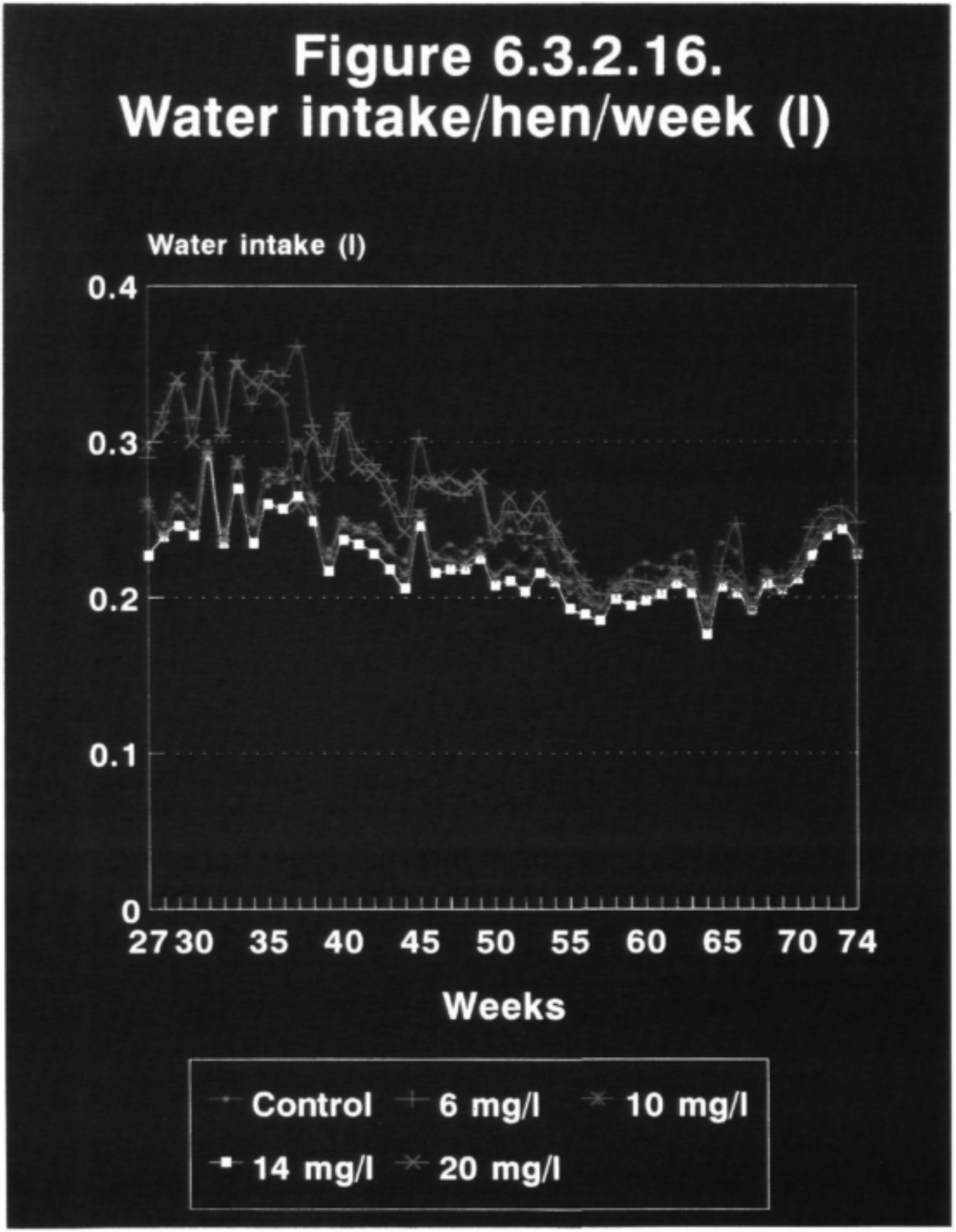
Table 6.3.2.16. (continued)

Weeks	0.29 mg/l	6 mg/l	10 mg/l	14 mg/l	20 mg/l	P value
69	0.213[a]	0.213[a]	0.206[a]	0.205[a]	0.206[a]	0.5960
70	0.217[a]	0.223[a]	0.215[a]	0.212[a]	0.213[a]	0.7067
71	0.221[b]	0.245[a]	0.235[ab]	0.227[b]	0.233[ab]	0.0043
72	0.244[a]	0.256[a]	0.245[a]	0.240[a]	0.249[a]	0.2346
73	0.250[a]	0.258[a]	0.250[a]	0.244[a]	0.250[a]	0.2011
74	0.223[a]	0.248[a]	0.229[a]	0.228[a]	0.229[a]	0.2700

Means with different superscripts are significantly different ($P < 0.05$) according to Tukey's Multiple Range Test.

From week 28 - 55 excluding a few isolated weeks, there were highly significant ($P < 0.0001$) differences between treatments. In almost all these weeks the 20 mg/l groups showed a much higher water intake than the control (Table 6.3.2.16 and Figure 6.3.2.16.).

Figure 6.3.2.16.
Water intake/hen/week (l)



6.4. CONCLUSION

The implications of the results obtained for water quality guideline formulation are that firstly, ingestion rates need to be calculated, and secondly, the production system must be taken into account in order to fully utilise the water source. This is important as it is the actual ingestion of the water quality variable that is relevant, and not only the concentration of the variable in the water source.

There were no significant differences between treatments regarding feed intake, feed conversion, liver weights, histopathology of the livers and kidneys, egg production or mortalities. The fluoride content of the femurs ($P = 0.0001$) was: Control - 573.69 mg/kg; 6 mg/l - 947.28 mg/kg; 10 mg/l - 1139.91 mg/kg; 14 mg/l - 1329.00 mg/kg; 20 mg/l - 1671.39 mg/kg. The breaking strength of the femurs were not influenced by fluoride treatment ($P = 0.5005$). The mean breaking strength for all groups was 24.041 kg. Carcass weights increased significantly ($P = 0.0332$) in the higher dosage groups, 0.29 mg/l = 2.1529 kg; 20 mg/l = 2.5523 kg. In the seventh, eighth and eleventh weeks the control groups had significant ($P < 0.05$) higher body weights than the 20 mg/l group. Over the rest of the growing period no significant differences occurred. Breaking strength of eggs increased in the high fluoride treatments ($P = 0.0681$).

Fatty changes in the livers, protein lakes in the liver and the amount of round cell infiltration in the kidneys were not fluoride linked, but rather linked to overfeeding due to the length of the trial. Subcapsular haematomas were only present in the treatment groups and not in the controls. Further research might be warranted in this respect.

The mean amount of eggs produced per hen per week were: 0.29 mg/l = 6.1335; 6 mg/l = 6.0348; 10 mg/l = 6.1327; 14 mg/l = 6.0770 and 20 mg/l = 6.0590. The 20 mg/l group produced more (203) soft eggs than the control group (133) and the control group produced more double yolked eggs (133), than the 20 mg/l group (95). These two indices of egg quality were subjectively appraised.

Fluoride treatments significantly ($P < 0.0001$) influenced the water intake of the hens. The 20 mg/l groups drank more water than the controls. This means that the fluoride effect will

be magnified because the groups that received the highest fluoride levels also drank the most water. It appeared that fluoride increased thirst or stimulates water intake and leads to a higher intake of fluoride. The effective ingestion of fluoride (mg) per hen per day was: 0,29 mg/l = 0.059 mg; 6 mg/l = 1.361 mg; 10 mg/l = 1.979 mg; 14 mg/l = 2.661 mg and 20 mg/l = 4.453 mg.

These results indicate that fluoride up to levels of 20 mg/l or an ingestion rate of 4.453 mg F per day in the drinking water of layers had no negative effect on production characteristics over a 74 week production period.

CHAPTER 7 THE EFFECT OF F IN THE DRINKING WATER ON GROWTH AND HEALTH OF BONSMARA STEERS TO MARKET WEIGHT and the EFFECT OF A F-SALINITY COMBINATION TREATMENT

7.1 INTRODUCTION

Tolerance to levels of F (water and dietary) vary between species (Wheeler and Fell, 1983). For this reason the results obtained with F in the drinking water on SAMM wethers in chapter 3 and chapter 4 cannot simply be extrapolated to cattle, although the previous work (chapter 3 and 4) suggests that testing at the lower levels (6 to 14 mg/l F), for the time/exposure frame of growth from weaned to market weight, is not required.

Furthermore, as explained in Chapter 3, it is the ingestion of F in mg/ day/ animal that is of importance and not merely the concentration of F in the drinking water. This value cannot be extrapolated from sheep to cattle, and these two factors necessitated a further trial using beef cattle as the experimental model.

The findings relating to the effect of salinity on bone F concentration dealt with in Chapter 4 can also not be assumed to be true for cattle, and therefore a F/salinity interaction was also incorporated in the trial design.

This chapter deals with the effects of F in the drinking water at 15 - 20 mg/l on the growth, water intake and health of Bonsmara steers to slaughter weight, the incidence and degree to which chronic fluorosis developed and the effect of A F-salinity treatment combination.

7.2 METHODS

Thirty three Bonsmara steers just weaned with an initial weight of 198.39 ± 17.47 kg were assigned to 3 groups using a complete randomized block design. Treatments (TRT) were as follows :

Group 1 = < 1mg/l F, <1000 mg/l TDS (control and fresh water treatment)
Group 2 = 15 - 20 mg/l F, <1000 mg/l TDS (F and fresh water treatment)
Group 3 = 15 - 20 mg/l F, >3000 mg/l TDS (F and saline treatment).

Water was reconstituted to TRT levels using NaF (AR-grade) and offered *ad libitum* in plastic drinking troughs and analyzed prior to and during the trial. Sodium fluoride was used as opposed to CaF_2 as it has approximately twice the dietary availability (Shupe, Miner, Harris and Greenwoord, 1962). Daily watering times were 07H00 - 08H00 and 15H00 - 16H00, with daily emptying and cleaning of the drinking troughs. Water intake was measured on a daily basis for 5 days per week, and calculated as a weekly average. A standard commercial feedlot ration was offered *ad libitum* (Appendix) for 124 days at which time an average slaughter weight of 359 ± 17.2 kg was attained.

Inspections were conducted for clinical signs of fluorosis on a monthly basis (Buck and Osweiler, 1976; Weatherell and Weidmann, 1959). The slaughter weight was recorded 1 day prior to slaughter with hot carcass weight and cold carcass weight recorded. Kidney and liver sections were collected for light microscopy from each steer and prepared as follows: cross-sections with a width of less than 5 mm were fixed by immersion in a 10% buffered formalin solution within 20 minutes post slaughter, whereafter the fixed tissues were processed routinely, embedded in paraffin wax, sectioned at 3 - 4 μm and stained with haematoxylin and eosin (Williams, 1990).

Differences in finishing live weights, slaughter weights (hot and cold), were analyzed by least squares means analysis of variance and multiple regression, using the general linear models procedure (GLM) on SAS (SAS, 1982). Differences in starting weight were corrected for by its inclusion as a covariate. Slaughter weight was included as covariate for the analysis of variance for hot carcass weight differences. Significance levels used were dependent on

the trial design. Most regression analysis were tested at the $P<0.05$ level, but for the least squares means analysis, stricter levels were used.

7.3 RESULTS

7.3.1 Growth and health

There were no significant differences ($P<0.05$) in starting live weights between the treatment groups (Table 7.1), hence any differences in growth could most probably be attributed to treatment effects.

Table 7.1 Differences in starting live weight between treatments of Bonsmara steers.

TRT[a]	Means (kg)	Standard Deviations	Significance values[b]		
			P	F	R^2
1	200.45	20.05	0.719	0.34	0.0221
2	194.72	21.62	Least squares means[c]		
3	200.00	10.74	TRT interactions		P-values
			1+2		0.4644
			1+3		0.9535
			2+3		0.5003

[a] TRT = treatment; TRT1 = Control group; TRT2 = F + TDS group; TRT3 = F group.

[b] + [c] Significance level $P<0.05$.

Treatment was found to have a significant effect on the final live weight recording (Table 7.2). The type III SS indicate that both treatment and starting live weight had a significant effect on the final live weight recording. The F treatment group and the F + TDS treatment group had a significantly heavier final live weight recordings ($P<0.05$) in comparison to the Control group (Table 7.2 - least squares means). The F + TDS treatment group recorded a heavier final live weight than the F group, although this was not significant ($P<0.5$).

Table 7.2 The effect of F treatment on final live weight in Bonsmara steers.

TRT[a]	Means (kg)	Standard Deviations	Significance values[b]			
				MODEL	TYPE III SS	
					TRT	STLW[d]
			P	0.0001	0.0016	0.0001
1	346.18	20.76	F	11.11		
2	374.27	31.31	R^2	0.53		
3	363.63	20.77	Least squares means[c]			
			TRT interactions		P-values	
			1+2		0.0004	
			1+3		0.0370	
			2+3		0.0727	

[a] TRT = treatment; TRT1 = Control group; TRT2 = F + TDS group; TRT3 = F group.

[b] + [c] Significance level $P<0.05$.

[d] STLW = starting live weight.

Live weight gain was found to be significantly affected by treatment at the $P<0.05$ level (Table 7.3). The type III SS indicate that starting live weight did not contribute significantly to live weight gain, whereas treatment did. Both the F treatment group and the F + TDS treatment group recorded significantly higher live weight gains than the Control group (least squares means values - $P<0.05$). The F + TDS treatment group recorded a higher live weight gain than the F treatment group, but was not significant at the $P<0.05$ level.

Table 7.3 The effect of F treatments on live weight gain in Bonsmara steers.

TRT[a]	Means (kg)	Standard Deviations	Significance values[b]			
				MODEL	TYPE III SS	
					TRT	STLW[d]
			P	0.0019	0.0007	0.8657
1	144.90	15.82	F	6.38		
2	179.54	16.75	R^2	0.397		
3	163.63	21.77	Least squares means[c]			
			TRT interactions		P-values	
			1+2		0.0002	
			1+3		0.0251	
			2+3		0.0531	

[a] TRT = treatment; TRT1 = Control group; TRT2 = F + TDS group; TRT3 = F group.

[b] + [c] Significance level $P<0.05$.

[d] STLW = starting live weight.

Treatment has a significant effect on hot carcass weight ($P<0.05$), with the F + TDS treatment group having a significantly higher hot carcass weight than the Control group (Table 7.4). Although the F + TDS treatment group recorded a higher hot carcass weight than the F treatment group, this was not significant. A similar finding was made with respect to the higher hot carcass weight recordings of the F treatment group compared to the Control group. There was similar allocation of grades for all three treatment groups (Appendix), with approximately 50% in each group graded as A_2, and 50% as A_3.

Table 7.4 The effect of F treatment on hot carcass weight of Bonsmara steers.

TRT[a]	Means (kg)	Standard Deviations	Significance values[b]			
				MODEL	TYPE III SS	
					TRT	STLW[d]
			P	0.0001	0.0509	0.0001
1	196.23	12.65	F	16.20		
2	202.12	15.08	R^2	0.626		
3	199.95	11.00	Least squares means[c]			
			TRT interactions		P-values	
			1+2		0.0157	
			1+3		0.2680	
			2+3		0.1581	

[a] TRT = treatment; TRT1 = Control group; TRT2 = F + TDS group; TRT3 = F group.

