# STUDIES ON WATER AND SALT METABOLISM IN SHEEP AND GOATS

A thesis submitted to the University of New England for the degree of

Doctor of Philosophy

b y

**DJONI PRAWIRA RAHARDJA**

The Department of Physiology

The University of New England

ARMIDALE - NSW

AUSTRALIA

April 1995

# DECLARATION

*I certify that the substance of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree.*

*I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.*

*April, 1995*

*Djoni Prawira Rahardja*

# ACKNOWLEDGMENTS

The study presented in this thesis was carried out in the Department of Physiology, the University of New England - Armidale, Australia, under financial support from the Australian Development Assistance Bureau. My thanks to the Government of Australia for giving me this opportunity.

I would like to express my personal and very deep appreciation to my supervisors, Mr V.J. Williams and Dr. I.R Godwin, for their excellent supervision, guidance, encouragement and friendship. Their knowledge and support were of great value for me before and throughout my Ph.D candidature. I am indebted to Mr. V.J. Williams, who so meticulously oversaw the progress of my study, kept the flow of activities reasonable, patiently sought answers, made many valuable suggestions and was always available to answer my questions.

My appreciation and thanks is extended to Dr. J.F. Hecker, who also supervised this study during Dr. Godwin's absence on sabbatical leave and, his valuable criticisms, discussion and suggestion for the report in Chapter III are greatly appreciated.

I extend my thanks to Mr. Frank Ball and Mr Simon Stachiw of the Department of Biochemistry and Nutrition for their valuable and skilled technical assistance in using, handling and counting radioactive materials and in handling the experimental animals. I also give thanks to Mr. Grahame Chaffey, Mr. Jim Sheedy and Ms D. Haynes of the Department of Physiology for their help with surgery and willing assistance in numerous ways.

My most heartfelt thanks go to all staff members and postgraduate students of the Department of Physiology for the warm social relations, which helped me in various situations.

Finally, a very special thanks to my supportive and considerate wife, Veronica, whom I met in "the city for all seasons", Armidale, who had the foresight to encourage me to finish this thesis, who supported me during the days of intensive work, and who created an environment in which I could devote the many hours required for thesis preparation. To all my sincerest thank you.

Djoni Prawira Rahardja

# ABBREVIATIONS

| | |
|---|---|
| | gradient colloid osmotic pressure |
| P | gradient hydrostatic pressure |
| ADH | anti diuretic hormone |
| ANP | atrial natriuretic peptide |
| ATP | adenosine triphosphate |
| Ca,Mg-ATPase | calcium,magnesium-dependent adenosine triphosphatase |
| $Ca^{+2}$ | calcium ion |
| cGMP | cyclic guanosine 3',5' monophosphate |
| $Cl^-$ | chloride ion |
| CNS | central nervous system |
| $CO_2$ | carbon dioxide |
| CrEDTA | chromium ethylene diamine tetra acetic acid |
| CT | connecting tubule |
| DCT | distal convoluted tubule |
| ECF | extra cellular fluid |
| EMW | estimated metabolic water |
| GBM | glomerular basement membrane |
| GFR | glomerular filtration rate |
| GIT | gastro intestinal tract |
| $H^+$ | hydrogen ion |
| $H_2O$ | dihydrogen oxide (water) |

| | |
|---|---|
| $HCO_3^-$ | bicarbonate ion |
| $HPO4^{-2}$ | mono hydrogen phosphate |
| ICF | intra cellular fluid |
| ISF | interstitial fluid |
| JGA | juxta glomerular apparatus |
| $K^+$ | potassium ion |
| N | nitrogen |
| Na,K-ATPase | sodium, potassium-dependent adenosine triphosphatase |
| $Na^+$ | sodium ion |
| NaCl | sodium chloride |
| NH3 | ammonia |
| ns | non significant |
| NSW | New South Wales |
| PEG | poly ethylene glycol |
| PGE2 | prostaglandins of the E series |
| RBF | renal blood flow |
| reab | reabsorption |
| rh | relative humidity |
| secr | secretion |
| SNGFR | single nephron glomerular filtration rate |
| TAL | thick ascending limb |
| TBW | total body water |
| TBWSp | tritiated body water space |

| | |
|---|---|
| TF/P | tubular fluid to plasma ratio of a certain substance (inulin) |
| TGF | tubulo glomerular feedback |
| TkAL | thick ascending limb |
| TnAL | thin ascending limb |
| TnDL | thin descending limb |
| TOH | tritiated water |
| W | live mass of animal |
| WTOR | water turnover rate |

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURE

# LIST OF PHOTOGRAPH

# SUMMARY

**1.** Water and salt metabolism in sheep and goats have been studied in a series of experiments reported in this thesis. The experiments were designed to investigate the effects of sodium chloride (salt) loading either as a single factor or in combination with different roughage diets, or with a thermal load. The first experiment on Merino sheep and Angora goats investigated the effects of continuous intraruminal salt infusion while fresh water was offered freely. This experiment was conducted in a thermoneutral environment (≈20°C), and the animals were fed mixed roughage containing 50% oaten chaff and 50% lucerne chaff. In the remaining experiments, the salt was in drinking water. A comparative study was undertaken in a thermoneutral environment to examine the combined effects of offering salt water to Merino sheep and Angora goats fed oaten, lucerne and mixed roughage chaff. The effects of simultaneous imposition of salt and thermal loads were studied in Merino sheep and Australian feral goats.

