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

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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.

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Table of contents

Abstract	ii
Acknowledgements	iii
Table of contents	iv
List of figures	vi
List of tables	vii
List of Photographs	ix
List of appendices	x
List of abbreviations	xi
CHAPTER 1. INTRODUCTION	1
CHAPTER 2. LITERATURE REVIEW	2
2.1 Principles of ensiling	2
2.2 Silage microbiology and LAB inoculants	5
2.3 Fermentation analyses	9
2.4 Moisture content and wilting	12
2.5 Factors affecting plant water-soluble carbohydrates	15
2.6 Buffering capacity and the WSC:BC ratio	20
2.7 Laboratory-scale silages	23
2.8 Expression of 'high lipid' technology in Arabidopsis and perennial ryegrass	27
2.9 Fatty acid and lipid metabolism during wilting and fermentation	33
2.10 Conclusion	39
2.11 Experimental objectives and hypothesis	40
CHAPTER 3. METHOD DEVELOPMENT	41
3.1 Layout of the experimental work	41
3.2 Small-scale ensiling	43
3.3 Addition of a commercial LAB inoculant	48
3.4 Controlled rapid wilt	51
3.5 Addition of glucose	54
3.6 Ensiling experiments 1-4 materials and methods	57
3.7 Ensiling experiments 1-4 results	67
3.8 Ensiling experiments 1-4 discussion	75
CHAPTER 4. TRANSGENIC VERSUS WILD TYPE SILAGE COMPARISON	79
4.1 Experiment 5 material and methods	79

4.2 Experiment 5 results	85
4.3 Experiment 5 discussion.....	95
CHAPTER 5. CONCLUSIONS	109
Literature cited	111
APPENDICES.....	119
<i>Appendix I Glasshouse abiotic growth environments.....</i>	<i>119</i>
<i>Appendix II Climatic data</i>	<i>121</i>
<i>Appendix III Plant material vegetative propagation and cutting history</i>	<i>123</i>
<i>Appendix IV Paddock section</i>	<i>124</i>
<i>Appendix V Biohydrogenation of fatty acids during in vitro rumen incubation</i>	<i>125</i>
<i>Appendix VI Example of automated spreadsheet for wilting calculations</i>	<i>127</i>
<i>Appendix VII Construct design, transformation and regeneration procedure.....</i>	<i>128</i>
<i>Appendix VIII Experiment 4 silage pH at intervals during the fermentation</i>	<i>129</i>

List of figures

Figure 2.1 The relationship between dry matter content (DM) and the pH required for anaerobic stability as proposed by (Leibensperger & Pitt, 1987) for two crops which possess a different water activity (a_w), for a given DM content. On account of their <u>lower</u> a_w legume silages require a smaller pH decline in order to inhibit clostridial growth.....	14
Figure 3.1 pH of glasshouse and paddock perennial ryegrass silage, wilted to 20% dry matter, with and without inoculant, at intervals during the fermentation. Values represent means \pm SD (n = 2). Time zero pH was estimated from the literature (Playne & McDonald, 1966).....	68
Figure 3.2 pH of transgenic containment glasshouse silage, wilted to 32% dry matter and inoculated, at intervals during the fermentation. Values represent means \pm SD (n = 2).....	70
Figure 3.3. pH of glasshouse and paddock perennial ryegrass silage, wilted to 40% dry matter and inoculated, at intervals during the fermentation. Values represent means \pm SD (n = 2).....	72
Figure 4.1 Dry biomass production from wild type, medium lipid and high lipid perennial ryegrass plants after 30 days regrowth. Bars represent means in grams of dry matter \pm SD (n = 18, 23, 24 for wild type, medium lipid and high lipid respectively).....	86
Figure 4.2 pH of wild type, medium lipid and high lipid perennial ryegrass silage, wilted to 37.5% dry matter and inoculated, with glucose added at 7.5% DM, at intervals during the fermentation. Values represent means \pm SD (n = 2).....	87
Figure 4.3 Changes in fatty acid content and composition in wild type, medium lipid and high lipid perennial ryegrass during wilting to 37.5% dry matter and then ensiling for 45 days. ‘Fresh’ and ‘Post-wilt’ bars represent measurements of a single subsample of the pooled and mixed plants from each genotype. ‘Silage’ bars represent means from multiple silos (n = 6).....	89
Figure 4.4 Changes in the fatty acid profile of fresh and ensiled wild type and high lipid perennial ryegrass during a 24 hour <i>in vitro</i> rumen incubation. Bars represent means \pm SD from two independent incubations and multiple silos (n=3). a) Unsaturated C18 fatty acids as a % of total fatty acids, b) Vaccenic acid as a % total fatty acids.....	91
Figure 4.5 Total gas production from fresh and ensiled wild type and high lipid perennial ryegrass during a 24 hour <i>in vitro</i> rumen incubation. Points represent means from a single incubation and multiple silos (n=3).....	92

