
High dietary salt during pregnancy in ewes alters the responses of offspring to an oral salt challenge

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By

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ABSTRACT

Most research to date has focused on non-pregnant sheep grazing saltbush to fill the summer/autumn feed gap in temperate regions of southern Australia. However, the summer/autumn period coincides with late pregnancy for autumn- or winter-lambing ewes, and feeding saltbush may reduce the amount and cost of supplementary feed that is required to meet the energy demands of late pregnancy. The challenge of dealing with a high-salt diet may be exacerbated during pregnancy since pregnancy is a salt-retaining physiological state, yet a high-salt intake requires an increase in mechanisms to excrete salt. The effect of high dietary salt on the developing foetus(es) has been studied in rodent models, but less so in sheep. Hence the aims of this thesis were to determine whether pregnant ewes can manage a high dietary salt content resembling that found in saltbush, and whether there are consequences to the offspring's physiological responses to ingested salt.

Merino ewes were synchronized for ovulation and artificially inseminated. To mimic the concentration of salt in animals grazing saltbush-based pastures in summer and autumn, a diet of 13% NaCl was fed from insemination through to parturition. It was found that pregnant ewes can be fed a 13% NaCl diet and manage the physiological conflict of high salt and pregnancy by decreasing their aldosterone concentrations and increasing their water consumption. There was no effect of high dietary salt on pregnancy rates, lamb birth weights, lamb survival or milk composition (fat and protein percentages).

A series of experiments were conducted to test if the high-salt intake of ewes during pregnancy was associated with a change in the dietary preference for salt and/or changes in physiological responses to ingested salt in the offspring ('S lambs' vs. control, 'C lambs'). C lambs and S lambs were exposed to short- and long-term preference testing to determine if there were differences in their voluntary selection for salt in their diet. There were no significant differences in dietary salt preference between C and S lambs.

The lambs were subjected to salt 'challenges' (oral dose of 40 g NaCl in 25% w/v solution) from 3-10 months of age and their water intake, urinary output, sodium excretion and hormone concentrations were measured over the ensuing 23 hours, and compared against counterparts dosed with an equal volume of water without salt. Following the initial salt challenge further experiments were conducted with slight alterations; water intake was manipulated immediately following the salt challenge; two consecutive salt challenges, 8 hours apart, were administered; and C and S lambs were offered salty water (1.5% NaCl) over a period of two days.

The results of these salt challenge experiments showed that C and S lambs excreted a salt load at a similar rate, but they differed in the magnitude of changes in water intake and hormone concentrations required to achieve sodium homeostasis. S lambs were able excrete sodium at the same rate as C lambs but without decreasing aldosterone concentrations to the same extent and whilst consuming 400 mL less water in the first two hours post challenge. The aldosterone results suggested a lowered responsiveness to aldosterone and the lower water consumption suggested an altered thirst threshold. The experiment in which water consumption was manipulated suggested that when the supply or access to fresh water is limited, the capacity to remove a salt load is likely to be less impaired in S lambs than C lambs; S lambs were able to excrete the salt load faster than the C lambs when the availability of drinking water was limited. From the experiment in which lambs were treated with two consecutive salt challenges, the rate of sodium excretion increased after the second dose, but there remained no difference in the rate of excretion between C and S lambs; all animals were able to excrete 95% of the administered dose of sodium within 23 hours. The final experiment in which animals were given salty water (1.5% NaCl) for a period of two days showed consistent results with the previous experiments for water consumption and aldosterone concentrations between C and S lambs. There was no difference in sodium excretion between

C and S lambs. A novel finding was a markedly lower voluntary feed intake in S lambs than C lambs. Although mechanisms for this are unknown, it may have profound effects on the productivity of the animals.

The experiments reported in this thesis provide new information of relevance to pregnant ewes grazing halophytic forages. It is apparent that they can withstand a high NaCl content typical, of a saltbush-based pasture. Further work is warranted to conclude whether high salt during pregnancy is (i) beneficial to the offspring in regards to a higher capacity to deal with excess salt under farming conditions and (ii) consistently associated with a lower voluntary feed intake of the offspring.

DECLARATION

I hereby declare that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, this thesis does not contain material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Serina Digby

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ABBREVIATIONS

µg:	Microgram
µL:	Microlitre
°C:	Degrees Celcius
ACE:	Angiotensin-converting enzyme
<i>ad libitum</i> :	Without restraint
ADF:	Acid Detergent Fibre
Ang II:	Angiotensin II
ANP:	Atrial natriuretic peptide
AQP2:	Aquaporin 2
AT ₁ :	Angiotensin II receptor 1
AT ₂ :	Angiotensin II receptor 2
AVP:	Arginine Vasopressin
BSA:	Bovine serum albumin
C:	Control lambs
cm:	Centimetres
CP:	Crude protein
cpm:	Cycles per minute
DM:	Dry Matter
DOC:	Desoxycorticosterone
EDTA:	Ethylenediamine tetraacetic acid
<i>et al</i> :	and others
g:	Grams
GPB:	Gelatin phosphate buffer
H ₂ O:	Water
HCl:	Hydrochloric acid
hrs:	Hour
IGF1:	Insulin like growth factor-1
IU:	International Units
IUGR:	Intrauterine growth retardation
kg:	Kilogram
L:	Litres
m:	Metre
M:	Molar
mg:	milligrams

mins:	Minutes
mL:	Millilitre
mm:	Millimetre
mmHg:	Millimetres of Mercury
mmol:	Millimolar
mOsm:	Milliosmole
mRNA:	Messenger RNA
n:	Number
Na ₂ HPO ₄ :	Disodium hydrogen phosphate
NaCl:	Sodium chloride (salt)
NaH ₂ PO ₄ .2H ₂ O:	Sodium dihydrogen phosphate (aqueos)
ng:	Nanograms
NRS:	Normal rabbit serum
NSB:	Non specific binding
P:	Probability
PBS:	Phosphate buffered saline
PCO ₂ :	Partial pressure of carbon dioxide
PEG:	Polyethylene Glycol
pg:	Picogram
PGI ₂ :	Prostacyclin
PMSG:	Pregnant Mare Serum Gonadotropin
PRA:	Plasma renin activity
RAS:	Renin-angiotensin system
S:	Salt lambs
SE:	Standard error
TC:	Total counts
V ₂ R:	Vasopressin 2 receptor
w/v:	Weight per volume