When salt was added to the drinking water, in addition to the interference of different taste preferences between or within species, it was presumed that some amounts of ingested salt water would bypass the reticulorumen through the oesophageal groove. An experiment was conducted to clarify the extent of oesophageal groove closure in the sheep and goats.

**2.** A comparison between Merino sheep and Angora goats showed that intraruminal infusion of 100 mmol NaCl/kg$W^{0.82}$/d resulted in a greater increase of fresh water intake by the sheep compared to the goats, and hence water turnover rate (WTOR). On the other hand, salt load decreased organic matter intake of mixed roughage chaff by both species, to the same extent, although it was higher for the goats compared to the sheep when no salt was infused. Under such conditions, there were some mechanisms by which the sheep and goats coped differently with the salt load.

* The digestibility of organic matter and nitrogen in the goats were significantly higher compared to the sheep. These responses to salt load were attributed to a faster outflow rate of rumen fluid in the sheep compared to that in the goats, even though there were no species differences when no salt was infused. Rumen fluid volumes (ml/kg $W^{0.82}$) in both species increased with salt load, but the volume in the sheep was higher compared to the goats with or without salt infusion. However, as percentages of body weight or tritiated body water space (TBWSp) there were no species differences.

* TBWSp (ml/kg $W^{0.82}$) in the goats increased significantly, whereas the increase in the sheep was not significant. As percentages of body weight, TBWSp in the goats was significantly higher compared to the sheep.

* Although there were no species differences in the proportions of water loss (%WTOR) through urination, defaecation or insensible loss, faeces from the goats was significantly drier compared to that from the sheep. In both species, the proportions of water loss through urination increased with salt infusion from 40 to 60% of WTOR, whereas faecal and insensible losses decreased from 19 to 9 and 40 to 30% respectively.

* Glomerular filtration rate in both species increased with salt load. It was higher in the sheep than in the goats. Sodium concentration in the urine from the goats was higher than that from the sheep.

* The effect of salt load on urinary nitrogen loss from the sheep and goats was similar, but as a proportion of digestible nitrogen, the loss from the sheep was greater .

3. Increasing input of salt either through continuous intraruminal infusion or through drinking water increased the intake of fluid by sheep and goats, but a greater increase resulted from intraruminal salt infusion. Under a thermoneutral environment (≈ 20°C) with the same diet of mixed roughage chaff, the input of salt through infusion or drinking water was about the same (≈40-50 mmol/kg$W^{0.82}$/d); but the increased fresh water intake by the infused sheep (≈100%) was markedly greater compared to the increased intake of water containing 1.35% sodium chloride (≈40-60%). There were some consequences in

gastrointestinal fluid movements outlined below which influenced the differences in the overall water and salt metabolism.

* The greater increase in fresh water intake by the infused sheep resulted in a greater reduction in organic matter intake (≈20-30%) compared to that of the sheep drinking salt water (≈11-19%).

* Apparent organic matter digestibility of the mixed roughage chaff in the infused sheep was significantly reduced (≈11%), whereas there was no significant effect of drinking salt water. These differences were attributed to the out-flow rate of rumen fluid (% rumen fluid volume/h) which was increased in the infused sheep, whereas no significant change resulted in the sheep drinking 1.35% salt water, although rumen fluid volume (ml/$kg^{0.82}$) tended to increase with both salt presentation in sheep. In addition to less water consumption by the sheep drinking salt water, no change in outflow rate of rumen fluid resulted partly from the bypassing of the reticulorumen of some of the ingested salt water.

* Drinking 1.35% salt water increased TBWSp in addition to an increase in WTOR, whereas in the infused sheep WTOR was double that in the control condition without a significant change in TBWSp. The increase TBWSp in the sheep drinking salt water was proportionately followed by the increases in the fluid volumes of extracellular and rumen compartments. Therefore, there were no significant alteration in the proportions of TBWSp distributed into the rumen and extracellular compartments.

* In both salt presentations, glomerular filtration rate (GFR) was relatively increased by more than 30%, but urine volume from the infused sheep was doubled that from the sheep drinking salt water. This paralleled the differences in water intake. Consequently, sodium concentration in the urine from the sheep drinking salt water was higher than from the infused sheep.