List of tables

Table 2.1 Typical (pre-ensiled) population counts of epiphytic bacterial and fungal groups (Pahlow et al., 2003).....	5
Table 2.2 Typical range for fermentation end products in 30-35% dry matter grass silage.....	9
Table 2.3 The impact of adding glucose on the silage fermentation.....	19
Table 2.4 The impact of different wilting procedures on total fatty acid (FA) content in perennial ryegrass (<i>Lolium perrene</i>) and Timothy (<i>Phleum pratense</i>).....	34
Table 3.1 pH of double-bagged, vacuum packed silos made from 20g and 50g wilted paddock-grown perennial ryegrass, with and without commercial inoculant. Values represent means \pm SD (n = 2). Day 0 pH was estimated from the literature (Playne & McDonald, 1966).....	46
Table 3.2 Wilting accuracy achieved during Experiments 1-5.	52
Table 3.3 Average rate of LAB application by weight to perennial ryegrass in Experiment 3.....	61
Table 3.4 Fermentation end products at day 22 of the fermentation of glasshouse and paddock perennial ryegrass silage, wilted to 20% dry matter, with and without inoculant. Values represent means \pm SD (n = 2). The pH values are presented as a reference point and represent measurements from different silage packets to those shown in Figure 3.1.....	69
Table 3.5 Pre-ensiling characteristics of glasshouse-grown and paddock-grown perennial ryegrass after 30 days regrowth.....	71
Table 3.6 Fermentation end products at day 40 of the fermentation of glasshouse and paddock perennial ryegrass silage, wilted to 40% dry matter and inoculated. Values represent means \pm SD (n = 2). The pH values are presented as a reference point and represent measurements from different silage packets to those shown in Figure 3.3.....	73
Table 3.7 Pre-ensiled nutritional characteristics of perennial ryegrass grown in the containment glasshouse; wild type, medium lipid and high lipid plants after 28 days regrowth.....	74
Table 4.1 Average rates of LAB and glucose application by weight to perennial ryegrass in experiment 5.....	80
Table 4.2 Pre-ensiled nutritional properties of wild type, medium lipid and high lipid perennial ryegrass after 30 days regrowth. Values represent measurements of a single subsample of the pooled and mixed plants from each genotype.....	85

Table 4.3 Fermentation end products at day 45 of the fermentation of wild type, medium lipid and high lipid silage, wilted to 37.5% dry matter and inoculated, with glucose added at 7.5% DM. Values represent means in g/kgDM \pm SD (n = 3).....	87
Table 4.4 Nutritional composition of wild type, medium lipid and high lipid perennial ryegrass silage, and percentage <u>decrease</u> in nutritional components from the freshly harvested material. Values represent means \pm SD (n = 3 for all nutritional components with the exception of; n=2 nitrates, and n = 6 fatty acids).....	88
Table 4.5 Total scVFAs, and the molar proportion of rumen fluid acetate, propionate, butyrate, and other scVFAs from fresh and ensiled wild type and high lipid perennial ryegrass during a 24 hour <i>in vitro</i> rumen incubation. Values represent means \pm SD from two independent incubations and multiple silage packets (n=3).....	94
Table 4.6 Containment glasshouse temperature data 27/12/2015-12/01/2016 ($^{\circ}$ C).....	97