[b] + [c] Significance level $P<0.05$.

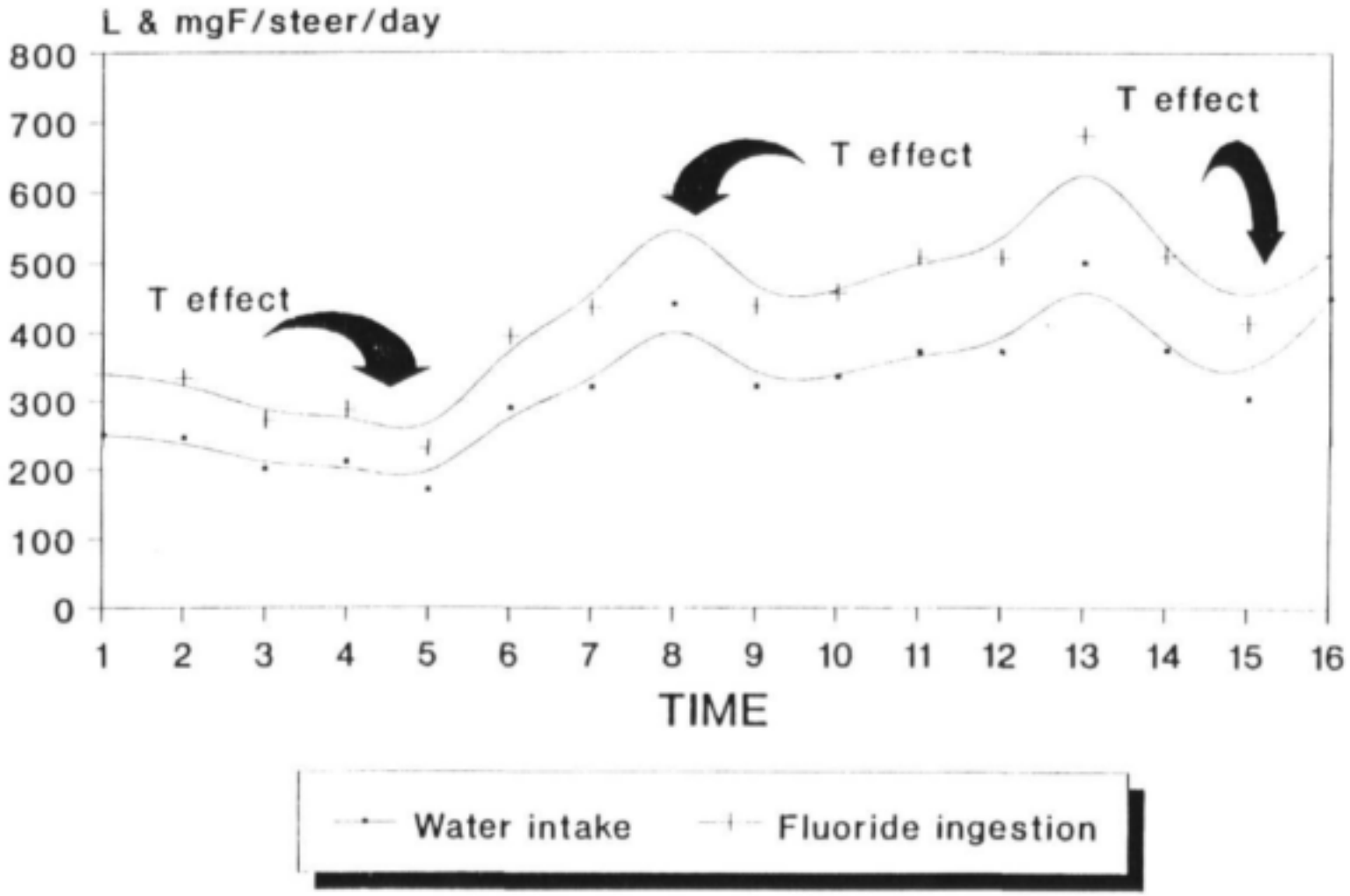
[d] STLW = starting live weight.

A significant Spearman correlation coefficient of 42.49 % for the variables live weight gain and treatment was found at the $P<0.05$ level.

There were no histological lesions found in the kidney and liver sections made, with all the treatment groups kidney and liver sections having a very similar microscopic appearance (Appendix). No visual macroscopic evidence of fluorosis was observed in any of the tissues examined.

The water intake increased with increases in body weight, with an average water intake of 29.28 l/ steer/ day, and an average ingestion of 350 mg F/ steer/ day for the first 60 days of the trial and 600 mg F/ steer/ day for the second 60 days of the trial (Figure 7.1).

Figure 7.1 Water intake and F ingestion in Bonsmara steers



7.4 DISCUSSION

The non significant differences between treatment groups for starting live weight enables the differences in growth recorded later between treatment groups to be attributed to the treatments themselves. This is so as all other factors were similar for the groups (housing, nutrition, etc.).

The type III SS for the effect of TRT on final live weight show starting live weight to have a significant effect. This is to be expected, and although there were no significant differences in starting live weight between treatment groups, it is incorporated as a covariate due to the obvious effect it has on individual steer final live weight. The type II SS for TRT effect on final live weight also indicates that TRT had a significant contribution to final live weight, with the F + TDS group having the highest value, then the F group and lastly the C group. Although only the F + TDS group were significantly higher than the C group, the indication is that the two groups receiving F in the drinking water performed better than the C group which did not receive F. This trend was also found for live weight gain differences between TRT groups, but with both the F + TDS and the F groups recording significantly higher live weight gains than the C group. The type III SS values indicated that starting live weight did not contribute significantly to the live weight gains, whereas TRT did, and therefore the differences in live weight gains are most likely due to the presence or absence of F in the drinking water.

A more accurate measurement of differences between TRT groups are the hot carcass weight values (no variance due to gut-fill at time of weighing). The similar trend found with respect to hot carcass weight differences between TRT groups once again indicates that the group receiving F + TDS performed better than the group not receiving F. The F group recorded a higher hot carcass weight than the C group and, although this was not significant, it indicates that the presence of F in the drinking water at 20 mg/l did not adversely affect the growth of the steers, but rather appeared to promote it. This is also indicated by the positive significant correlation found between live weight gain and TRT. The similar grades attained for the treatment groups indicate a similar stage of maturation at time of slaughter (differences would have vast implications for a feedlot scenario).

The absence of histological lesions in the kidney and liver sections made, and the lack of macroscopic fluorotic lesions in the tissues viewed, indicate that the presence of F in the drinking water did not have an adverse effect in terms of the manifestation of fluorosis. Were the steers not slaughtered but continually exposed to F ,this would most probably have occurred for the F group first, and then the F + TDS group (based on findings in Chapter 4), but the growth results and the histopathological results would indicate that this would not occur on the dosage level used in the trial, within the time frame taken to reach market weight.

As explained in Chapter 3, it is not the dosage or concentration of F in the drinking water as such that is the crucial factor, but rather the ingestion in mg/day. This enables the extrapolation of the effects observed in the trial to other environments, using the guideline of 350 mg F/ steer/ day from weaned (approximately 200 kg live weight) and gradually increasing to an ingestion of 600 mg F/ steer/ day for the final stage to market weight. The stages were approximately 60 days each in this trial, but would be less in a feedlot scenario (not more than 110 days total), and hence due to the shorter time/ dose / exposure interaction a higher ingestion would probably be found over the final stages, but would not result in adverse effects manifesting themselves in terms of growth. Similarly, a longer time /dose/ exposure interaction might occur in extensive conditions, but would seldom be more than 130 days.

The results seem therefore to indicate that the philosophy of rather using a concentration of a water quality variable in conjunction with the production system, species and environment, all of which determine the effect of the concentration on the animal, as opposed to just a concentration as a guideline, is correct. Using the current guidelines available, the water source used in this trial would have been classed unfit at 2 mg/l F (international) and at 6 mg/l F (local), but for the time/ dose/ exposure interaction from weaned to market weight, 20 mg/l F was used without any adverse effects on health or growth. This philosophy is particularly relevant for water quality variables that are potentially toxic and specifically those which are cumulative in nature.

7.5 CONCLUSION

Similar findings to those made with SAMM wethers were made with Bonsmara steers with respect to a drinking water concentration of 20 mg/l F not having an adverse effect on health or growth from weaned to market weight. The absence of histopathological and macroscopic lesions further indicate that due to the chronic nature of fluorosis and production system in question (mutton and beef production - weaned to market weight), sheep and cattle may be exposed to F concentrations far in excess of the recommended guidelines without the risk of adverse effects on production or health.

The F/TDS interaction referred to in Chapter 4 would seem to be applicable to beef steers. This is borne out by the F + TDS group having significantly higher live weight gains, final weights and hot carcass weights than the C group. This highlights the need to take into account the relevant synergistic and antagonistic interactions between water quality variables (in conjunction with environmental and nutritional interactions) in the assessment of the impact a given water source will have for a given livestock watering scenario.

The conclusion is thus that beef steers may be allowed access to a water source under conditions that result in the ingestion of approximately 350 mg F/ steer/ day from weaned to an ingestion of approximately 600 mg F/ steer/ day in the latter half of growth to market weight (less than 130 days total exposure). In this range of F ingestion there should be no adverse effects on health or growth, nor any possible consumer product hazard. A TDS concentration of 3000 - 6000 mg/l can possibly allow for higher concentrations of F to be ingested.

CHAPTER 8 THE EFFECTS OF CHLORIDE AND SULPHATE SALTS AT VARIOUS TOTAL DISSOLVED SOLIDS LEVELS ON THE PALATABILITY OF WATER FOR FRIESLAND STEERS

8.1 INTRODUCTION

The following points have been outlined in previous chapters:

- the water quality variables F, Cl, SO_4, and TDS had the highest incidence of potential toxicity in the assessment of subterranean water samples (Chapter 2);
- the water quality variables Cl, SO_4 and TDS are involved with the acceptability of water primarily via their effect on palatability (Chapter 4);
- taste appears to be the limiting factor in most water sources with high concentrations of the variables, due to the resulting adverse effects on livestock production via reducing the water intake, hence feed intake and performance, rather than kidney function being the limiting factor (Chapter 4);
- some waters which were classed as fit for livestock were refused by livestock, and the opposite was also found to occur (Chapter 2);
- sheep tolerated highly saline waters after a slow incremental adaptation period, without any adverse effects on health, growth or kidney function (Chapter 4);
- significant decreases in water intake were found with manipulation of varying ratios of Cl:TDS:SO_4 ratios over varying TDS concentrations and within set TDS concentrations (Chapter 4).

Due to the higher sensitivity of cattle to salinity as opposed to sheep, extrapolation of results obtained in Chapter 4 and hence guideline recommendations, cannot be assumed to be true for cattle, particularly with reference to specific concentration ratios and levels.

Contrary to the chemical treatment pattern used in Chapter 4 for the sheep, where it was found that the sheep exhibited an ability to adapt to highly saline waters with slow incremental adaptation, in this trial the animals were only given saline treatments for a 24 hour period and allowed a 2-3 day recovery period on fresh water. This was in order to gain

an accurate assessment of the initial response to a chemical water treatment that was not influenced by previous long term exposure to a similar chemical treatment.

The aim of the trial was to investigate a possible existence of a " zone of preference" for Cl:SO_4:TDS ratios over increasing saline ranges as shown in Figure 8.1. The ratio lines for each of the TDS concentrations represent possible combinations of Cl:SO_4 ratios for that specific TDS range. As the TDS concentration increases the location of the optimal water intake would be the "zone of preference" and would conceivably change as the TDS concentration increases.

Having the knowledge of where the "zone of preference" lies would enable a more accurate assessment of the effect of a water source on the palatability and thus acceptance of that water source to a sensitive ruminant model.

This chapter deals with the effect of Cl and SO_4 salts on the palatability of water for Friesland steers.

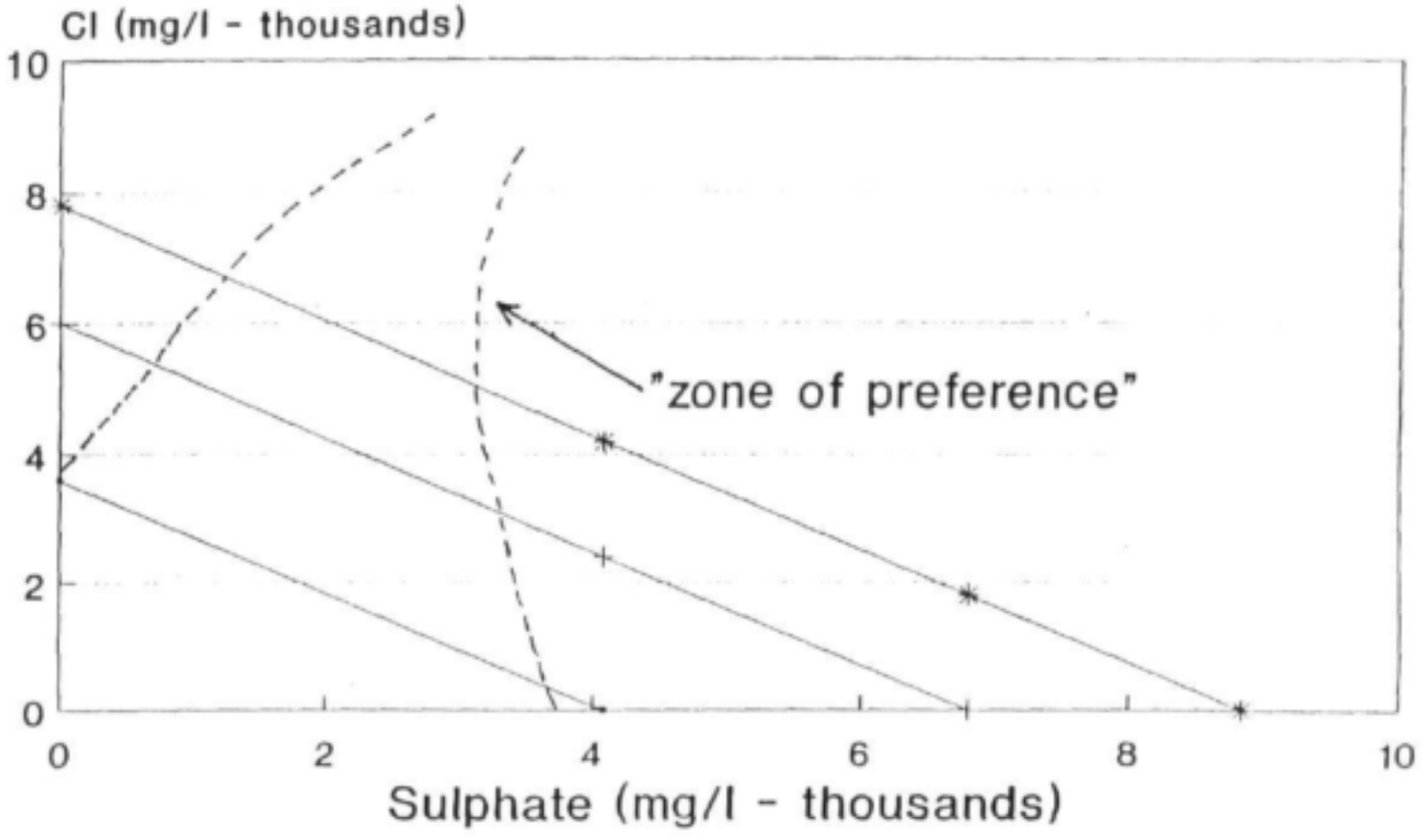


Figure 8.1 Theoretical position of the "zone of preference"

8.2 METHODS

Due to the higher sensitivity of dairy cattle as opposed to beef cattle, dairy cattle were used as the experimental animals. Ten Friesland steers (180 - 220 kg live weight) were used as the experimental animals.