**4**. In a thermoneutral environment (≈ 20°C), the Merino sheep and Angora goats responded differently to the intake of 1.35% salt water and organic matter intakes of oaten, lucerne and mixed roughage chaff. On three occasions with different roughages

offered, the intakes of salt water by the sheep were markedly greater compared to the goats, and the outflow rate of rumen fluid was significantly faster in the sheep than in the goats. Both species decreased their organic matter intakes of oaten and mixed chaff when salt water was offered. However, the sheep and the goats behaved differently when lucerne chaff was fed while offering salt water: The sheep increased their intake, whereas the goats decreased their intake significantly.

* In the sheep, there was a positive significant relationship between water intake and outflow rate of rumen fluid regardless of whether drinking fresh water or salt, or eating different roughages. The intake of salt water was markedly greater than fresh water.

* In the goats, however, this positive relationship was not significant: The effect of drinking salt water on the outflow rate varied with roughage differences in accord with the variation in the intake of salt water: Not significant with oaten chaff, increased with mixed roughage chaff, and decreased with lucerne chaff.

* In such conditions, organic matter digestibility of the three roughages in the goats tended to be decreased with drinking salt water, particularly when lucerne chaff was fed. In the sheep, however, it only occurred when oaten chaff was fed, whereas for the other two roughages it tended to increase.

**5.** In a thermoneutral environment, the proportion of water lost through urination, defaecation and insensible losses were not significantly affected by species differences, diets or between drinking fresh or salt water. They were 40-50, 15-25 and 30-35% respectively.

* A comparison between the Merino sheep and Australian feral goats when salt water was offered in a hot environment (≈ 40°C) showed that both these species have similar proportions of water loss through urination (45-50%), defaecation (5-9%) and insensible loss (45-47%), even though the intake of salt water by the sheep was markedly greater, compared to the intakes by the feral goats. Drinking fresh water by both species decreased

the proportion of water loss through urination to 29-31%, whereas the insensible loss increased to 60-62%, with no significant changes in the faecal water loss occurred.

**6.** In the sheep, increasing temperature from 20 to 40°C increased the intake of water either as fresh or salt water, but the greatest increase occurred when salt water was drunk.

* A similar proportion of decreasing organic matter intake (11-15%) resulted from increasing temperature from 20 to 40°C or drinking salt water in either temperatures. However, when salt and thermal loads were imposed simultaneously, the decrease in organic matter intake was about 25-30%. Under the same environmental temperature (40°C), drinking salt water by the Australian feral goats did not significantly affect their organic matter intake.

* At 40°C, the intake of salt water by the goats was markedly less than the intake by the sheep, although organic matter intake was about the same.

* Increasing temperature from 20 to 40°C did not significantly decrease the outflow rate of rumen fluid in the sheep drinking fresh water. However, when salt and thermal loads were imposed simultaneously, the outflow rate decreased significantly, whereas rumen fluid volume increased significantly. In addition to a possible greater proportion of ingested salt water bypassing the reticulorumen, this condition in the sheep was partly due to a decreased influx of endogenous water into the reticulorumen, because there was no significant effect of drinking salt water on the endogenous water influx in a thermoneutral environment (20°C). The outflow rate of rumen fluid in the Australian feral goats drinking salt water at 40°C responded similarly.

* Organic matter digestibility in both species increased significantly with drinking salt water. This was compensated for by the decrease in organic matter intake of the sheep.

* TBWSp in both species was increased significantly with drinking salt water at 40°C. However, the goats with less intake of 1.35% salt water had a proportionalally greater increase (24%) compared to the sheep (10%). As in a thermoneutral environment, the increased TBWSp in both species was proportionate to the increased fluid volumes in the

rumen and extracellular compartments. In addition, the proportion of TBWSp distributed into the rumen and extracellular compartments were relatively the same in both the Merino sheep and feral goats, and there were no significant effect of thermal and salt loads. A similar proportion of rumen fluid volume was also found in the sheep and Angora goats when salt was infused intraruminally with free access to fresh water. The proportion of TBWSp distributed into the rumen and extracellular fluid compartment in Merino sheep, Angora and feral goats were 18-23 and 30-32% respectively.

* TBWSp in the sheep drinking fresh water was also increased with increasing the temperature from 20 to 40°C.

**7**. The kidney was the main route for excreting the excess input of sodium. Urinary sodium excretion as a percentage of sodium intake increased with increased input of sodium.

**8**. There were two cases of diarrhoea observed during the studies. This occurred in two sheep in the first 2-3 days of drinking 1.35% salt water at 40°, when the intakes of salt were 3780 and 2730 mmol/d. However, later in the experiment these two sheep randomly consumed the same amount or more salt without any diarrhoea.