List of photographs

Photograph 2.1 A recently cut ryegrass-clover sward being field-wilted in a swath prior to ensiling on a New Zealand (Manawatu) dairy farm.....	13
Photograph 3.1 Steps for ensiling perennial ryegrass by double bagging and vacuum packing.....	44
Photograph 3.2 Preliminary silo assessment; 50g paddock-grown perennial ryegrass silage packets.....	45
Photograph 3.3 Layered, chopped and wilted perennial ryegrass during the inoculation procedure. The small atomiser bottles were used for inoculation in Experiments 2-5.	50
Photograph 3.4 Two wilting procedures. Left & center; transgenic perennial ryegrass being wilted in separate ovens within bags or plastic trays in the containment glasshouse ovens (Experiment 4). Right; non-containment glasshouse-grown perennial ryegrass being wilted in steel trays (Experiment 3).	52
Photograph 3.5 Top left; section of paddock during harvest. Top right; glasshouse plants after cutting. Bottom left; 5 x glasshouse tillers (left) and 5 x paddock tillers (right). Bottom right; 20g glasshouse-grown silos (bottom) and paddock-grown perennial ryegrass silos (top) at day 22 of fermentation.....	57
Photograph 3.6 Experiment 3 harvest. Left; paddock-grown perennial ryegrass during harvest. Right; two glasshouse-grown perennial ryegrass plants prior to harvest.....	60
Photograph 3.7 Buffering capacity measurement equipment.....	63
Photograph 3.8 Silage subsampling protocol. Left; open silage packet defrosting. Right; mixing packet contents prior to subsampling.....	65
Photograph 4.1 Left; perennial ryegrass plants at the beginning of the regrowth for experiment 5 (14/12/2015). Right; plants at the end of the regrowth, immediately before harvest (13/01/2016). Top; wild type. Center; medium lipid. Bottom; high lipid.....	81
Photograph 4.2 Perennial ryegrass silage packets at day 1 of fermentation. Left; wild type. Center; medium lipid. Right; high lipid.....	88

List of appendices

Appendix I Glasshouse abiotic growth environment.....	119
Appendix II Climatic data.....	121
Appendix III Plant material vegetative propagation and cutting history.....	123
Appendix IV Paddock section.....	124
Appendix V Biohydrogenation of fatty acids during in vitro rumen incubation.....	125
Appendix VI Example of automated spreadsheet for wilting calculations	127
Appendix VII Construct design, transformation and regeneration procedure.....	128
Appendix VIII Experiment 4 silage pH at intervals during the fermentation.....	129

List of abbreviations

AA ; acetic acid	ME ; metabolisable energy
ACCCase ; acetyl-coA carboxylase	MJ ; Mega joules
ACP ; acyl carrier protein	ML ; medium lipid
ADF ; acid detergent fibre	N ; nitrogen
a_w ; water activity	NDF ; neutral detergent fibre
BA ; butyric acid	NH₃ ; ammonia
BC ; buffering capacity	NO₃⁻ ; nitrate
BH ; biohydrogenation	NPN ; non-protein nitrogen
cfu/g ; colony forming units per gram	NSC ; non-structural carbohydrate
CLA ; conjugated linoleic acid	NZ ; New Zealand
CO₂ ; carbon dioxide	OMD ; organic matter digestibility
CP ; crude protein	PAR ; photosynthetically active radiation
DAC ; days after cutting	PC2 ; physical containment level 2
DAG ; diacylglycerol	PRG ; perennial ryegrass
DAS ; days after sowing	PUFA ; polyunsaturated fatty acid
DGAT ; diacylglycerol acyl transferase	Rubisco ; ribulose-1, 5-bisphosphate carboxylase
DGAT1 ; diacylglycerol O-acyltransferase 1	scVFA ; short chain volatile fatty acid
DM ; dry matter	TAG ; triacylglycerol
DMD ; dry matter digestibility	VA ; vaccenic acid
DOMD ; dry organic matter digestibility	VFA ; volatile fatty acid
DW ; dry weight	VOC ; volatile organic compound
ER ; endoplasmic reticulum	WAC ; weeks after cutting
FA ; fatty acid	WSC ; water soluble carbohydrates
FAME ; fatty acid methyl ester	WT ; wild type
FFA ; free fatty acids	16:0 ; palmitic acid
GE ; gross energy	16:1 ; palmitoleic acid
HL ; high lipid	18:0 ; stearic acid
iWUE ; intrinsic water use efficiency	18:1 ; oleic acid
LA ; lactic acid	18:2 ; linoleic acid
LAB ; lactic acid bacteria	18:3 ; linolenic acid
LD ; lipid droplet	