Housing and health

The steers were housed indoors, and haltered to an open system, individual water and feeding trough system. The halters had approximately 3 meters of free - movement lead attachment. This method was used in preference to housing the animals in crates as it allowed for greater movement and easier handling and cleaning operations. The condition of the steers was constantly monitored.

Adaptation period

A 6 week adaptation period was required for the steers to become accustomed to the housing facility, halters and to being handled (this was required for cleaning and administering the chemical treatments). The steers were adapted to a standard grower ration which was fed *ad lib*. Feeding and cleaning was carried out twice per day.

Water treatments

The water treatments were administered using NaCl and Na_2SO_4 (AR - grade). The steers were allowed free access to the treatments for 24 hours. Following the treatments the steers received fresh water *ad lib* for 2-3 days before the next treatment was administered.

The water treatments given depended on the response of the steers to the previous treatment. If the response to a specific variable concentration was a significant decline in water intake, the responsible variable was not administered in an increased concentration.

The following treatments were given:

(a) Increasing SO_4 concentrations;

(b) Increasing Cl concentrations;

(c) Ratios with a TDS concentration of 6000 mg/l;

(d) Ratios with a TDS concentration of 10 000 mg/l;

(e) Ratios with a TDS concentration of 13 000 mg/l.

The treatments are presented in Table 8.1.

Similar statistical analysis were used as in Chapter 4.

Table 8.1 Chemical treatments administered in the drinking water to Friesland steers.

Chemical treatments	TDS (mg/l)	Cl (mg/l)	SO_4 (mg/l)	RATIOS Cl:TDS	RATIOS SO_4:TDS
Increasing SO_4					
TRT 1	1470	0	1000	0	0.68
2	4411	0	3000	0	0.68
3	5882	0	4000	0	0.68
4	8332	0	5666	0	0.68
Increasing Cl					
TRT 1	1666	1000	0	0.6	0
2	5000	3000	0	0.6	0
3	10000	6000	0	0.6	0
4	13000	7800	0	0.6	0
Ratios at 6000 TDS					
TRT 1	6000	0	4080	0	0.68
2	6000	3600	0	0.6	0
3	6000	2717	1000	0.354	0.167
4	6000	1835	2000	0.306	0.333
5	6000	953	3000	0.159	0.500
Ratios at 10000 mg/l					
TRT 1	10000	6000	0	0.6	0
2	10000	4000	2267	0.4	0.227
3	10000	3352	3000	0.335	0.3
4	10000	5117	1000	0.512	0.1
Ratios at 13000 mg/l					
TRT 1	13000	7800	0	0.6	0
2	13000	5859	2200	0.451	0.169
3	13000	5859	2200	0.451	0.169
4	13000	4200	4080	0.323	0.314

8.3 RESULTS

(a) Increasing SO_4 concentrations:

Treatments 1 - 4 were found to have a significant effect on water intake at the $P<0.05$ level (Table 8.2). Treatment 4, with a SO_4 concentration of 5666 mg/l was found to result in a significant decrease in water intake compared to the other treatments (least squares means). The other treatment interactions were not significantly different.

Table 8.2 Effect of increasing SO_4 concentrations in the drinking water on water intake in Friesland steers.

TRT[a]	Means (l)	Standard Deviations	Significance values[b]	
			MODEL	
			P	0.0101
			F	4.35
			R^2	0.266
1	23.7	5.355	Least squares means[c]	
2	21.6	5.146		
3	26.4	4.115	TRT interactions	P-values
4	15.7	10.832	1+2	0.4994
			1+3	0.3862
			1+4	0.0135
			2+3	0.1276
			2+4	0.0632
			3+4	0.0013

[a] TRT = treatment: TRT1 - 1000 mg/l SO_4; TRT2 - 3000 mg/l SO_4; TRT3 - 4000 mg/l SO_4; TRT4 - 5666 mg/l SO_4.

[b] significance level $P<0.05$;

[c] significance level $P<0.05$.

(b) Increasing Cl concentrations:

Treatments 1 - 4 were found to have a significant effect on water intake at the $P<0.05$ level (Table 8.3), with TRT 4 yielding a significantly lower water intake than TRT 2 and TRT3.

Table 8.3 Effect of increasing Cl concentrations in the drinking water on water intake in Friesland steers.

TRT[a]	Means (l)	Standard Deviations	Significance values[b]
			MODEL
			P 0.0475
			F 2.91
			R^2 0.192
1	25.6	5.189	Least squares means[c]
2	27.5	6.023	
3	29.1	10.877	TRT interactions P-values
4	19.4	9.430	1+2 0.4300
			1+3 0.3413
			1+4 0.1005
			2+3 0.8723
			2+4 0.0182
			3+4 0.0122

[a] TRT = treatment: TRT1 - 1000 mg/l Cl; TRT2 - 3000 mg/l Cl; TRT3 - 6000 mg/l Cl; TRT4 - 7800 mg/l Cl.

[b] significance level $P<0.05$;

[c] significance level $P<0.05$.

(c) Ratios at 6000 mg/l TDS:

The treatments administered were found to have a significant effect on water intake ($P<0.05$). Treatment 1, with a maximum SO_4:TDS ratio at the 6000 TDS level of 0.68, was found to differ significantly with TRT 2 - 5, recording the lowest water intake. None of the other treatment interactions differed significantly.

Table 8.4 Effect of manipulating CL:TDS:SO_4 ratios at a TDS concentration of 6000 mg/l on water intake in Friesland steers.

TRT[a]	Means (l)	Standard Deviations	Significance values[b]
			MODEL
			P 0.0064
			F 4.11
			R^2 0.267
1	28.40	5.0376	Least squares means[c]
2	36.70	3.945	
3	36.80	5.3083	
4	34.50	5.9675	TRT interactions P-values
5	34.70	6.1833	1+2 0.0012
			1+3 0.0800
			1+4 0.0142
			1+5 0.0115
			2+3 0.9668
			2+4 0.3625
			2+5 0.4047
			3+4 0.3413
			3+5 0.3845
			4+5 0.9337

[a] TRT = treatment: TRT1 - 44080 mg/l SO_4; TRT2 - 0 mg/l SO_4; TRT3 - 1000 mg/l SO_4; TRT4 - 2000 mg/l SO_4; TRT5 - 3000 mg/l SO_4.

[b] significance level $P<0.05$;

[c] significance level $P<0.05$.

(d) Ratios at 10000 mg/l TDS:

Water intake was significantly affected by TRT at the $P<0.05$ level (Table 8.5). Treatment 1, with the maximum Cl:TDS ratio at 10000 mg/l TDS of 0.6, recorded a significantly higher water intake than TRT 3 and TRT 4. Treatment 2 differed significantly from TRT3, where the SO_4:TDS ratio increased from 0.227 to 0.3, having a higher water intake than TRT 3.

Table 8.5 Effect of manipulating CL:TDS:SO_4 ratios at a TDS concentration of 10000 mg/l on water intake in Friesland steers.

TRT[a]	Means (l)	Standard Deviations	Significance values[b]
			MODEL
			P 0.0105
			F 3.73
			R^2 0.2249
1	41.41	7.795	Least squares means[c]
2	35.8	8.080	
3	26.6	9.465	
4	32.8	9.9330	TRT interactions P-values
			1+2 0.1757
			1+3 0.0005
			1+4 0.0366
			2+3 0.0212
			2+4 0.4402
			3+4 0.1145

[a] TRT = treatment: TRT1 - 0 mg/l SO_4; TRT2 - 2267 mg/l SO_4; TRT3 - 3000 mg/l SO_4; TRT4 - 1000 mg/l SO_4;

[b] significance level $P<0.05$;

[c] significance level $P<0.05$.

(e) Ratios at 13000 mg/l TDS:

The treatments administered had a significant effect on water intake (Table 8.6), with the treatments 2, 3 and 4 all having significantly lower water intakes compared to TRT1, which had the lowest SO_4:TDS ratio of 0.

Table 8.6 Effect of manipulating CL:TDS:SO_4 ratios at a TDS concentration of 13000 mg/l on water intake in Friesland steers.

TRT[a]	Means (l)	Standard Deviations	Significance values[b]
			MODEL
			P 0.0261
			F 3.46
			R^2 0.2239
1	28.8	14.109	Least squares means[c]
2	18.1	8.020	
3	17.3	5.596	
4	18.3	7.101	TRT interactions P-values
			1+2 0.0143
			1+3 0.0089
			1+4 0.0160
			2+3 0.8484
			2+4 0.9619
			3+4 0.8111

[a] TRT = treatment: TRT1 - 0 mg/l SO_4; TRT2 - 2200 mg/l SO_4; TRT3 - 2200 mg/l SO_4; TRT4 - 4080 mg/l SO_4;

[b] significance level $P<0.05$;

[c] significance level $P<0.05$.

The resultant effect of the ratio manipulations on the zone of preference is shown in Figure 8.2.

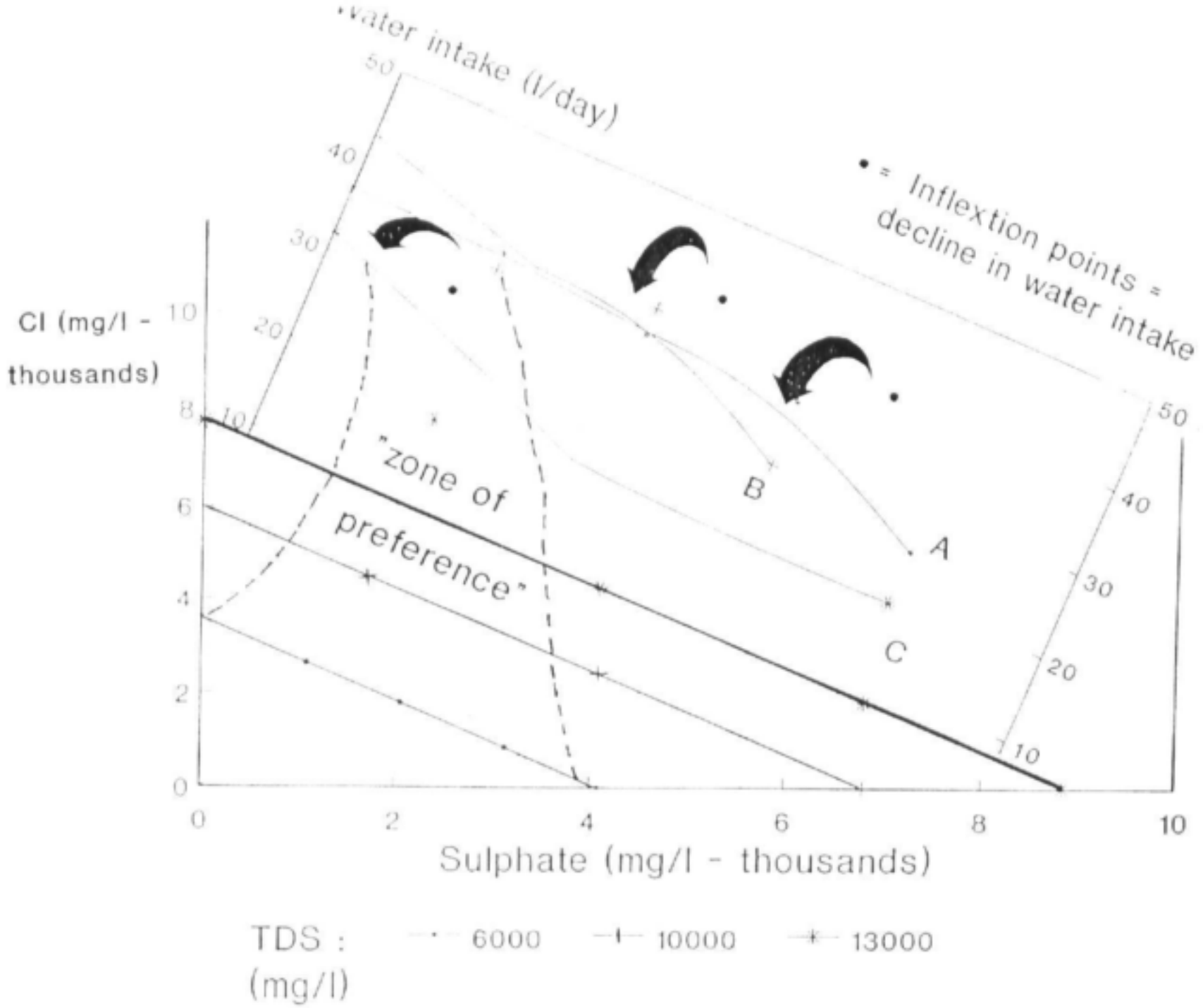


Figure 8.2 Effect of ratio change on water intake

(f) Health

During the adaptation period one of the steers tended to bloat in response to the ration, but this ceased after 2 days. There were no other incidents for the remainder of the trial.

8.4 DISCUSSION

8.4.1 Increasing SO_4 and Cl concentrations:

Sulphate was tolerated until a level of 5666 mg/l SO_4 (TDS = 8332 mg/l), where a significant decrease in water intake occurred. Prior to this level water intake increased with the increase in salinity, suggesting that the steers responded to the increase in salinity without experiencing an adverse effect on palatability. The Cl treatments resulted in an adverse effect on the palatability of the water at a Cl level of 7800 mg/l, with that level not being significantly different from a TDS level of 1666 mg/l from TRT 1, despite the increase in salinity to the 13000 mg/l TDS of TRT 4. Similarly to the SO_4 treatments, water intake increased with increased salinity until TRT 4.

8.4.2 Ratio manipulations:

For the 6000 mg/l TDS level ratio manipulations only the highest SO_4:TDS ratio of 0.68 resulted in a significant decrease in water intake. As it is unlikely under natural conditions to encounter a water source with such a high ratio it would seem that for a TDS level of 6000 mg/l a SO_4 ratio of *greater than* 0.5 is the upper limit of reasonable risk. For Cl:TDS ratios however, it would seem that the maximum ratio of 0.6 could be used safely in this TDS range.

For the 10000 mg/l TDS level this upper limit of reasonable risk would appear to occur at a lower SO_4:TDS ratio of 0.3. The highest Cl:TDS ratio of 0.6 appears to not have any adverse effect on water intake, having recorded a significantly higher intake than the SO_4:TDS ratio of 0.1. The significant increase in water intake from the SO_4:TDS ratio of 0.1 to 0.227 highlights the variation in intakes that can occur for a set TDS level depending on the ratios present.

For the 13000 mg/l TDS level the zone of preference would seem to lie at a SO_4:TDS ratios of lower than 0.153. Ratios greater than this (tested to 0.313) would therefore not be recommended for cattle watering.

8.4.3 Summary

The resulting shift of the zone of preference (Figure 8.2) indicates that the zone shifts towards the left, favouring Cl, as the TDS level increases. Otherwise stated, the ratio of SO_4:TDS at which an adverse effect on palatability can be expected decreases as the TDS level increases.

