Development of novel strategies for improving protein utilisation in forage-fed ruminants

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Inefficient converters of plant biomass

- Rumen fermentation: Retains 15-30%
- Faeces: 25-30%
- Grass protein: 100%
- Urea loss: 50-55%
What is polyphenol oxidase (PPO)?

You’re probably quite familiar with it....

The same enzyme occurs in red clover

In the plant proposed role of PPO as anti-feedant

Causes complexing of proteins with quinones to reduce digestibility (insects) (conversion of plant phenolics to highly reactive quinones)

Can protein complexing occur in down boli to protect ingested plant protein?
Bolus capture and storage

Grasses
- Cocksfoot
- Timothy
- Ryegrass

Legume
- Red clover cv milvus (Wild type)
- Low PPO mutant
1. Effect of transport

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sample point</th>
<th>%N</th>
<th>%DM</th>
<th>Protein (mg/gDM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildtype</td>
<td>Field</td>
<td>23.1</td>
<td>14.5</td>
<td>107.2</td>
</tr>
<tr>
<td></td>
<td>Farm</td>
<td>15.7</td>
<td>13.2</td>
<td>94.0</td>
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<tr>
<td>Mutant</td>
<td>Field</td>
<td>22.9</td>
<td>14.3</td>
<td>102.6</td>
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<td>Farm</td>
<td>14.7</td>
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<td>106.7</td>
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</table>

\[ P = 0.827 \quad \text{and} \quad \text{sed.} = 2.7 \quad \text{and} \quad df = 9 \]

2. Comparison of boli from wild type and mutant (with removal of cow*cow effect)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sample point</th>
<th>%N</th>
<th>%DM</th>
<th>Protein (mg/gDM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildtype</td>
<td>Bolus</td>
<td>2.6</td>
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\[ P = 0.705 \quad \text{and} \quad \text{sed.} = 0.2 \quad \text{and} \quad df = 2 \]
PPO loss during cutting, transportation and down bolus creation in Red clover wild type and mutant

![Graph showing the comparison of PPO loss between wildtype and mutant Red clover during different stages of processing.](image)

- **Activated PPO**
- **Total PPO**
# Proximate analysis and protein recovery in grass species

## 1. Effect of transport

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sample point</th>
<th>%N</th>
<th>%DM</th>
<th>Protein (mg/gDM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocksfoot</td>
<td>Field</td>
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<tr>
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<td>Field</td>
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<td>Farm</td>
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\[
P = 0.167, \quad \text{sed.} = 3.1, \quad \text{df.} = 12
\]

## 2. Comparison of grass boli (with removal of cow*cow effect)

<table>
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<th>Sample point</th>
<th>%N</th>
<th>%DM</th>
<th>Protein (mg/gDM)</th>
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<tbody>
<tr>
<td>Cocksfoot</td>
<td>Bolus</td>
<td>1.5</td>
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<tr>
<td>Ryegrass</td>
<td>Bolus</td>
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<td>Bolus</td>
<td>1.4</td>
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\[
P = 0.216, \quad \text{sed.} = 0.1, \quad \text{df.} = 9
\]
Activated PPO loss during cutting, transportation and down bolus creation in grass species
Conclusions

- PPO in WT and mutant red clover is physically deactivated during transport and bolus formation

- PPO in grasses is physically deactivated during transport and bolus formation

- Lack of difference in recovered protein likely due to insufficient PPO activity in fed material
Can we minimise transport losses?

- Transport for 12 miles from field plot to farm causes loss of up to 80% of total PPO activity.

- Tested transportation at cold temperature.

- Losses in active PPO decreased from 68% (ambient) to 36% (cold).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wildtype</th>
<th>Active PPO</th>
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</thead>
<tbody>
<tr>
<td>Ambient</td>
<td>Field</td>
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<td></td>
<td>Farm</td>
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<td>Cold (4°C)</td>
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- Investigate further effects on protein recovery in boli.
Forward plans

- Qualitative protein analysis
  Although protein content the same is there a difference in protein complexing?

- Bolus formation experiments
  Minimise transport to minimise PPO activity losses
  - Grass and red clover field plots at farm site

- Mock mastication experiments
  Glass house wild type and mutant red clover

- Identify PPO degradative protease(s)
Sponsored by:

Academic expertise
Dr Alison Kingston-Smith
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Dr Michael Lee
Dr Eun Joong Kim

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Vince Theobold
Martin Langden
Naomi Ellis
Teri Davies
Dave Leemans