**Supplementary Material - *animal* Journal**

**Nitrogen and fatty acid rumen metabolism in cattle offered high or low polyphenol oxidase red clover silage.**

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**Supplementary Material (five pages)**

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**Supplementary Material S1.** Materials and Methods

***Sward establishment and maintenance***

Red clover (cv. Milvus and cv. Aa4512 (selected to be low Polyphenol oxidase; PPO); 0.7 ha each) were sown at a rate of 6.5 kg/ha on May 07, 2009 at Gogerddan, Aberystwyth, Ceredigion, UK. Perennial ryegrass (cv. AberDart; 2 ha) was sown at a rate of 35 kg/ha at the same location. Fertiliser phosphorus pentoxide 89 kg/ha and potassium oxide 89 kg/ha were applied to both clover plots on May 06 2009. Fertiliser ammonium nitrate 41 kg/ha was applied on February 26 2010 and ammonium nitrate 76 Kg/ha, phosphorus pentoxide 29 kg/ha and potassium oxide 40 kg/ha was applied March 31 2010 to the grass plots.

**Supplementary Material S2.** Discussion

***Silage quality***

As the quantity of seed available of the low polyphenol oxidase (PPO) red clover cultivar was only sufficient to sow 0.7 ha (Supplementary Materials and Methods) the decision was taken to run the two experiments using consecutive years’ silage, but consequently producing different silages in terms of their chemical composition. When ensiling big bales, the aim is to achieve a DM between 35 to 45%. In the current trial the DM of red clover (RC) silages in Experiment 1 and perennial ryegrass (PRG) in Experiment 2 achieved this target. However, the DM of PRG in Experiment 1 exceeded the optimum and the RC silages in Experiment 2 were under the optimum DM, both of which can influence quality.High DM silage can result in poor conservation due to poor compaction and reduced ability to remove oxygen from the clamp/bale. The resultant dry crop increased the need for a bacterial inoculant which resulted in well preserved silage despite the high DM content. The high DM resulted in relatively high pH with low fermentation acid concentrations. Low DM silage can increase effluent losses however the range for RC silage in Experiment 2 is inside the target of 20 – 25% to reduce effluent loss but below the target of big bale (35%) which is more associated with bale weight and number of bales produced per ha than preservation quality. The high level of water soluble carbohydrate (WSC) in PRG for both Experiments was related to the high sugar grass selected (AberDart) and the use of bacterial inoculant, which also resulted in relatively high levels of WSC in RC silage in Experiment 1 (Harrison et al., 1994; Merry et al., 2006). However, the RC silages in Experiment 2 showed low levels of WSC which may reflect a more mature plant (also shown in the higher fibre content) and the lower DM which inhibited homofermentative lactic acid fermentation resulting in a slower pH decline, greater proportion of heterofermentative acetate formation (lower pka than lactic acid) and a subsequently greater depletion of WSC.

Harrison, J. H., R. Blauwiekel, and M. R. Stokes. 1994. Fermentation and utilization of grass silage. Journal of Dairy Science 77, 3209-3235.

Merry R. J., M. R. F. Lee, D. R. Davies, R. J. Dewhurst, J. M. Moorby, N. D. Scollan, and M. K. Theodorou. 2006. Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 1. In vitro and in vivo studies of nitrogen utilization. Journal of Animal Science 84, 3049-3060.