The philosophy that at different TDS levels, different ratios of Cl and SO_4 to TDS are acceptable, would therefore seem to be correct. More importantly, the argument that using single values of water quality variables as guidelines is incorrect, would also seem to be correct. This is illustrated in Figure 8.2, which attempts to indicate that as the ratios move away from the zone of preference, the following occurs:

- the risk of an adverse effect on palatability (and hence on production) occurring increases;
- the importance of a slow, small incremental adaptation period becomes greater;
- management of the water source becomes more critical;
- synergistic and antagonistic factors become more important as a method of utilizing the water source optimally (ie. nutritional status, water demand due to physiological stage and production requirements).

By using the above philosophy as a guideline, as opposed to the guideline tables that are currently in use, a water source is not declared unfit for stock watering based solely on a concentration of one or more water quality variables. Rather, the variables that exert a significant effect on palatability are used in a guideline which predicts a preferable combination of those variables, and affords the manager of the water source with the possibility of still using the source by making use of management tools, either in the form form of adapting the stock to water or manipulating factors affecting the water needs of the animal. The above mentioned method creates an awareness of potential hazards associated

with a particular water source, and therefore indicates the need to monitor the animals water, feed intake and performance.

The risk with using waters with combinations that fall far to the right of the zone of preference is not only that concerning a reduced water and therefore feed intake, but also a physiological hazard associated with SO_4. This is in the form of diarrhoea, and is also influenced by the type of SO_4 salts present in the water. The guidelines therefore need to bring this to the attention of the water user, and these factors must also be taken into account, along with the other factors such as nutritional status, water needs, production level etc., when assessing the suitability or possibility of using a water source which is potentially hazardous.

The risk with using waters with combinations are to the left of the zone of preference is primarily one of reduced water intake due to an adverse effect on palatability, as Cl exerts an adverse effect on palatability at a level far below that at which it has an adverse effect on kidney function (Chapter 4).

8.5 CONCLUSION

Knowledge of the palatability of a water source is important in classing a water source as fit for use or not fit for use, not only for predicting a decline in water intake, but also for attempting to estimate possible solutions and risks involved with highly saline waters. The use of a "zone of preference" in a guideline system using the three water quality variables with a high incidence of potentially toxic levels, namely TDS, Cl and SO_4, is a more accurate method of predicting the effect of a water source on livestock and offering possible solutions to water sources with high concentrations of these variables.

This is relevant for southern African livestock production as in many of the extensive regions the water sources available have salinity levels in excess of the levels currently recommended in the guidelines used for livestock watering. The use of the ratios of Cl:TDS:SO_4 for specific TDS levels to predict the effect of a water source on livestock watering would appear to be correct, as evidenced by the significant decreases in water intake by Friesland steers exposed to ratio manipulations in this trial. Although the results are in terms of the specific ratio values and TDS levels species specific, the rationale behind the results would appear to be valid, and therefore find application for livestock in general.

This philosophy has formed the basis for further research in developing an index system for water quality guidelines for livestock watering. The title of the follow - up project funded by the Water Research Commission is " An investigation into the quality of water for livestock production with emphasis on subterranean water and the development of a water quality guideline index system" (file nr: K5/644/0/1).

CHAPTER 9 WATER QUALITY MONITORING AND INDEX SYSTEM

9.1 WATER QUALITY MONITORING SYSTEM

Everett (1980) defined monitoring as "... a scientifically designed surveillance system of continuous measurements and observations, including evaluation procedures". Everett (1980) points out that the U.S. Environmental Protection Agency has defined four types of monitoring. These are :

a) Ambient trend monitoring

b) Source monitoring

c) Case preparation monitoring (legal procedures)

d) Research monitoring.

It is principally a) and d) that are referred to in this chapter.

9.1.1 A NATIONAL GROUND WATER QUALITY MONITORING SYSTEM

The reader is referred to the Water Research Commission report (WRC Project No. K5/482) titled " The development of a strategy to monitor groundwater quality on a national scale" by Parsons and Tredoux, 1993. A similar philosophy is stated in terms of establishing a network system and the development of an index system for water quality in that they should start at a low level and work towards the ideal, with improvements being knowledge-based. The implementation of the proposed monitoring system is vital to the development of the index system for water quality as it provides data needed to formulate guidelines for local conditions.

9.1.2 ASPECTS OF CONSIDERATION IN MONITORING

The U.S.Geological Survey's National Stream Quality Accounting Network (NSQAN) (Steele, 1974; Hawkinson, Ficke and Saindon, 1977; Briggs and Ficke, 1978; Smith, Hirsch and Slack, 1982) is an example of a broad scale frequency measurement of water quality

variables. The advantages are cost related and influenced by the following factors (Sanders *et al.*, 1987):

(a) availability of continuous measurement instrumentation,

(b) ease of sampling and preservation of samples,

(c) cost of analysis,

(d) relative importance to specified objectives,

(e) inherent seasonal variability, and

(f) field logistics.

The statistical requirements determine the second consideration and need to be identified prior to network implementations.

The obtaining of a representative sample is superimposed on the problem of insuring against chemical change. There are guidelines for the methods of sampling (Sanders *et al.*, 1987; Hem, 1970), but the sampling site variation that can occur has been illustrated in Chapter 2. Strictly speaking the sample from the borehole is necessary, and has as such first priority, but experience from the field trips made would suggest that samples from the reservoir and drinking trough (in decreasing order of priority) are also of valuable use. Should a problem be suspected with a water source then all 3 sites should be sampled, but generally the first 2 will do. This is due to the reservoir not being cleaned on a regular basis (loss of water incurred and cracking of reservoir in the sun prevent regular cleaning), but the drinking trough can (and should be) cleaned daily. The sample from the reservoir should not be taken from the top (scoop or from overflow pipe) but rather from the point of entry into the drinking trough as some water quality variables vary significantly with depth.

The design of monitoring networks and analysis of data (in this case for use by the livestock producer) both need information regarding the variance of water quality variables over time as these have been shown to be important. Seasonal variation implies that the approximate level of a water quality variable can be predicted for a given year. A conceptual illustration is given in Figure 9.1.

Mean annual water quality variables can often be considered to be random, daily, weekly and even monthly may be serially correlated.

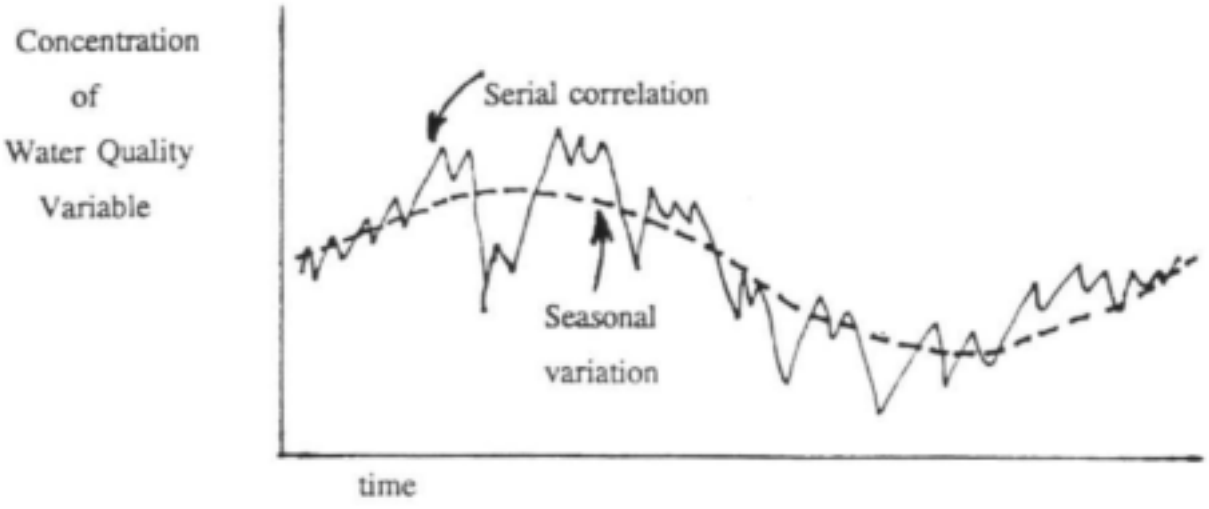


Figure 9.1 Conceptual illustration of seasonal variation and serial correlation (Sanders *et al.*, 1987).

Trend monitoring makes use of existing wells which are incorporated into the design of a regional ground water information gathering programme (Miller, 1981). The knowledge of trends can be of valuable assistance to the livestock producer in determining his sampling frequency and site, and be of assistance in his stock watering management. Trends are often discovered through retrospective evaluation of data. The trends which are determined may be in time, across space or both. An example of this is the F, TDS , SO_4 and Cl variables that have been identified as main variables of concern (Chapter 2) which was made possible by a retrospective review of data available. Trends through time were not possible due to the amount and nature of the data used, but are evident across space to a limited extent in the regions investigated. Both trend analysis (across space and time) are lacking for the period 1987 - 1990. Correct sampling sites, methods, frequency and strategies are needed. Having identified the variables of concern (Chapter 2) further knowledge of trends across time and space would be valuable.

A water quality index should provide quantitative measurements for water quality that are understood by decision making laymen (Landwehr, 1974). The weighting of the water quality variables is usually determined subjectively. An example of a water quality index is a sequence of "weighted" water quality variables

$$WQI = \Sigma^{n}_{i=1} w_i q_i$$

where WQI = water quality index

w_i = weighted factor of i^{th} water quality variable

q_i = quality level of i^{th} water quality variable

n = number of water quality variables

w_i = weighting term depends on importance of the particular water quality variable

q_i = quality level subjective measure of ranges of water quality variable from 0 - 100 (least to most desirable).

An important factor is the possible loss of trend identification when using a WQI, and here a weighted linear combination of water quality variables (although not an index) can be used to detect significant changes in water quality for a region with very short length of records (Koch, Sanders and Morel-Seytoux, 1982; Koch, Sanders and Morel-Seytoux, 1980).

9.2 WATER QUALITY INDEX SYSTEM

The scarcity in the relative abundance of water, the increase in pollution levels, intensity of livestock production systems and their expected production levels and economic returns have placed more emphasis on the essential need for good water quality as a prerequisite for livestock watering. Unfortunately, livestock producers tend to underestimate the impact of water quality on livestock even though it is significant for both intensive and extensive production systems, albeit for different reasons. Furthermore, the only guidelines available are international guidelines and local guidelines which are based largely on the former with few locally established variables.

The variation in the environment in which livestock production is practised is one of the main factors to consider. Quite often ideal or desirable water quality is not available for the majority of the time in many of the extensive regions, and therefore water quality less than

the "ideal" must be acceptable. The maximum permissible level for many variables change accordingly. In this case, the lowest or poorest quality that can be used before steps are needed to improve it must be known. In many intensive systems the producer requires water of a high quality and anything other than ideal is not acceptable as it could result in suboptimal production. Here the most beneficial combination of water quality variables must be known and the maximum permissible levels will be related to the economic goals of the specific systems. Furthermore, for each of the above mentioned extremes, changes in conditions for example, environmental or market induced changes can alter the specific variables and their values that are relevant. During drought conditions for example, the main objective may be the maintenance of the nucleus herd, and in this instance the animals need only survive and not perform on the water source. In certain intensive systems the life span of certain stock may be short enough to prevent certain variables from having an adverse effect on the animals production, whereas in many extensive regions the exposure time is longer and the cumulative effects change the maximum permissible levels of certain variables.

Water quality guidelines can also change dramatically for different livestock species mainly due to the different environments in which they exist and different tolerance levels related to the physiology of the species. Different water quality variables, variable levels and hence guidelines can also apply to the different stages of a given species, be they physiological differences or man induced differences, both of which relate to growth phases, production types and levels (intensive or extensive).

The interactions between various variables in a water source are of the utmost importance due to the synergistic and antagonistic effects they exert. So too are the interactions between the water source and the other factors that cumulatively affect the animal important. These include mainly pasture and climatic conditions usually pertaining to extensive systems and production systems such as intensive swine/poultry/feedlot producers where the environment is controlled to a certain extent.

These factors give rise to different conditions of use for water quality guidelines. Some of the guidelines can be flexible and others not, for example, toxic heavy metals have a fairly

constant effect and are not as flexible as salinity. This gives rise to the formation of guidelines envisaged which must incorporate all these facets in the form of an index system.

9.2.1 STRATEGY FOR DEVELOPING GUIDELINES

The approach to be taken centres around (A) identifying the main types of livestock production and the water source common to them (subterranean or surface),(B) identifying the main variables of relevance in these water sources and their impact and (C) developing guideline levels for the respective livestock production systems in the format of an index system.

From a realistic point of view the guideline systems cannot accommodate all the various facets of relevance and a compromise is needed in this respect. So too must the system be practical in that while incorporating all the facets possible it must be user friendly for the farmer.

An index system lends itself to these problems as the format envisaged will enable it to be flexible, with more or less factors being incorporated, depending on the production system and the producer's financial and practical ability to manage a water source. This implies that the system must offer various recommendations for the same water source and use depending on the aspects mentioned.

9.2.1.1 IDENTIFICATION OF LIVESTOCK PRODUCTION SYSTEMS AND WATER SOURCES COMMON TO THEM.

This phase can be carried out by data assimilation of the main types of livestock production systems and the water sources used by them.

(i) Database

(ii) Extensive and intensive systems

(iii) Specific systems

(a) Dairying

(b) Beef - extensive/intensive feedlotting

(c) Sheep - extensive/semi-intensive wool & meat

(d) Poultry - intensive layers,broilers, geese, turkeys

(e) Pigs - intensive & semi- intensive

(f) Goats - extensive mohair & meat, semi intensive milch goats

(g) Horses - extensive ranching, intensive stabling, breeding & working/sporting horses

(h) Aquaculture

(i) Ostriches - extensive and semi-intensive

(j) Intensive small livestock

(k) Game - monogastrics & ruminants

(l) Communal water sources for human & livestock use - surface and subterranean

9.2.1.2 SELECTION OF WATER QUALITY VARIABLES

The importance of this step is made clear when reviewing international standards where it is seen that different variables are listed as being relevant due mainly to the different environments of the respective countries. This occurs within a country as well and so variables of relevance can differ for different environs within RSA.

This phase thus rests on the successful completion of the previous phase, with the main obstacle being the lack of accurate and recent data on water samples from the water sources. Due to the changes in water quality for a given source over time and the increase in pollution

levels, a water quality monitoring system is vital. A recommendation would be made to farmers to participate in this programme by monitoring the water quality on their respective farms at a frequency based on the variance within the water source. Water samples should preferably be taken from more points than just the borehole for example, such as the reservoir and drinking trough.

Once a clear view is obtained of the variables present, they must then be evaluated to identify which variables are of primary importance. This is done mainly through the use of already existing standards to assess the potential risk of toxicity for the variables occurring. This goes hand in hand with the livestock production system using the water source, as different variables are of relevance for different systems and species. The problem area here is the lack of specific guidelines for these variable factors and the obvious danger is that as local standards are not as developed and international guidelines must therefore be used, the possibility of incorrectly assessing the importance of specific variables based on these guidelines is present.

The completion of this phase would result in specific variables being singled out as having the most significant impact on the livestock production system for the water source in question.

