Effects of willow (Salix spp.) browse upon ewe reproduction and rumen microbiology under drought feeding conditions

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New Zealand

Dipti Wilhelmina Pitta
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Massey University
INSTITUTE OF VETERINARY, ANIMAL AND BIOMEDICAL SCIENCES

Institute of Veterinary, Animal and Biomedical Sciences
Private Bag 11 222,
Palmerston North,
New Zealand
T: 64 6 350 4525
F: 64 6 350 5714
www.massey.ac.nz

20 April, 2007.

CERTIFICATE OF REGULATORY COMPLIANCE

This is to certify that the research carried out in the Doctoral Thesis entitled "Effects of willow (*Salix spp.*) browse upon ewe reproduction and rumen microbiology under drought feeding conditions", in the Institute of Veterinary, Animal and Biomedical Sciences at Massey University, New Zealand and AgResearch (Grasslands), New Zealand:

a. is the original work of the candidate, except as indicated by appropriate attribution in the text and/or in the acknowledgements.

b. that the text, excluding appendices does not exceed 100,000 words;

c. all the ethical requirements applicable to this study have been complied with as required by Massey University and AgResearch that has a particular association with this study, and relevant legislation.

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Candidate: Dipti W. Pitta	Chief Supervisor: Professor Tom N. Barry
Signature: Dipti Wilhelmina Pitta	Signature: TN Barry
Date: 3/09/2007	Date: 12/9/2007.

Massey University
INSTITUTE OF VETERINARY, ANIMAL AND BIOMEDICAL SCIENCES

Institute of
Veterinary, Animal and
Biomedical Sciences
Private Bag 11 222,
Palmerston North,
New Zealand
T: 64 6 350 4525
F: 64 6 350 5714
www.massey.ac.nz

CANDIDATE'S DECLARATION

This is to certify that the research carried out for my Doctoral thesis entitled "Effects of willow (*Salix spp.*) browse upon ewe reproduction and rumen microbiology under drought feeding conditions", in the Institute of Veterinary, Animal and Biomedical Sciences at Massey University, New Zealand is my own work and that the thesis material has not been used in part of whole for any other qualification.

Candidate: Dipti W. Pitta

Signature: Dipti Wilhelmina Pitta

Date: 3/09/2007

Massey University

INSTITUTE OF VETERINARY, ANIMAL AND BIOMEDICAL SCIENCES

Institute of
Veterinary, Animal and
Biomedical Sciences
Private Bag 11 222,
Palmerston North,
New Zealand
T: 64 6 350 4525
F: 64 6 350 5714
www.massey.ac.nz

SUPERVISOR'S DECLARATION

This is to certify that the research carried out for the Doctoral thesis entitled "Effects of willow (*Salix spp.*) browse upon ewe reproduction and rumen microbiology under drought feeding conditions", was done by Dipti W. Pitta in the Institute of Veterinary, Animal and Biomedical Sciences at Massey University, New Zealand. The thesis material has not been used in part of whole for any other qualification, and I confirm that the candidate has pursued the course of study in accordance with the requirements of the Massey University regulations.

Chief Supervisor: Professor Tom N. Barry

Signature: T N Barry.

Date: 12/9/2007.

ABSTRACT

A series of grazing experiments were conducted in the summer/autumn of 2003 and 2004 at Massey University's Riverside dryland farm near Masterton in Wairarapa on the East Coast of NZ, to study the effects of grazing willow fodder blocks (6,000 stems/ha) upon the production and reproductive performance of ewes relative to ewes grazing drought pastures. Drought pastures were simulated in this study and included short drought pasture and long drought pasture. Pasture with a low pre-grazing mass of approximately 1500 kg DM/ha, a dead matter content of >50 % and a sward height of 5-7 cm was defined as short drought pasture typical of drought conditions. Long drought pasture was similar to pasture growing in the willow fodder blocks, with a pre-grazing pasture mass of >4000 kg DM/ha, a sward height of > 30cm and a dead matter content of 30–60 %. Willow fodder blocks were established on low-lying wet, marshy areas of the farm that had very low or zero productivity in the undeveloped state. Pasture development in the fodder blocks was noticed with the growth of unsown grasses and legumes, as the areas dried up following the planting of willow stakes, due to evapotranspiration from the trees. Forage in the willow fodder blocks included both trees and pasture that was grown under the trees. The nutritive value of short drought pasture was low with an ME of 8 MJ/kg DM; long drought pasture ranged between 8-10 MJ ME/kg DM; willow pasture contained 8 MJ ME/kg DM in 2003 and 10 MJ ME/kg DM in 2004. The nutritive value of edible willow tree (<5 mm diameter) was superior to drought pasture with an ME of >10 MJ/kg DM. The concentrations of the secondary compounds such as condensed tannins (CT; 30-40 g/kg DM) and phenolic glycosides (PG; 15-35 g/kg DM) were higher in willow

trees compared to their concentrations (CT; 2-3 g/kg DM) and (PG; 2-9 g/kg DM) in control drought pastures.