**Table S1**. *Amino acid compositions (g/kg DM) of the dietary silages offered to steers in experiment 1*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | RC+1 | RC-1 | PRG1 | SED |
| Aspartic acid | 22.6 | 24.4 | 6.05 | 2.18 |
| Threonine | 5.28 | 5.61 | 2.81 | 0.330 |
| Serine | 5.37 | 5.74 | 2.63 | 0.375 |
| Glutamic acid | 10.4 | 11.4 | 4.55 | 0.797 |
| Proline | 10.0 | 11.8 | 4.73 | 0.784 |
| Glycine | 5.03 | 5.41 | 3.02 | 0.278 |
| Alanine | 6.89 | 7.20 | 4.39 | 0.333 |
| Valine | 7.02 | 7.52 | 3.91 | 0.420 |
| Methionine | 1.40 | 1.51 | 0.99 | 0.061 |
| Isoleucine | 5.79 | 6.13 | 3.07 | 0.360 |
| Leucine | 9.38 | 10.1 | 5.25 | 0.567 |
| Tyrosine | 3.03 | 3.40 | 1.28 | 0.254 |
| Phenylalanine | 5.83 | 6.26 | 3.18 | 0.361 |
| Histidine | 2.48 | 2.62 | 1.13 | 0.177 |
| Lysine | 7.93 | 8.37 | 4.04 | 0.511 |
| Arginine | 3.93 | 4.61 | 2.41 | 0.251 |
| Total | 112 | 122 | 53.5 | 8.00 |

1 RC+ = High Polyphenol oxidase (PPO) Red clover silage; RC- = Low PPO Red clover silage; PRG = Perennial Ryegrass silage.

**Table S2**. *Fatty acid compositions (g/kg DM) of the dietary silages offered to steers in experiment 1*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | RC+1 | RC-1 | PRG1 | SED |
| Fatty acids |  |  |  |  |
| C12:0 | 0.03 | 0.03 | 0.05 | 0.025 |
| C14:0 | 0.08 | 0.08 | 0.12 | 0.006 |
| C16:0 | 3.35 | 3.64 | 2.95 | 0.097 |
| C16:1n-7 | 0.03 | 0.02 | 0.06 | 0.013 |
| Phytanic acid | 0.04 | 0.04 | 0.04 | 0.005 |
| C18:0 | 0.49 | 0.49 | 0.33 | 0.025 |
| C18:1n-9 | 0.43 | 0.51 | 0.48 | 0.023 |
| C18:2n-6 | 3.85 | 4.18 | 2.60 | 0.095 |
| C18:3n-3 | 7.65 | 9.00 | 6.85 | 0.003 |
| C20:0 | 0.18 | 0.20 | 0.14 | 0.007 |
| BOC1 | 0.21 | 0.21 | 0.17 | 0.017 |
| Total | 17.8 | 20.1 | 14.8 | 0.63 |

1 RC+ = High Polyphenol oxidase (PPO) Red clover silage; RC- = Low PPO Red clover silage; PRG = Perennial Ryegrass silage; BOC = Branched and Odd Chain fatty acids.

**Table S3**. *Amino acid compositions (g/kg DM) of the dietary silages offered to dry dairy cows in experiment 2*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | RC+1 | RC-1 | PRG1 | SED |
| Aspartic acid | 11.5 | 12.4 | 11.7 | 0.38 |
| Threonine | 5.70 | 6.09 | 5.78 | 0.205 |
| Serine | 5.09 | 5.43 | 5.25 | 0.115 |
| Glutamic acid | 12.7 | 13.3 | 12.9 | 0.49 |
| Proline | 5.86 | 6.33 | 5.98 | 0.148 |
| Glycine | 6.46 | 6.83 | 6.53 | 0.164 |
| Alanine | 7.66 | 8.04 | 7.81 | 0.362 |
| Valine | 6.73 | 7.16 | 6.85 | 0.204 |
| Methionine | 2.30 | 2.46 | 2.36 | 0.132 |
| Isoleucine | 6.01 | 6.50 | 6.22 | 0.209 |
| Leucine | 9.07 | 9.64 | 9.29 | 0.248 |
| Tyrosine | 3.83 | 4.34 | 4.09 | 0.152 |
| Phenylalanine | 6.44 | 6.84 | 6.62 | 0.156 |
| Histidine | 2.81 | 2.94 | 2.87 | 0.059 |
| Lysine | 7.66 | 8.23 | 7.80 | 0.321 |
| Arginine | 4.64 | 5.00 | 4.84 | 0.158 |
| Total | 104 | 112 | 107 | 3.4 |

1 RC+ = High Polyphenol oxidase (PPO) Red clover silage; RC- = Low PPO Red clover silage; PRG = Perennial Ryegrass silage.