9.2.1.3 VARIABLE VALUES

Internationally established values can be of use as broad guidelines, but as is evident when reviewing the guidelines, different countries have different values for the same variables. This is so as the variables pertaining to certain variables differ between countries. Due to the fact that variables of little significance have proportionately less research directed at them, many variable values are based on assumptions, and extrapolations are made across environments and species that are often inaccurate. A further problem is that the present guidelines seldom, if ever, take interactions between variables into account which result in inaccurate assessments of the effect certain levels have on livestock.

The above mentioned points lead towards the preference of an index system as opposed to the present tables which give single values for variables. The determination of the values must therefore relate to the effect of the specific variables on the relevant livestock, production system and physiological stages concerned. As mentioned some will be more flexible than others and so vary over a wider range.

The starting point to arrive at these values is to work with the variables that have been identified as posing the largest concern in their specific situations.

Literature surveys are then conducted on these variables allowing the planning of biological trials to take place which will investigate mainly the following:

(i) Testing the impact of variables at the levels commonly occurring and at the upper range of these levels on growth to slaughter weight. This is the first region that the index system must assess the variables impact on, with one of the main considerations the presence or absence of possibility of the variables accumulating in the soft tissue and so posing a hazardous health problem to consumers. This is an important phase as often levels used as guidelines are conservative as the variables effect may have detrimental effects over a period of years -in this case since the stock are slaughtered the maximum permissible level changes.

(ii) Long term trials on testing the effects on reproduction, health and performance. Impact determination can be made via in-depth physiological trials over a shorter period that would elucidate possible problem areas that might occur in later stages and thus require longer term research, are the variables cumulative or not etc.

(iii) The affect of variables on the palatability of the water. This is one of the principle determinations the index system must make, as often the problems experienced with low water quality is that the adverse effects are indirectly caused by a decreased water intake which leads to decreased feed intake and

thus performance as opposed to a direct adverse physiological effect.

(iv) The levels at which variable effects are ionic as opposed to osmotic. This is more concentrated on the less flexible ranges in which variables tend to have toxic effects irrespective of the other variables present in the water or environment.

(v) The calculation of how the above mentioned levels change with variation in mainly the following:

(a) Temperature, humidity, evaporative effects

(b) Energy, protein and mineral ratios of rations and pastures (different rations and seasons)

(c) Interactions between variables (synergistic and antagonistic interactions)

This is of paramount importance as the acceptable levels of variables depend on the effects of the variables on the animal which depend on the animal's consumption of the variables. Anything that changes the water intake or absorption and excretion of that variable will change the acceptable level, and it is on this point that the index system concentrates.

(vi) Manipulation of water quality with management procedures (principally adaptation and watering techniques) and administration of alleviatory chemicals.

9.2.2 FORMAT OF GUIDELINES

The envisaged format will cater for mainly two scenarios. The first deals with variables that possess toxic properties which have an adverse effect via certain physiological pathways. The second scenario concerns those variables (or combinations of variables) which result in an indirect adverse effect on production due to mainly their effect on the palatability of the water. The first scenario deals thus primarily with ionic effects of specific variables as opposed to the second which deals with palatability effects of combinations of variables. In both cases the water intake is of major importance, but in the first it is the correlation between water intake and the ingestion of the ion that is relevant, whereas in the second case the correlation between water intake, feed intake and thus performance is of relevance (Forbes, 1968).

The following sections illustrate briefly the envisaged water quality index system (fluoride is used as example). The index is still in its formative stages and is thus not complete.

9.2.2.1 POTENTIALLY TOXIC VARIABLES

Water intake is first predicted by the use of tables. Table 9.1 is a brief example of the factors that are included. Table 9.2 gives a guideline that could be used in estimating water intake. Once water intake is known an estimate of the ingestion of the variable in question can be made. This is needed as it is not the level of the variable in the water source that is the critical factor, but the water intake and thus ingestion of the ion with the synergistic and antagonistic factors that influence the uptake and metabolism of the ion that are the critical factors.

The following illustrates this first step.

WI = x(Litres/day) - (by use of Table 9.1)

y = (WI)(mg/l of WQV)

where y = estimate of ingestion of variable in mg or g per day

WQV = water quality variable.

The estimate obtained (y) will then be used in a Table 9.3 to assess the suitability of the water for a defined use. The combination of the variables that fall into this category will then be incorporated into a regression that also takes into account the categories of variables that affect the palatability of the water to yield a value **WQI** that will be used to assess the water sample for fitness for use.

Table 9.1 Factors of importance in estimating water intake.

Temperature and Humidity cold wet . . . . . . hot dry	Estimated Water Intake (litres/d)
Synergists and Antagonists Presence of Ca, Mg Salinity - low < 1000 ppm medium 2-10000 ppm high 10 - 20000 ppm > 20000 ppm Protein % of ration = Fat % of ration = Energy level of ration = LE ME HE Other (liveweight)	Estimated effect synergistic /antagonistic
Production level (may be included in Table 5.2 - depends on variable)	Estimated Water Intake (litres/d)

Table 9.2 Average daily water intakes for livestock (Ensminger *et al.*, 1990).

Species Age (weeks)	Body Weight (kg)	Condition	Water Intake (l/d)
Cattle 4	51	growing	0.3-5.7
8	69	growing	5-7
12	93	growing	8-9
16	119	growing	11-13
20	148	growing	15-17
26	189	growing	7-23
60	354	growing	23-30
84	464	pregnant	30-38
1-2 yrs	454-545	fattening	30-34
2-8 yrs	545-726	lactating	38-95
2-8 yrs	545-726	grazing	17-34
Sheep	9	growing	1.9
	23	growing	1.5
	68-91	grazing	1.9-5.7
	68-91	grazing (salty)	8
	68-91	hay+grain	0.4-3
	68-91	good pasture	<1.9
Swine	14	growing	1-4
	27-36	growing	2.6-4.5
	36-57	growing	4-7.5
	91-180	maintn.	5.7-13
	91-180	pregnant	15-19
	91-180	lactating	19-25

Table 9.2 (cont).

Chickens 1-3		growing	2.7-5
3-6		growing	5.7-11.3
6-10		growing	11.3-15.2
9-13		growing	15-19
mature		non-laying	19
mature		laying	19-28
mature (90°F)		laying	34
Horses mature			45

Table 9.3 Fitness for use - Variable = F.

Animal : sheep (specify breed)

Production System: (intensive or extensive)

y (WI.mg/lF) mg/d	Slaughter	Reproduction	Genetic material 40 - 45 kg
*	s	s	s
*	s	s	s (after sw)
*	s	s (after sw)	q
	s	q (after sw)	ns
	q	ns	ns
	ns	ns	ns

s = suitable

q = questionable

ns = not suitable

Fitness for use is defined as s = suitable, q = questionable and ns = not suitable. The questionable use implies that the water could be used for a limited period of time depending on the variable concerned. The not suitable for use implies that watering the specified

livestock with this water source should be avoided at all times.

The various categories in Table 9.3 will depend on the animal, production system and variable concerned. The categories must be motivated and important points need to be presented for the specific variable. An example of this is given with reference to F :

(i) Slaughter weight :

In this case there is no danger of F being deposited in the soft tissue and thus being a health hazard for human consumption at the levels listed. If growth to the target weight is not adversely affected by a level then the level is suitable, even if F is accumulating in the hard tissue at this level and might result in adverse effects over a longer period of time.

(ii) Production system:

The higher levels will be different for the various types of production systems. An intensive system has a shorter time period needed to reach the target weight and thus a higher level would be suitable as opposed to an extensive system where the exposure to the ion would be longer. In the intensive system the increased water intake would not result in a significantly higher ingestion of the ion as a higher percentage of moisture is obtained from the feed and the sheep tend to drink with a fuller rumen with a less acidic rumen environment, all of which is conducive to less F absorption from the digestive system. The increased protein % of the ration that is usually found in intensive systems (particularly feedlots) also has a synergistic effect. There are many other dietary effects that may cumulatively have a significant effect and these are referred to in Chapter 3.

(iii) Genetic material:

Younger animals in the growth phase are more susceptible to F than older animals whose skeleton and dentition has already been formed, and they have thus conservative levels applied to them. There are specific variables that have noted affects on the reproductive processes and the nature of these will determine the fitness for use.

(iv) Reproductive stages :

Certain variables cross the placental wall and are deposited in the foetus during formation. If the amount deposited increases significantly with increased levels in the drinking water then fitness for use levels will alter accordingly. The same philosophy applies to the levels in milk and the absorption of the variable by the suckling young. In the case of F it appears that neither placental transfer nor milk F concentration are significantly altered in sheep with increasing levels in the drinking water (for sheep and cattle - the opposite is true for poultry with respect to egg production).

During certain stages of pregnancy and lactation animals may become more tolerant or susceptible to specific variables. In the case of dairy cattle the large fluctuation in water intake between the dry period and lactation result in significantly different amounts of F ingestion and thus exposure of the cow to F. A large variation in F ingestion is more deleterious to animals than a consistent intake and thus the fitness for use will alter accordingly.

Not all variables will be as complex as F in this case as some are less flexible and more toxic. For these primarily dietary and environmental effects will be of importance as it is water intake and thus the ingestion of the ion which is the most important factor in setting a suitable level for fitness for use. Table 9.3 would thus in certain instances have less categories or only one category. The production system and breed may not need specification for some variables.

9.2.2.2 VARIABLES THAT INFLUENCE PALATABILITY

Here a mathematical equation could be used to estimate the water intake based on the various factors present that affect palatability of the water for the specific animal.

The equation would be of the following structure :

Animal = (specify animal)

$(y) = q + a(x_1) + b(x_2) + K_i$

where,

(y) = value that will be used in Table 9.4 to predict water intake

a + b = weighted coefficients for variables $x_1 + x_2$ (slope)

$x_1 + x_2$ = ratios of variables that are the primary factors that affect palatability, such as Cl/TDS and SO_4/TDS

K_i = single factors such as temperature, production level

q = regression constant (intercept).

Table 9.4 Estimation of water intake.

(y) value	Estimated Water Intake	
0	x litres/d	s
*	*	s
*	*	s
*	*	s
10	*	ns

A (y) value of 0 - 5 would be required for TDS < 10000mg/l. This is to satisfy the physiological need for water intake (osmotic effects) for a specific TDS level. For TDS levels of 10 000mg/l to 20 000mg/l the (y) value required would be between 5 - 9. Above a TDS level of 20 000mg/l the (y) value required would be 10. This is unsuitable (salinity too high) and water samples and equations that yielded (y) values of 10 would be classed as not suitable for use. This cut-off point could vary for species, environment and production systems, and each scenario would have a cut-off point determined for it.

This estimated water intake will then be used to assess the fitness for use by using tables such as Table 9.2 to judge whether or not the estimated intake is sufficient for the animals requirements.

9.2.2.3 COMBINATION OF TOXIC AND PALATABILITY EFFECTS

Both effects must then be combined to form an index that assesses the overall suitability of the water source. The following method is suggested :

$$WQI = ax + bz \ldots$$

where,

WQI = water quality index value (0 - 100)

a + b = weighted coefficients for independent variables x + z

x = value for toxic effects

z = value for palatability effects

For the toxic effects, x could be calculated as follows:

x = 1-10

where,

1-5 = ns

6-7 = q

8-10 = s (demarcations for ns, q and s are variable specific)

For palatability effects, z a similar method could be used:

z = 1 -10

where,

1-3 = adverse palatability effects

4-6 = possible adverse palatability effects

7-10 = no adverse palatability effects.

The less toxic the sample, the higher the x - value. The less unpalatable the sample, the higher the y - value.

The end value for the WQI will be between 0 - 100. For specific species, environments and production systems certain WQI values will be suitable, others questionable, and others not

suitable.

This system does not only produce the one value that assesses the suitability of the water source (WQI), but also produces fitness for use values at various stages throughout the calculation of the toxic variables and palatability variables used to calculate the WQI value. This enables the livestock producer to see the nature and source of the adverse effects that may be expected were stock allowed to drink from the water source. Using the Tables 9.1 - 9.4 in conjunction with Table 9.5 the producer can apply management skills to change the acceptability of the water source should the WQI value be ns.

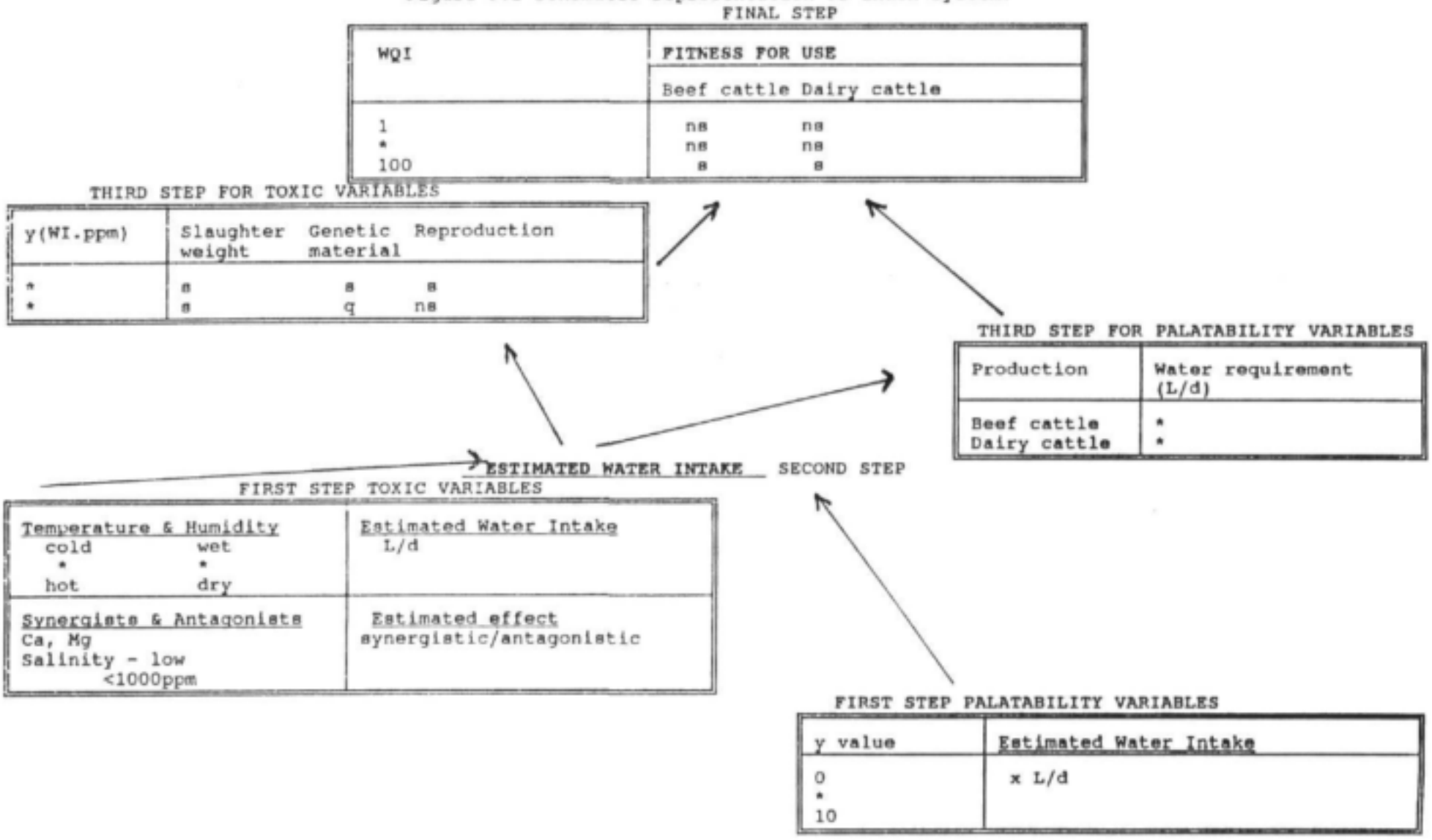
This allows for the manipulation of poor water (within limits) and thus greater usage of the water source. By making allowances for manipulations (watering management, licks, alleviators, adaptation, and diet manipulation are a few examples) after a WQI value is known, the index system can be used by those who do not have the ability to the necessary management practices, while those who do can apply the necessary measures within their capabilities.