Experiments involving short drought pasture, long drought pasture and willow fodder blocks as treatment groups were grazed by ewes for 10 weeks in regular breaks from mid February to early May. Ewes were mated during this period and were joined together after mating and grazed on normal pasture until weaning. Live weight (LW) change and body condition score (BCS) were recorded throughout the experiments, whilst reproductive performance of ewes was measured as the number of lambs recorded at ultrasound pregnancy scanning, lambing, docking and weaning. Measurements on wool production were also recorded at weaning.

In 2003, experimental ewes grazed control drought pastures (short and long) and willow fodder blocks (restricted and full access) as treatment groups (n=100 ewes/group; Chapter 2). Ewes grazing short drought pasture had an allowance of 0.8 kg DM/ewe/d whilst ewes with restricted access had an allowance of 0.8 kg DM/ewe/d from drought pasture and 0.4 kg DM/ewe/d from willow fodder blocks. Ewes in full access treatment group had no access to pasture but were confined to willow fodder blocks at an allowance of 2.0 kg DM/ewe/d, which was the same allowance given to long drought pasture ewes. Ewes grazing short drought pasture lost weight at approximately 100g/d and recorded a low reproductive rate (90 lambs weaned/100 ewes mated) with a high proportion of single lamb births. Live weight loss was significantly reduced to 40 g/d in ewes grazing willow fodder blocks (full access) with a 20% units increase in reproductive rate due to more multiple births ($P<0.05$). Ewes grazing long drought pasture performed intermediate to ewes with full access to fodder blocks and ewes grazing short drought pasture, whilst ewes with

restricted access performed similar to ewes grazing short drought pasture. In 2004 (Chapter 3), the restricted access to willow fodder blocks treatment was eliminated from the study and the number of ewes was increased to 165 ewes per treatment group. Performance of ewes grazing short drought pasture was similar to that of ewes grazing short drought pasture in 2003, with ewes loosing live weight (40g/d) and a low reproductive rate (90 lambs weaned/100 ewes mated) whilst ewes grazing long drought pasture gained LW (54 g/d) and had a higher reproductive rate ($P<0.05$). Ewes grazing willow fodder blocks performed better than ewes grazing short drought pasture by maintaining LW and their reproductive rate was intermediate to ewes grazing short and long drought pasture.

In 2005, a short grazing trial with rumen fistulated sheep was conducted to study the effect of supplementing willow to ewes grazing drought pastures upon plasma amino acid concentrations (Chapter 4) and upon the microbiology of the rumen (Chapter 5 and 6). Grazing occurred during summer/autumn for 10 weeks with two treatment groups; control (short drought pasture; n=7) at an allowance of 0.8 kg DM/ewe/d and ewes grazing short drought pasture at 0.8 kg DM/ewe/d plus a supplement of fresh willow at 1.4 kg fresh willow/ewe/d (n=7). Blood samples for the quantification of plasma amino acids were collected at week 5 and 10, with LW and BCS measured at fortnightly intervals. Short drought pasture in this experiment had a low pasture mass (2000 kg DM/ha) and a low nutritive value (8 MJ/kg DM), whilst willow had a higher ME of 10 MJ/kg DM. Both groups of ewes lost live weight at the rate of 50 g/d. Plasma concentration of 3 methyl histidine (3-MTH; 88 vs 127 μ mole/L) at week 5 and non essential amino acids (NEAA; 1082 vs 1417 μ mole/L) at week 5 and (1155 vs 1324 μ mole/L) at week 10, were substantially lower ($P<0.05$) in

willow supplemented ewes than control ewes. It was concluded that the increased reproductive rate from willow supplementation in ewes grazing drought pasture might be partly explained by reduced body protein catabolism, besides also increasing plasma branched chain amino acids (BCAA) and essential amino acids (EAA) concentrations.

