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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List of abbreviations

AA; acetic acid
ACCase; acetyl-coA carboxylase
ACP; acyl carrier protein
ADF; acid detergent fibre
a_w; water activity
BA; butyric acid
BC; buffering capacity
BH; biohydrogenation
cfu/g; colony forming units per gram
CLA; conjugated linoleic acid
CO_2; carbon dioxide
CP; crude protein
DAC; days after cutting
DAG; diacylglycerol
DAS; days after sowing
DGAT; diacylglycerol acyl transferase
DGAT1; diacylglycerol O-acyltransferase 1
DM; dry matter
DMD; dry matter digestibility
DOMD; dry organic matter digestibility
DW; dry weight
ER; endoplasmic reticulum
FA; fatty acid
FAME; fatty acid methyl ester
FFA; free fatty acids
GE; gross energy
HL; high lipid
iWUE; intrinsic water use efficiency
LA; lactic acid
LAB; lactic acid bacteria
LD; lipid droplet
ME; metabolisable energy
MJ; Mega joules
ML; medium lipid
N; nitrogen
NDF; neutral detergent fibre
NH_3; ammonia
NO_3^-; nitrate
NPN; non-protein nitrogen
NSC; non-structural carbohydrate
NZ; New Zealand
OMD; organic matter digestibility
PAR; photosynthetically active radiation
PC2; physical containment level 2
PRG; perennial ryegrass
PUFA; polyunsaturated fatty acid
Rubisco; ribulose-1, 5-bisphosphate carboxylase
scVFA; short chain volatile fatty acid
TAG; triacylglycerol
VA; vaccenic acid
VFA; volatile fatty acid
VOC; volatile organic compound
WAC; weeks after cutting
WSC; water soluble carbohydrates
WT; wild type
16:0; palmitic acid
16:1; palmitoleic acid
18:0; stearic acid
18:1; oleic acid
18:2; linoleic acid
18:3; linolenic acid