Figure 9.2 shows a schematic presentation of the entire index system.

Table 9.5 Water Quality Index System (combined).

WQI	FITNESS FOR USE	
	Beef Cattle	Dairy Cattle
1	ns	ns
"	ns	ns
"	ns	ns
"	ns	ns
"	ns	ns
"	q	ns
"	q	ns
"	s	ns
50	s	ns
"	s	q
"	s	s
"	s	s
"	s	s
"	s	s
100	s	s

Figure 9.2 Schematic representation of index system.



CHAPTER 10 FUTURE RESEARCH PROPOSALS AND GENERAL CONCLUSIONS

10.1 FURTHER RESEARCH PROPOSALS

10.1.1 Water quality monitoring system

A water quality monitoring system is recommended that is on-going to verify the status of F, TDS, Cl, SO_4, NO_3 and other variables that are potentially hazardous to livestock. This recommendation is not limited to the designated region referred to in this study, but should incorporate a country wide data base. Seasonal fluctuations and the true concentrations that livestock are exposed to in the drinking trough are two of the most important points that the water quality monitoring system should provide. The design of the water quality monitoring system will depend on factors mentioned in Chapter 9.

10.1.2 Research regarding fluoride

Mechanistic trials are needed to investigate :

(i) NaCl - F interaction: The mechanism for the significant negative correlation found in the second trial is not clear. A decrease in absorption of F from the digestive tract is apparently not responsible (Chapter 3). Storage of F by bone tissue is the primary mechanism for removal of F from body fluids. Kidney excretion is secondary as F has a high affinity for bone. After the apatite crystal and fluorapatite has formed F cannot be removed without resorption of the crystal. If Ca or Vit D are limiting factors then the increase in osteoblast activity caused by F may result in poorly mineralized matrix. Sheep excrete 50-90% of dietary F in the urine. This proportion decreases as the F level increases. The amount of F entering the nephrons/unit time is dependant on the glomerular filtration rate and upon plasma [F] (Whitford and Pashley, 1979). The renal clearance of F exceeds that of Cl and is less than creatine (Rosenberg, Oikkonen, Neuvonen and Leander, (1981); Jarnberg, Ekstrand and Ehrnebo, 1983). Diuresis experiments have yielded conflicting results, but a general conclusion is that F clearance is positively correlated with urine flow.

The urinary pH may be important in determining the proportion of F that is resorbed in the tubules (pH < 5.6 resorption of F is > 95%, pH > 5.6 yields an increase in F excretion). The pH dependence involves the formation of increased amounts of HF as the internal environment becomes more acidic, therefore alkaline urine yields a relatively higher F ion concentration compared with HF, thus the F ions remain within the tubules and their urinary clearance is thus high.

If the above is true, implications of mild chronic acid/base disturbances would have an effect on the retention of F (mild alkalosis would be less susceptible to F than mild acidosis). Aldosterone is suppressed by salt, and Na diuresis is induced by a low rate of resorption of Na in the proximal tubule and osmotic retention of water in the collecting duct by Na. An increase in the salinity of the drinking water can result in Na diuresis. The absorption of Na in the proximal tubule is reduced with an increase in salinity so that extra Na in the distal tubule retains water and produces diuresis that removes both salt and water. High potassium loading of the tubules and increased urea infiltration leads to an increased water turnover. When sheep ingest saltbush, which is high in K and Mg (alkali-ash) the K is removed as $KHCO_3$ or K_2CO_3 and the pH of the urine increases to levels between 6 and 9.3. As mentioned sheep and goats may respond to Na receptors in the CSF as a mechanism for diluting the plasma and increasing the Na concentration in the urine.

The mechanism described above could offer an explanation for the observation in Trial 2 with the saline treatments and bone [F], and further investigation into the validity of this mechanism would not only elucidate the alleviatory effects seen in the regions visited of fluorosis, but would also open up an interesting field of study concerning the intakes of various grasses and bushes in the NW Cape region and the influence thereof on urinary pH and renal F clearance.

(ii) Alleviators: The use of boron as an alleviator of fluorosis and the ability to alter the effects of high F levels in the drinking water by use of diet manipulations and watering techniques are areas that need further investigation.

(iii) Species differences: The response of other livestock types to high levels of F for growth to market weight should be investigated (primarily for index system categories).

10.1.3 TDS, Cl, SO_4 and NO_3

(i) A trial based on the results of the second trial investigating the response to unpalatable salts with set ratios and different TDS levels with larger intervals of concentration changes is needed.

(ii) The ability of primarily flavouring agents and a higher energy level to alter the response found in point (i) above should be investigated.

(iii) The effect of NO_3 in the drinking water on digestibility of certain forages, and research regarding the adaptation of ruminal microflora to increasing NO_2 concentrations in the rumen.

10.1.4 Extensive region trials

The findings of the trials mentioned above need to be verified under field conditions. This will enable the formulation of the index system to be such that it is relevant to field conditions and will lend itself to use by the livestock producer.

10.2 GENERAL CONCLUSIONS

- Guideline levels for water quality variables and the specific variables considered to be relevant differ between countries and emphasize the need for each country to have own relevant variables and levels of acceptability.

- The need and importance of ground water as a source of water is increasing.

- The water quality variables F, TDS, Cl, SO_4 and NO_3 were identified as the variables of main concern for livestock watering based on their incidence of potential toxicity in the designated regions (NW & NE Transvaal and NW Cape) and southern African region. Research emphasis regarding livestock watering is placed on them.

- The importance of nitrate and nitrite in ground water appears to increasing as worldwide trends seem to indicate rising ground water levels of nitrates.

- The present system of assessing the suitability of water for livestock appears to be inadequate, largely as a result of its lack in ability to account for species differences, livestock production system differences, synergistic and antagonistic factors, and environmental influences.

- A F ingestion of approximately 96 mg/ day/sheep (25 kg live weight) and approximately 122 mg/ day/ sheep (42 kg live weight) over 107 day exposure period to market weight did not adversely affect the growth or health of SAMM wethers.

- The variables Cl and SO_4 affected the palatability of water negatively (decrease in water intake for SO_4 and water and feed intake for Cl)) at ratios of 0.57 - 0.6 for SO_4:TDS and 0.4-0.6 for Cl:TDS. Sulphate appeared to have an adverse affect on the palatability of the water at lower levels than Cl. The adverse affects on palatability did not have significant affects on live weight or growth to market weight, or health of SAMM wethers (TDS levels of 12000-16000 mg/l for SO_4 treatment and 20000 mg/l for Cl treatment).

- Broilers tolerated F in the drinking water (20 mg/l) over a 49 day growth period without any adverse effect on production or health (average ingestion of 18.4 mg/l).

- Layers tolerated F in the drinking water (20 mg/l) over a 72 week laying period without any adverse effect on growth or production (results for production up to week 35 analysed). Carcass weight tended to increase with increasing F dosage.

- Taste, and not kidney function, appears to be the major factor in determining the suitability of saline water for livestock.

- Fluoride in the drinking water at 15 mg/l in the second trial yielded the same results as F in the drinking water (0-20 mg/l) in the first trial.

- A strong significant negative correlation was found between increase in salinity of the drinking water and bone F concentration (metacarpal bone).

- Bonsmara steers tolerated F in the drinking water (20 mg/l) without any adverse effect on growth or health (ingestion of approximately 350 mg/ day/ steer initially, to approximately 600 mg/ day/ steer over final growth stage) over a 130 day exposure period.

- A TDS concentration of 3000 - 6000 mg/l appeared to improve the final carcass weight of steers exposed to F in the drinking water at 20 mg/l.

- Varying ratios of Cl:TDS:SO_4 with constant and increasing levels of TDS were found to have significant effects on the palatability of water for Friesland steers. The results suggest that a "zone of preference" can be calculated to predict the animals response to certain Cl and SO_4 salts, and to thus determine the adaptation period needed and effect on production.

- The need for a water quality monitoring system that continually monitors ground water quality so that reliable, recent data that can be used for statistical purposes can

be obtained which is representative of the variable levels which livestock are exposed to is emphasized as this information is lacking and necessary to formulate an index system for the different livestock production systems and environments.

- An index system that caters for toxic and palatability water quality variables and the different livestock and production systems is envisaged that will adequately assess the suitability of water for livestock production.

- Further information is still needed (geohydrological and physiological mechanistic research concerning the water quality variables) to formulate the water quality index system.

- Research needs and relevant factors have been identified for water quality variables and these entail primarily toxic variables that influence the health and physiology of the animal, and palatability variables that influence the water intake of a water source, interactions between these variables, species and environmental factors.

- Although the findings in this investigation relate to ground water a similar philosophy applies to surface water, the factors being more complex due to the increased role of microorganisms and the larger fluctuations on a daily basis in water quality variable levels.

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CHAPTER 11 APPENDIX

In this chapter histopathologic reports, water analyses and various statistical analyses are presented.

trial 1

Mr J Meyer
Faculty of Agriculture
University of Pretoria

Universiteit van Pretoria
Privaatsak X04 0110 Onderstepoort RSA Telekx 3-22723 SA
Teleg PUNIV Tel (012) 554101 Faks (012) 5460290

Fakulteit Veeartsenykunde
Department of Pathology

HISTOPATHOLOGY REPORT

Your reference: -
Our reference: S168.92
Date submitted: 27 January 1992

Owner: departmental
Address: as above

Species: sheep
Breed: -
Sex: -
Age: -
Colour: -
Name/ ID: -

Specimen(s): kidneys (A to E) in formalin.

RESULT: None of the kidneys submitted show any histological abnormalities.

Comment: -

Yours sincerely

Dr M.C. Williams MMedVet(Path)
Senior Lecturer: Department of Pathology
18 February 1992

Key words: sheep, kidneys, normal.

trial 1

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEII
Privaatsak X79
PRETORIA
0001

21/06/91

WATER ONTLEDINGSVERSLAG NO: W 78-90/91

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		194.8
Fluoried (19)	0.01	0.18	Natrium (23)	1.62	37.3
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.20	7.7
Nitraat (62)	0.03	1.9	Kalsium (20)	1.25	25.0
Chloried (35.5)	0.74	26.3	Magnesium(12.16)	0.85	10.3
Sulfaat (48)	0.80	38.4	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	2.10	128.1			
Totaal, Subtotaal	3.68	194.8	Totale	3.92	275.1
			Min		64.1 *
			Totale Opgeloste Stowwe		211.1
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	2.10	105
Na Bikarbonaat(84)	0.00	0	Perm Hardheid(50)	0.00	0
Alkaliteit (50)	2.10	105			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.66	8.00	1.58	34.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/Sak X79, Pretoria

VERW:

DATUM: 91/06/20

KWITANSIE NO:

NAMENS: T Meyer

W.N.N.R. WATER SOURCE 2

Sender No: No 1

R 39.00 Comp 1.

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiek Bottel

LAB NOMMER: 78-90/91

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

trial1

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEI:
Privaatsak X79
PRETORIA
0001

21/06/91

WATER ONTLEDINGSVERSLAG NO: W 79-90/91

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		201.1
Fluoried (19)	0.01	0.18	Natrium (23)	1.68	38.7
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.19	7.5
Nitraat (62)	0.03	1.9	Kalsium (20)	1.25	25.0
Chloried (35.5)	0.83	29.5	Magnesium(12.16)	0.85	10.3
Sulfaat (48)	0.80	38.4	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	2.15	131.2			
Totaal, Subtotaal	3.82	201.1	Totale	3.97	282.5
			Min		65.6 *
			Totale Opgeloste Stowwe		216.9
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	2.10	105
Na Bikarbonaat(84)	0.05	4	Perm Hardheid(50)	0.00	0
Alkaliteit (50)	2.15	108			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.60	7.99	1.64	35.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/Sak X79, Pretoria

VERW:

DATUM: 91/06/20

KWITANSIE NO:

NAMENS: S. meyer

W.N.N.R. WATER SOURCE 1

Sender No: No 2

R 39.00 Group 1

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiek Bottel

LAB NOMMER: 79-90/91

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

trial 1

trt–2

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

~~VERW No: W2/5~~

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEIIN
Privaatsak X79
PRETORIA
0001

14/08/91

WATER ONTLEDINGSVERSLAG NO: W 195-90/91

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		173.0
Fluoried (19)	0.32	6.15	Natrium (23)	1.90	43.8
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.17	6.5
Nitraat (62)	0.02	1.2	Kalsium (20)	1.04	20.8
Chloried (35.5)	0.54	19.2	Magnesium(12.16)	0.86	10.5
Sulfaat (48)	0.51	24.5	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	2.00	122.0			
Totaal, Subtotaal	3.39	173.0	Totale	3.97	254.6
			Min		61.0 *
			Totale Opgeloste Stowwe		193.6
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.90	95
Na Bikarbonaat(84)	0.10	8	Perm Hardheid(50)	0.00	0
Alkaliteit (50)	2.00	100			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.81	8.10	1.95	31.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B

P/Sak X79, Pretoria

NAMENS: J. Meyer

U. Pretoria

VERW:

DATUM: 2/8/91

Sender No: No 2.1

KWITANSIE NO:

R 39.00

Group 2.
Replicate 1.