To investigate the effects of willow supplementation on rumen microbes, rumen samples were collected during the 2005 experiment with fistulated ewes over a 10 week period. The study involved the use of a molecular technique (Chapter 5), denaturing gradient gel electrophoresis (DGGE), to compare the rumen microbial populations between the control and supplemented ewes and a cultivation technique (Chapter 6) to study the effect on rumen bacteria of ewes grazing drought pastures with and with out willow supplementation. DGGE analysis of the V3 region of 16*S* ribosomal RNA genes in DNA extracted from samples of rumen contents taken fortnightly over a 10 week feeding period showed a distinct difference in banding patterns between treatment groups which progressively developed over time, showing rumen microbial adaptation to willow supplementation. However, phylogenetic analysis of the DNA sequences retrieved from the DGGE bands from willow-supplemented and control ewes did not cluster by treatment group. It was deduced that willow supplementation induced a change in rumen bacterial populations through selecting sub-populations of organisms already present in the rumen. The changes in the rumen bacterial populations is attributed to the ability of these bacteria to metabolise secondary compounds in willow such as phenolic glycosides and flavanoid monomers and their ability to resist the inhibitory effects of condensed tannins.

The cultivation study involved enumeration, isolation and purification of bacterial colonies on Complete Carbohydrate, Salicin, Xylan, Cellulose and Willow media followed by full characterisation of a representative set of pure bacterial cultures. Total bacterial counts on the above media at week 5 and week 10 were generally lower in willow-supplemented ewes compared to control ewes and the 16*S* rRNA gene sequences of the majority of isolates characterised from both Salicin and Xylan media, were most closely related to species from the *Pseudobutyrivibrio* genus. Isolates from Willow medium clustered as two distinct groups. One group (mostly isolated from control ewes) was made up of mainly of organisms not usually associated with the rumen and probably represent non-resident organisms that are passing through the rumen. The other group of bacteria were mainly retrieved from willow-supplemented ewes and were most closely related to species of the *Olsenella* genus. Compared to bacteria isolated on Salicin and Xylan media, isolates on Willow medium showed little ability to ferment various carbohydrates or trypticase (hydrolysed protein) but were able to utilise secondary compounds from willow.

It was concluded that willow fodder blocks are useful sources of supplementary fodder for mating ewes during drought situations. Both the field and microbiological studies showed adaptation to the willow supplementary diet, including the detection of *Olsenella*-like bacteria for the first time in the rumen. It is suggested that the principal purpose of the rumen investigation is the degradation of secondary compounds present in willow.

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LIST OF ABBREVIATIONS

BCAA	Branched chain amino acid
BCS	Body condition score
CA	Chlorogenic acid
CC	Complete carbohydrate
CP	Crude protein
Cr2O3	Chromium sesquioxide
CT	Condensed tannins
d	day
DGGE	Denaturing gradient gel electrophoresis
DM	Dry matter
DMI	Dry matter intake
DOMD	Digestible organic matter
DON	Deoxy Nivalenol
DSMD	Days of soil moisture deficit
EAA	Essential amino acid
ELISA	Enzyme linked immunosorbent assay
ENSO	El Nino - Southern oscillation phenomenon
EU	European union
FM	Flavanoid monomer
FV	Feeding value
GDP	Gross domestic product
GLM	Generalised linear model
ha	hectare
HCL	Hydrochloric acid
hd	Head
HPLC	High performance liquid chromatography
HT	Hydrolysable tannins
IPO	Interdecadal pacific oscillation index
IVDMD	Invitro dry matter digestibility
LHV	Lower heating value

LIG	Lignin
LW	Live weight
LWC	Live weight change
MAF	Ministry of agriculture and forestry
ME	Metabolisable energy
MTH	Methyl histidine
MW	Molecular weight
NAN	Non ammonia nitrogen
ND	Not determined
NDF	Neutral detergent fibre
NE	Net energy
NEAA	Non-essential amino acid
NH3	Ammonia
NIV	Nivalenol
NIWA	National institute for water and atmospheric research
NV	Nutritive value
NZ	New Zealand
OM	Organic matter
OMD	Organic matter digestibility
OR	Ovulation rate
P	Probability
PED	Potential evapotranspiration deficit
PEG	Poly ethylene glycol
PG	Phenolic glycoside
SAS	Statistical analysis system
SE	Standard error
SMD	Soil moisture deficit
SOI	Southern oscillation index
TLC	Thin layer chromatography
UDP	Undegradable dietary protein
VFI	Voluntary feed intake