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**Novel genetic engineering technology which increases  
leaf lipid content modifies the ensiling properties of  
perennial ryegrass**

A thesis presented in partial fulfillment of the requirements for  
the degree of

Masters

In

Agricultural science

at Massey University, Palmerston North

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**2016**

## Abstract

A novel strategy to increase the metabolisable energy (ME) yield of pastures has been the development of a genetic engineering technology which increases the leaf lipid content and biomass production of perennial ryegrass (PRG). Outdoor plot/feeding trials of genetically engineered crops are prohibited under the current New Zealand (NZ) regulatory framework. However, this high lipid PRG may become available to farmers and eventually be used to make silage, which could fulfill an important role as a high ME, inexpensive, supplementary feed for livestock. Ensiling preserves a crop's nutrients at a high moisture content and at a low pH, by microbial fermentation of plant sugars into lactic acid under anaerobic conditions.

In a preliminary investigation into the ensiling biochemistry of this high lipid PRG, glasshouse-grown materials were wilted and inoculated, and then ensiled on a miniature scale. A series of method development ensiling experiments revealed that non-transgenic PRG grown in glasshouse conditions during the NZ spring/summer was very difficult to ensile naturally, due to its low water soluble sugar to buffering capacity ratio. In order to generate well-preserved silage in the main experiment, glucose was added (post-harvest) to a non-transgenic PRG genotype (WT) and two transgenic PRG genotypes containing 'medium' and 'high' leaf lipid levels (ML and HL).

The HL plants produced 51% more dry biomass than WT during the regrowth period. Pre-ensiled HL had 31% higher fatty acid content, 70% higher nitrate content and a 17% lower water soluble sugar to crude protein ratio than WT. ML was intermediate. The glasshouse growth environment resulted in an atypical overall PRG nutritional composition. WT, ML and HL underwent a similar fermentation, and nutrients were well-preserved. The nutritional differences in the ensiled material largely reflected those in their fresh counterparts, although a longer wilt caused greater overall digestible nutrient losses in HL. In an *in vitro* rumen incubation experiment the fatty acids in HL silage exhibited less complete biohydrogenation than in fresh and ensiled WT. Experiments using a range of high lipid PRG lines grown in a range of environments will be needed to validate these results.

## Acknowledgements

I would like to thank the Plant Biotechnology group at AgResearch for their past and ongoing science, which created the motivation for this project, my academic supervisors Nick Roberts, Jennifer Burke, Allan Hardacre and Cory Matthew for their advice regarding the direction that the experimental work and writing took, Somrutai Winichayakul for providing lab training and help with data interpretation, Fliss Jackson and the Massey University Nutrition Laboratory staff for performing the majority of nutritional analyses, Hong Xue for performing additional nutritional analysis, Stephan Muetzel for providing the training required for the use of the *in vitro* rumen incubation system, Sarah Lewis for performing much of the post-*in vitro* incubation analytical work, Editha Meeking, Anne Allen and Kim Richardson for providing plants and for assistance with watering and plant maintenance, John Koolaard for statistical advice and coding, Trevor Holloway for providing a section of paddock, Zulfi Jahufer for advice on ryegrass reproduction, and Samra Arshad and Siripat Ngoennet for assistance with practical work.

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## List of abbreviations

<b>AA</b> ; acetic acid	<b>ME</b> ; metabolisable energy
<b>ACCase</b> ; acetyl-coA carboxylase	<b>MJ</b> ; Mega joules
<b>ACP</b> ; acyl carrier protein	<b>ML</b> ; medium lipid
<b>ADF</b> ; acid detergent fibre	<b>N</b> ; nitrogen
<b>a<sub>w</sub></b> ; water activity	<b>NDF</b> ; neutral detergent fibre
<b>BA</b> ; butyric acid	<b>NH<sub>3</sub></b> ; ammonia
<b>BC</b> ; buffering capacity	<b>NO<sub>3</sub><sup>-</sup></b> ; nitrate
<b>BH</b> ; biohydrogenation	<b>NPN</b> ; non-protein nitrogen
<b>cfu/g</b> ; colony forming units per gram	<b>NSC</b> ; non-structural carbohydrate
<b>CLA</b> ; conjugated linoleic acid	<b>NZ</b> ; New Zealand
<b>CO<sub>2</sub></b> ; carbon dioxide	<b>OMD</b> ; organic matter digestibility
<b>CP</b> ; crude protein	<b>PAR</b> ; photosynthetically active radiation
<b>DAC</b> ; days after cutting	<b>PC2</b> ; physical containment level 2
<b>DAG</b> ; diacylglycerol	<b>PRG</b> ; perennial ryegrass
<b>DAS</b> ; days after sowing	<b>PUFA</b> ; polyunsaturated fatty acid
<b>DGAT</b> ; diacylglycerol acyl transferase	<b>Rubisco</b> ; ribulose-1, 5-bisphosphate carboxylase
<b>DGAT1</b> ; diacylglycerol O-acyltransferase 1	<b>scVFA</b> ; short chain volatile fatty acid
<b>DM</b> ; dry matter	<b>TAG</b> ; triacylglycerol
<b>DMD</b> ; dry matter digestibility	<b>VA</b> ; vaccenic acid
<b>DOMD</b> ; dry organic matter digestibility	<b>VFA</b> ; volatile fatty acid
<b>DW</b> ; dry weight	<b>VOC</b> ; volatile organic compound
<b>ER</b> ; endoplasmic reticulum	<b>WAC</b> ; weeks after cutting
<b>FA</b> ; fatty acid	<b>WSC</b> ; water soluble carbohydrates
<b>FAME</b> ; fatty acid methyl ester	<b>WT</b> ; wild type
<b>FFA</b> ; free fatty acids	<b>16:0</b> ; palmitic acid
<b>GE</b> ; gross energy	<b>16:1</b> ; palmitoleic acid
<b>HL</b> ; high lipid	<b>18:0</b> ; stearic acid
<b>iWUE</b> ; intrinsic water use efficiency	<b>18:1</b> ; oleic acid
<b>LA</b> ; lactic acid	<b>18:2</b> ; linoleic acid
<b>LAB</b> ; lactic acid bacteria	<b>18:3</b> ; linolenic acid
<b>LD</b> ; lipid droplet	