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiese Houer

LAB NOMMER: 195-90/91

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

trial 1

trt—3

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEIIN
Privaatsak X79
PRETORIA
0001

14/08/91

WATER ONTLEDINGSVERSLAG NO: W 196-90/91

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		170.0
Fluoried (19)	0.54	10.18	Natrium (23)	2.05	47.2
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.14	5.3
Nitraat (62)	0.02	1.2	Kalsium (20)	1.09	21.8
Chloried (35.5)	0.48	17.0	Magnesium(12.16)	0.66	8.0
Sulfaat (48)	0.47	22.6	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	1.95	119.0			
Totaal, Subtotaal	3.46	170.0	Totale	3.94	252.4
			Min		59.5 *
			Totale Opgeloste Stowwe		192.9
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.75	88
Na Bikarbonaat(84)	0.20	17	Perm Hardheid(50)	0.00	0
Alkaliteit (50)	1.95	98			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.89	8.09	2.19	32.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/Sak X79, Pretoria

NAMENS: J.Meyer

U. Pretoria

VERW:

DATUM: 2/8/91

Sender No: No 3.1

KWITANSIE NO:

R 39.00

Comp 3.
Replicate 1

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiese Houer

LAB NOMMER: 196-90/91

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF:...

trial 1

trt—4

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEIIN
Privaatsak X79
PRETORIA
0001

14/08/91

WATER ONTLEDINGSVERSLAG NO: W 197-90/91

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		178.5
Fluoried (19)	0.76	14.51	Natrium (23)	2.27	52.2
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.13	5.3
Nitraat (62)	0.02	1.2	Kalsium (20)	1.04	20.8
Chloried (35.5)	0.50	17.8	Magnesium(12.16)	0.66	8.0
Sulfaat (48)	0.48	23.0	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	2.00	122.0			
Totaal, Subtotaal	3.76	178.5	Totale	4.10	264.8
			Min		61.0 *
			Totale Opgeloste Stowwe		203.8
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.70	85
Na Bikarbonaat(84)	0.30	25	Perm Hardheid(50)	0.00	0
Alkaliteit (50)	2.00	100			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.84	8.10	2.46	34.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/Sak X79, Pretoria

VERW:

DATUM: 2/8/91

KWITANSIE NO:

NAMENS: J.Meyer

U. Pretoria

Sender No: No 4.1

R 39.00

Group 4
Replicate 1

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiese Houer

LAB NOMMER: 197-90/91

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

trial 1

water source

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEII
Privaatsak X79
PRETORIA
0001

13/09/1991

WATER ONTLEDINGSVERSLAG NO: W 226- 91/92

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		146.4
Fluoried (19)	0.00	0.00	Natrium (23)	1.01	23.1
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.14	5.5
Nitraat (62)	0.11	6.8	Kalsium (20)	1.10	22.0
Chloried (35.5)	0.48	17.0	Magnesium(12.16)	0.70	8.5
Sulfaat (48)	0.52	25.0	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	1.60	97.6			
Totaal, Subtotaal	2.71	146.4	Totale	2.95	205.5
			Min		48.8 *
			Totale Opgeloste Stowwe		156.7
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.60	80
Na Bikarbonaat(84)	0.00	0	Perm Hardheid(50)	0.20	10
Alkaliteit (50)	1.60	80			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.59	8.16	1.06	29.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/sak X79, Pretoria

VERW:

DATUM: 91/09/10

KWITANSIE NO:

NAMENS: J. Meyer

Universiteit Pretoria

Sender No: No 1

R39.00

Group 1

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiek Houer

LAB NOMMER: 226- 91/92

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

trial 1

trt — 2

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEIING
Privaatsak X79
PRETORIA
0001

13/09/1991

WATER ONTLEDINGSVERSLAG NO: W 227- 91/92

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		175.9
Fluoried (19)	0.34	6.49	Natrium (23)	1.64	37.7
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.19	7.4
Nitraat (62)	0.07	4.3	Kalsium (20)	1.10	22.0
Chloried (35.5)	0.62	22.0	Magnesium(12.16)	0.75	9.1
Sulfaat (48)	0.63	30.2	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	1.85	112.9			
Totaal, Subtotaal	3.51	175.9	Totale	3.68	252.1
			Min		56.4 *
			Totale Opgeloste Stowwe		195.7
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.85	93
Na Bikarbonaat(84)	0.00	0	Perm Hardheid(50)	0.00	0
Alkaliteit (50)	1.85	93			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.04	8.11	1.70	36.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

NAMENS: J. Meyer

P/sak X79, Pretoria

Universiteit Pretoria

VERW:

DATUM: 91/09/10

Sender No: No 2

KWITANSIE NO:

R39.00

Group 2.

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiek Houer

LAB NOMMER: 227- 91/92

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

trial 1

trt-3

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEIING
Privaatsak X79
PRETORIA
0001

13/09/1991

WATER ONTLEDINGSVERSLAG NO: W 228- 91/92

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		185.7
Fluoried (19)	0.61	11.50	Natrium (23)	1.85	42.6
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.17	6.5
Nitraat (62)	0.10	6.2	Kalsium (20)	1.15	23.0
Chloried (35.5)	0.53	18.8	Magnesium(12.16)	0.75	9.1
Sulfaat (48)	0.63	30.2	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	1.95	119.0			
Totaal, Subtotaal	3.82	185.7	Totale	3.92	267.0
			Min		59.5 *
			Totale Opgeloste Stowwe		207.5
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.90	95
Na Bikarbonaat(84)	0.05	4	Perm Hardheid(50)	0.00	0
Alkaliteit (50)	1.95	98			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.34	8.07	1.90	37.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/sak X79, Pretoria

VERW:

DATUM: 91/09/10

KWITANSIE NO:

NAMENS: J. Meyer

Universiteit Pretoria

Sender No: No 3

R39.00

Group 3

BESONDERHEDE VAN WATER MONSTER

KENMERK VAN HOUER: Plastiek Houer

LAB NOMMER: 228- 91/92

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+H2'+...

trial 1

trt — 3

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEIING
Privaatsak X79
PRETORIA
0001

27/01/1992

WATER ONTLEDINGSVERSLAG NO: W 341- 91/92

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		229.8
Fluoried (19)	0.67	12.67	Natrium (23)	1.46	33.5
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.25	9.7
Nitraat (62)	0.13	8.1	Kalsium (20)	1.35	27.0
Chloried (35.5)	1.31	46.5	Magnesium(12.16)	1.00	12.2
Sulfaat (48)	1.10	52.8	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	1.80	109.8			
Totaal, Subtotaal	5.01	229.8	Totale	4.05	312.2
			Min		54.9 *
			Totale Opgeloste Stowwe		257.3
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.80	90
Na Bikarbonaat(84)	0.00	0	Perm Hardheid(50)	0.55	28
Alkaliteit (50)	1.80	90			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.46	8.04	1.34	50.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/sak X79, Pretoria

VERW:

DATUM: 92/01/15

KWITANSIE NO:

NAMENS: J.A. Meyer

Universiteit Pretoria

Sender No: 23 e

R39.00

(Group 3 – Replicate 2)
– evaporation effects
midday
Sample

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiese Bottel

LAB NOMMER: 341- 91/92

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

trial 1
trt—4

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEII
Privaatsak X79
PRETORIA
0001

27/01/1992

WATER ONTLEDINGSVERSLAG NO: W 338- 91/92

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		187.3
Fluoried (19)	0.84	15.98	Natrium (23)	1.35	31.0
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.20	8.0
Nitraat (62)	0.12	7.4	Kalsium (20)	1.05	21.0
Chloried (35.5)	0.93	33.0	Magnesium(12.16)	0.95	11.6
Sulfaat (48)	0.82	39.4	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	1.50	91.5			
Totaal, Subtotaal	4.21	187.3	Totale	3.55	258.9
			Min		45.8 *
			Totale Opgeloste Stowwe		213.1
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.50	75
Na Bikarbonaat(84)	0.00	0	Perm Hardheid(50)	0.50	25
Alkaliteit (50)	1.50	75			

pH	pHS	NAV	Electriese Geleidingsvermoe
8.06	8.22	1.35	42.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/sak X79, Pretoria

VERW:

DATUM: 92/01/15

KWITANSIE NO:

NAMENS: J.A. Meyer

Universiteit Pretoria

Sender No: 4*

R39.00

Group 4
middem sample.

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiese Bottel

LAB NOMMER: 338- 91/92

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

trial 1
trt—5

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEI
Privaatsak X79
PRETORIA
0001

27/01/1992

WATER ONTLEDINGSVERSLAG NO: W 339- 91/92

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		202.2
Fluoried (19)	1.27	24.05	Natrium (23)	1.42	32.7
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.21	8.1
Nitraat (62)	0.12	7.4	Kalsium (20)	1.10	22.0
Chloried (35.5)	0.95	33.7	Magnesium(12.16)	1.00	12.2
Sulfaat (48)	0.82	39.4	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	1.60	97.6			
Totaal, Subtotaal	4.76	202.2	Totale	3.73	277.2
			Min		48.8 *
			Totale Opgeloste Stowwe		228.4
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.60	80
Na Bikarbonaat(84)	0.00	0	Perm Hardheid(50)	0.50	25
Alkaliteit (50)	1.60	80			

pH	pHS	NAV	Electriese Geleidingsvermoe
8.33	8.18	1.39	45.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/sak X79, Pretoria

VERW:

DATUM: 92/01/15

KWITANSIE NO:

NAMENS: J.A. Meyer

Universiteit Pretoria

Sender No: 5*

R39.00

Group 5 – evaporative effects – midday sample

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiese Bottel

LAB NOMMER: 339- 91/92

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

t rial 1
trt — 4

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEIIN
Privaatsak X79
PRETORIA
0001

27/01/1992

WATER ONTLEDINGSVERSLAG NO: W 336- 91/92

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		219.6
Fluoried (19)	0.97	18.38	Natrium (23)	1.43	32.8
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.23	9.0
Nitraat (62)	0.20	12.4	Kalsium (20)	1.20	24.0
Chloried (35.5)	1.10	39.1	Magnesium(12.16)	1.10	13.4
Sulfaat (48)	0.96	46.1	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	1.70	103.7			
Totaal, Subtotaal	4.93	219.6	Totale	3.96	298.8
			Min		51.9 *
			Totale Opgeloste Stowwe		246.9
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.70	85
Na Bikarbonaat(84)	0.00	0	Perm Hardheid(50)	0.60	30
Alkaliteit (50)	1.70	85			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.37	8.12	1.33	48.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/sak X79, Pretoria

VERW:

DATUM: 92/01/15

KWITANSIE NO:

NAMENS: J.A. Meyer

Universiteit Pretoria

Sender No: 2.4 e

R39.00

Group 4 Replicate 2
Evaporation effects - middag sample

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiese Bottel

LAB NOMMER: 336- 91/92

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HP+...

trial 1
trt — 5

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

VERW No: W2/5

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEIIN
Privaatsak X79
PRETORIA
0001

13/09/1991

WATER ONTLEDINGSVERSLAG NO: W 230- 91/92

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		211.0
Fluoried (19)	1.35	25.58	Natrium (23)	2.53	58.1
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.16	6.3
Nitraat (62)	0.09	5.6	Kalsium (20)	1.15	23.0
Chloried (35.5)	0.62	22.0	Magnesium(12.16)	0.80	9.7
Sulfaat (48)	0.62	29.8	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	2.10	128.1			
Totaal, Subtotaal	4.78	211.0	Totale	4.64	308.2
			Min		64.1 *
			Totale Opgeloste Stowwe		244.2
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	1.95	98
Na Bikarbonaat(84)	0.15	13	Perm Hardheid(50)	0.00	0
Alkaliteit (50)	2.10	105			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.32	8.04	2.56	42.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

P/sak X79, Pretoria

NAMENS: J. Meyer

Universiteit Pretoria

VERW:

DATUM: 91/09/10

Sender No: No 5

KWITANSIE NO:

R39.00

Group 5 - Evaporative effects
- midday sample

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiek Houer

LAB NOMMER: 230- 91/92

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

RATION FOR SAMM WETHERS FOR TRIAL 2 (tested chemical equivalent for protein = 14.6 (balanced to 14.66))

Table 3.5. MODERATELY HIGH ENERGY DIET (25kg wethers)

FEEDSTUFF	INCLUSION RATE	ME (MJ/kg)	PROT. (g/kg)	Ca (g/kg)	P (g/kg)	CF (g/kg)	PRICE R
Maize meal	31.84%	12.93	103	0.2	2.0	23	360.00
Kalori 3000	7.96%	11.4	40	8.9	0.8	8	625.50
Tef	42.52%	7.5	60	1.1	1.0	329	200.00
Cottonseed	15.02%	11.73	467	2.5	11.5	168	720.00
Urea	0.52%		2880				550.00
Feed lime	0.60%			380.0			335.00
$NaHCO_3$	0.50%						1874.00
DiCaP	0.50%			260.0	180.0		885.20
Salt (NaCl)	0.54%						80.00
TOTAL	100.00%	10.18	146.6	5.2	3.7	173	R376.26

trial 2

water source

REPUBLIEK VAN S.A
ADMINISTRASIE VOLKSRAAD
DEPARTEMENT VAN LANDBOU-ONTWIKKELING

~~VERW NO: W2/5~~

KANTOOR

NAVORSINGSINSTITUUT VIR GROND EN BESPROEIING
Privaatsak X79
PRETORIA
0001

1991/92

WATER ONTLEDINGSVERSLAG NO: W 428 91/92

RESULTATE:	me/l	dpm		me/l	dpm
			Subtotaal		200.4
Fluoried (19)	0.00	0.00	Natrium (23)	0.56	12.8
Nitriet (46)	0.00	0.00	Kalium (39.1)	0.01	0.5
Nitraat (62)	0.29	18.0	Kalsium (20)	1.40	28.0
Chloried (35.5)	0.72	25.6	Magnesium(12.16)	1.55	18.8
Sulfaat (48)	0.09	4.3	Yster (28)	0.00	0.0
Karbonaat (30)	0.00	0.0			
Bikarbonaat(61)	2.50	152.5			
Totaal, Subtotaal	3.60	200.4	Totale	3.52	260.5
			Min		76.3 *
			Totale Opgeloste Stowwe		184.2
Na Karbonaat(53)	0.00	0	Tyd Hardheid (50)	2.50	125
Na Bikarbonaat(84)	0.00	0	Perm Hardheid(50)	0.45	23
Alkaliteit (50)	2.50	125			

pH	pHS	NAV	Electriese Geleidingsvermoe
7.57	7.87	0.46	31.0 mS/m by 25 grade C

VAN: Direkteur N.I.G.B.

NAMENS: J.A. Meyer

P/Sak X79, Pretoria

Universiteit Pretoria

VERW:

DATUM: 92/03/03

Sender No: 1 M

KWITANSIE NO:

R 39.00 WATER SOURCE

B E S O N D E R H E D E V A N W A T E R M O N S T E R

KENMERK VAN HOUER: Plastiese Bottel

LAB NOMMER:W 428 91/92

* Korreksie vir enige vlugtige stowwe, HCO3/2 of.. HCl+HNO3+HF+...

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE :MWI TRT 1-4 - FOR TRIAL 2

DUNCAN GROUPING	MEAN	N	TRT
A	5.6440	70	3
A	5.4832	70	1
B	4.9143	70	4
C	4.0466	70	2

(Means with the same letter are not significantly different)

TRT 1 = Control
2 = Fluoride
3 = Chloride
4 = Sulphate

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: MWI - CONTROL GROUP DAYS (1-70)

DUNCAN GROUPING			MEAN	N	WEEK
	A		7.0514	7	8
	B		6.2343	7	7
C	B		5.6114	7	9
C	B		5.5750	7	10
C	D		5.2771	7	2
C	D		5.1829	7	3
C	D		5.1686	7	4
C	D		5.1543	7	6
C	D		4.8657	7	5
	D		4.7114	7	1

(Means with the same letter are not significantly different)

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: MWI - FLUORIDE GROUP DAYS (1-70)

DUNCAN GROUPING			MEAN	N	WEEK
	A		4.5286	7	2
	A		4.4686	7	1
B	A		4.3400	7	3
B	A	C	4.2686	7	4
B	A	C	4.0743	7	6
B	A	C	3.9914	7	7
B		C	3.7771	7	5
B		C	3.7200	7	10
		C	3.6629	7	8
		C	3.6343	7	9

(Means with the same letter are not significantly different).

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: MWI - CHLORIDE DAYS (1-70)

DUNCAN GROUPING			MEAN	N	WEEK
	A		6.8857	7	8
B	A		6.1893	7	7
B	A	C	6.0429	7	2
B	A	C	6.0143	7	5
B		C	5.6686	7	4
B		C	5.3229	7	3
B		C	5.2279	7	6
		C	5.0714	7	9
		C	5.0286	7	10
		C	4.9886	7	1

(Means with the same letter are not significantly different)

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: MWI - SULPHATE GROUP DAYS (1-70)

DUNCAN GROUPING		MEAN	N	WEEK
	A	5.4914	7	2
B	A	5.0743	7	3
B	A	5.0371	7	1
B	A	5.0171	7	6
B	A	5.0000	7	7
B	A	4.8657	7	4
B		4.7629	7	10
B		4.7057	7	8
B		4.6229	7	5
B		4.5657	7	9

(Means with the same letter are not significantly different).

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: MWI - CHLORIDE GROUP DAYS (1-70)

DUNCAN GROUPING	MEAN	N	DAYS
A	6.2200	3	5
A	6.0280	5	2
A	5.3833	3	3
A	4.9900	2	4
A	4.9760	5	1
A	4.7000	2	6

(Means with the same letter are not significantly different)

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: MWI - CONTROL DAYS (1-40)

DUNCAN GROUPING		MEAN	N	DAYS
	A	5.5167	3	3
B	A	5.2680	5	2
B	A	5.0867	3	5
B	A	4.6900	5	1
B	A	4.6700	2	4
B		4.2700	2	6

(Means with the same letter are not significantly different)

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: MWI - SULPHATE GROUP - DAYS (1-70) - (BY TRT)

DUNCAN GROUPING		MEAN	N	DAYS
	A	5.763	3	3
B	A	5.453	3	7
B	A	5.380	3	5
B	A	4.898	5	1
B	A	4.814	5	2
B	A	4.325	2	8
B	A	4.025	2	4
B		3.015	2	6

(Means with the same letter are not significantly different)

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: MWI - CHLORIDE GROUP - DAYS (40-70) - (BY TRT)

DUNCAN GROUPING			MEAN	N	DAYS
	A		7.4425	4	5
B	A		6.9750	4	6
B	A	C	6.5375	4	4
B	A	C	6.4525	4	7
B	A	C	6.3075	4	3
B	A	C	6.1050	4	2
B	D	C	5.5725	4	1
	D	C	5.3100	4	8
	D	C	5.2375	4	9
	D	C	5.1440	5	10
	D		4.4380	5	11

(Means with the same letter are not significantly different)

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: MWI - SULPHATE GROUP - DAYS (1-40)

DUNCAN GROUPING	MEAN	N	DAYS
A	5.6000	3	3
A	5.4780	5	2
A	5.4633	3	5
A	5.0500	5	1
A	4.2200	2	6
A	4.1050	2	4

(Means with the same letter are not significantly different)

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: MWI - FLUORIDE FOR -(SELECTED INTERVALS)

DUNCAN GROUPING	MEAN	N	DAYS
A	4.6080	5	2
A	4.5060	5	1
A	4.2800	2	7
A	4.1533	3	3
A	3.8800	2	4
A	3.8100	3	5
A	3.4300	2	6

(Means with the same letter are not significantly different)

PEARSON CORRELATION COEFFICIENTS FOR SULPHATE GROUP - DAYS (40-70)

	TDS	CL	SO4
MWI	0.23502 0.5753	0.43690 0.2791	0.45477 0.2576

* Significance level p<0.05.

MEANS AND STANDARD DEVIATIONS FOR THYROID GLAND WEIGHT AND HOT CARCASS WEIGHT FOR TRIAL 2

LEVEL OF TRT	N	THY		HCM	
		MEAN	SD	MEAN	SC
1	5	1.55	0.16	25.04	1.89
2	4	1.47	0.54	22.45	3.49
3	5	1.45	0.23	25.08	2.22
4	5	1.20	0.32	24.56	1.48

TRIAL 2 : THYROID GLAND WEIGHT AND HOT CARCASS WEIGHT VALUES

Sheep nr	TRT	THYROID GLAND WEIGHT (g)	HOT CARCASS WEIGHT (kg)
18	1	1.470	26.0
4	1	1.700	23.0
1	1	1.756	27.4
3	1	1.424	23.2
19	1	1.403	25.6
16	2	0.960	19.4
11	2	1.139	22.2
17	2	1.629	20.8
15	2	.	.
13	2	2.169	27.4
9	3	1.276	25.6
6	3	1.464	24.6
21	3	1.712	28.0
22	3	1.664	21.8
20	3	1.176	25.4
8	4	0.944	22.6
12	4	1.226	25.0
2	4	0.985	23.8
7	4	1.106	24.8
14	4	1.745	26.6

TRT = 1 = CONTROL GROUP
2 = CHLORIDE GROUP
3 = SULPHATE GROUP
4 = FLUORIDE GROUP

TRIAL 2 : [F] IN METACARPAL BONE SAMPLES AND REAL F INTAKE

TRT	[F] in bone (ppm)	Real F intake (g/70 days)
2	787.0	4446.5
2	707.1	4446.4
2	938.7	4272.1
2	1030.9	4272.5
2	665.9	3699.3
2	638.5	3699.0
2	982.4	4883.1
2	861.8	4550.1
2	841.1	4550.5
2	802.1	7581.5
1	526.5	7581.4
1	573.3	6308.3
1	741.3	6308.4
1	586.8	5131.3
1	613.0	5131.4
1	649.9	3625.4
1	689.3	3625.3
1	688.7	5680.8
1	682.5	5680.9
1	858.1	

TRT - 1 = SALINE WATER
2 = FRESH WATER

TRIAL 2 : Means and standard deviations for Liveweights (1-9)

Liveweight	TRT	Mean	S.D.
LW1	1	32.8	1.10
	2	32.2	3.56
	3	32.8	1.30
	4	32.8	1.30
LW2	1	33.6	1.95
	2	33.6	4.51
	3	34.0	1.58
	4	34.2	2.38
LW3	1	36.0	2.55
	2	35.8	4.20
	3	36.2	1.64
	4	36.0	2.73
LW4	1	38.0	2.74
	2	37.6	5.94
	3	38.6	1.95
	4	38.8	3.49
LW5	1	39.2	2.38
	2	38.6	5.12
	3	39.8	2.16
	4	39.4	3.04
LW6	1	41.4	3.36
	2	40.4	4.83
	3	41.6	2.88
	4	41.6	2.70
LW7	1	42.4	3.65
	2	40.3	4.57
	3	42.6	2.60
	4	43.2	3.19
LW8	1	43.8	3.70
	2	41.8	4.99
	3	46.0	3.81
	4	45.4	2.88

LW9	1 2 3 4	48.0 46.0 49.2 47.8	4.06 6.78 4.27 2.28

N = 5 per TRT
TRT = 1-4 (as above)

AVERAGE FEED INTAKE FOR TRIAL 2

AVERAGE FEED INTAKE FOR TRAIL 2																				
OB	SHEEP NUMBER																			
	18	4	1	3	19	16	11	17	15	13	9	6	21	22	20	8	12	2	7	14
1	2.02	2.06	2.34	1.59	2.20	1.61	1.77	2.20	2.40	2.41	2.04	2.06	2.04	2.42	2.12	1.64	2.31	2.07	2.36	2.37
2	2.08	2.13	2.59	2.02	2.15	1.83	2.21	2.35	2.33	2.34	2.26	2.21	2.15	1.85	2.46	1.86	2.34	2.22	2.05	2.51
3	2.01	2.38	2.41	1.73	1.63	1.17	2.03	1.28	1.96	2.16	2.09	2.02	2.53	1.86	2.49	1.71	2.16	2.20	2.21	2.51
4	1.96	1.89	2.20	1.70	1.82	1.23	1.80	1.51	1.80	1.98	2.05	1.90	2.42	1.63	2.21	1.57	2.10	1.95	1.88	2.25
5	1.84	1.73	2.13	1.59	2.02	1.48	1.64	1.72	1.42	1.49	1.88	1.67	2.18	1.35	1.88	1.45	1.92	1.63	1.65	1.98
6	2.02	1.86	2.12	1.65	1.89	1.28	1.32	1.61	.	1.58	2.18	1.85	1.76	1.46	2.03	1.57	1.99	1.78	1.99	2.15
7	2.01	1.90	2.05	1.80	1.90	1.45	1.50	1.78	.	1.48	2.02	1.90	1.96	1.57	1.82	1.68	1.92	1.65	2.02	2.20
8	1.65	1.72	1.86	1.67	1.98	1.38	1.69	1.82	.	1.76	2.10	1.82	2.25	1.42	1.96	1.88	2.05	1.74	1.94	1.98
9	1.73	1.66	1.98	1.69	1.91	1.34	1.80	1.78	.	1.85	2.16	1.95	2.55	1.45	1.25	1.84	2.14	1.85	1.78	2.00
10	2.34	1.74	2.20	1.85	2.04	1.68	1.86	1.79	.	2.15	2.03	2.23	2.43	1.74	2.31	1.95	2.29	2.18	1.84	2.01

TREATMENTS:

CONTROL	CHLORIDE	SULPHATE	FLUORIDE
18	16	9	8
4	11	6	12
1	17	21	2
3	15	22	7
19	13	20	14

Trial 2 : Blood samples

CHLORIDE (MMOL/L) BLOOD SAMPLES

Sheep nr	SAMPLE 1	2	3	4	5	6
1	103	108	103	106	107	107
2	102	106	105	109	106	108
3	104	104	102	111	103	107
4	104	101	104	110	101	182
5	-	-	-	106	106	108
6	106	108	106	111	107	114
7	96	100	107	109	108	125
8	105	107	109	108	107	119
9	101	107	106	108	104	103
10	-	-	-	109	102	116
11	105	103	101	102	98	125
12	102	107	106	107	104	116
13	105	105	103	110	103	104
14	103	103	107	107	111	109
15	102	105	108	-	-	-
16	107	102	99	105	104	105
17	107	110	99	113	-	101
18	105	108	105	111	103	108
19	105	106	110	108	100	105
20	102	105	109	110	108	123
21	100	105	110	107	104	105
22	101	102	112	108	108	146

Trial 2 : Urine samples

CHLORIDE and CREATININE (MMOL/L) URINE SAMPLES

Sheep no.	SAMPLE 1		2		3	
	Cl	Creat	Cl	Creat	Cl	Creat
1	362	5..7	354	5.4	626	6.9
2	107	2.4	173	3.8	154	5.4
3	261	5.2	325	6.2	486	2.3
4	275	7.0	290	4.1	450	1.4
5	.	.	.	.	.	.
6	134	1.8	284	5.0	357	11.9
7	75	1.3	.	.	202	7.4
8	.	.	180	4.3	228	8.2
9	312	7.0	413	7.3	324	10.1
10	.	.	.	.	.	.
11	369	6.6	411	2.2	392	2.0
12	91	1.5	122	2.3	.	.
13	234	1.9	336	1.7	419	2.6
14	93	1.2	.	.	133	3.5
15	344	5.8	319	2.4	.	.
16	288	6.5	275	1.8	412	5.4
17	364	1.5	484	3.8	524	4.7
18	260	2.5	282	1.9	392	4.8
19	.	.	394	5.5	438	2.6
20	190	2.3	85	1.0	341	4.1
21	.	.	115	1.5	304	3.9
22	69	1.4	276	5.9	240	5.2

trial 2

Ref.: S1746.92 to S1754.92

Department of Pathology
Fax no. (012) 546 0047
14 September 1992

Mr James Meyer
Faculty of Agriculture
University of Pretoria

HISTOPATHOLOGY REPORT

Your reference: Sheep 3,5,7,8,11,16,19,20 & 22. Our reference: S1746.92 to S1754.92
Date submitted: 26 August 1992

Owner: Faculty of Agriculture

Species: Sheep (X 9)

Specimen(s): Livers and kidneys in formalin

RESULT: Four of the sheep showed varying grades of chlamydial pyelitis of the kidneys. This was most severe in Sheep no. 7, moderate in Sheep nos. 5 and 11 and mild in Sheep no. 22. It was absent in Sheep nos. 3, 8, 16, 19 and 20. Mild stileziasis was found in the livers of two of the experimental animals (Sheep nos. 19 and 20). The parenchymal cells and other histologic features of all the livers and kidneys were indistinguishable i.e. no other lesions were present in these organs.

Comment: The finding of chlamydial infection and stileziasis in some of the animals can be regarded as more or less incidental. The chlamydial pyelitis may have interfered, to some extent, with normal urinary tract function.

Yours sincerely

Dr M C Williams
Senior lecturer: Dept. of Pathology
14 September 1992

Cost of this investigation: R20 x 9 = R180 + 10% VAT = R308:00
Invoice No.:

Key words: sheep, experimental fluorine, chlamydia, pyelitis.

trial 2

sheep – 15
choride group

Verw.: PM625.92

Universiteit van Pretoria
Privaatsak X04 0110 Onderstepoort RSA Teleks 3 22723 SA
Teleg PUNIV Tel (012) 554101

Fakulteit Veeartsenykunde
Departement Patologie
Faksnr (012) 546 0047
10 Junie 1992

Mnr T de Bruyn
Bestuurder Kleinvee-eenheid
Universiteit van Pretoria

PATOLOGIEVERSLAG

Ons verwysing: PM625.92
Datum ontvang: 10 Junie 1992

Eienaar: Universiteit van Pretoria
Adres: Hatfield
Tel: (H) (W) 437311 uitbr 232

Diersoort: Ovine Geslag: Manlik ID/ Naam: Nr 15 Rooi oorplaatjie

Monster: Karkas

UITSLAG: Matige edeem van die brein. Matige nefrose. Erge kongestie van die milt.
Haemonchus contortus 1+; Trichuris ovis 1+

Histopatologie: Matige brein edeem veral in die hippokampus en serebellum en plek-plek in grysstof van serebrum. Erge nefrose (degenerasie en nekrose van kronkelbuisselle met hialien druppel degenerasie en proteïen druppels in die lumens) met heelwat neutrofiele interstisiëel in nierkorteks teenwoordig. Die nier medulla was erg kongestief gewees. Matige fokaal gedissemineerde nekrose van die lewer.

Kommentaar: Die brein letsels kan wees as gevolg van sout vergiftiging of tiamien (vit B1) tekort - m.a.w. ligte, akute serebrokortikale nekrose. Die oorsaak van die nier letsels is nie duidelik nie. Hulle mag a.g.v. skok wees. Die lewer letsels kan toksies van aard wees. Daar was geen teken van 'n infeksiesiekte nie.

Die uwe

M.G. Collett MMedVet(Path)
Mede Professor : Departement Patologie
17 Junie 1992

Koste van hierdie ondersoek: R35-00 + ~~10% BTW~~ = ~~R38-50~~
Faktuurnommer: ~~verwys~~. Interdepartementeel ASN 03389
Key words: Sheep, suspected cerebrocortical necrosis, nephrosis, salt poisoning?, Haemonchus contortus.

An investigation into the quality of water for animal production

NH Casey • JA Meyer • C Coetzee • WA van Niekerk

Report to the Water Research Commission
by the
Department of Animal and Wildlife Sciences
University of Pretoria

WRC Report No 301/1/96

Disclaimer

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