# Meta-analyses comparing the nutritional composition of perennial ryegrass (*Lolium perenne*) and plantain (*Plantago lanceolata*) pastures

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## Abstract
Studies comparing livestock responses to diets containing plantain with traditional ryegrass/clover diets suggest differences in the nutritional composition between the species might explain some of the variation in results. To explore this theory, a meta-analysis was performed to compare the nutritional composition of plantain and perennial ryegrass pastures, and define key differences between the species. Standardised effect sizes (plantain vs ryegrass pastures), expressed as correlation coefficients ($r$) with values from -1 to +1, and treatment means from 34 studies were determined using a weighted random effects model. Pastures containing plantain had lesser herbage dry matter content than ryegrass pastures across all seasons (mean 13.4 vs. 19.4%, $P<0.001$; $r<-0.65$). Structural fibre content was consistently smaller (mean 32.1 vs. 43.4% DM), but non-structural fibre content larger in plantain compared with ryegrass pastures (large effect sizes of $r>0.70$). There was no difference in total herbage nitrogen concentration (g/100 g DM; $r<0.03$). However, plantain pastures contained less soluble and degradable nitrogen compared with ryegrass pastures ($r<-0.78$). Herbage digestibility did not differ significantly between pastures ($r=-0.13$; $P=0.168$). Generally, the effect of plantain on nutritional composition was consistent across seasons. Experiments and models investigating the effects of pasture mixes on environmental nitrogen losses ought to include detailed nitrogen and carbohydrate fractions data for accurate interpretation and prediction.

**Keywords:** chemical composition, digestibility, fibre, mixed pasture, nitrogen

## Introduction
The forage herb, plantain (*Plantago lanceolata*) has potential to reduce the environmental impact of intensive livestock systems. Studies of livestock fed perennial ryegrass (*Lolium perenne*; ryegrass) diets including plantain have consistently demonstrated reduced urine nitrogen (N) concentration, a key determinant of N leaching risk (Di & Cameron 2007), at similar or improved levels of production compared to livestock fed diets without plantain (Box et al. 2017; Minneé et al. 2017; Bryant et al. 2018). It is generally accepted that N intake influences N excretion (Kebreab et al. 2001), however, in the studies cited above, N intake did not wholly explain the variation in urinary N concentration. It is therefore necessary to understand how plantain differs compositionally to ryegrass, and how consistently these differences are expressed under seasonal variation.

Further, as farmers adopt the use of plantain to reduce N leaching, methods to account for the plantain in the farm nutrient budgets are required. Direct measurement of nutrient loss is impractical and is generally replaced by simulation modelling. The models should be based on clear understanding of the mechanisms that result in reduced N leaching and use accurate inputs. While there are several models available that are applicable to pastoral systems (Cichota & Snow 2009), their predictions will depend on the parameter values used to represent the nutritional composition of ryegrass-based and plantain-based pastures. Confidence in model outputs will be improved if model inputs are based on a standardised set of parameter values drawn from as much available data as possible. To our knowledge, extensive evaluation of the nutritional composition of modern cultivars has not been conducted previously.

The objective of the study reported here was to quantify the nutritional composition of plantain pastures relative to perennial ryegrass pastures with the aim of defining key differences between the species that may explain variation in N excretion, and to provide robust data for simulation studies involving the use of plantain.

## Materials and Methods
### Data
A database of key forage quality variables was created from raw data sets obtained from DairyNZ, Massey University, Lincoln University, and published literature. The literature search was performed using the Lincoln University library in October 2018, using the search term ‘plantain’ with ‘chemical composition’, ‘agronomic evaluation’, ‘nutritive value’ or ‘nitrogen concentration’. Inclusion criteria used to refine the data sets in the data base were that the experiment compared

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plantain monoculture or mixed swards containing plantain with perennial ryegrass monoculture or traditional swards containing a mixture of perennial ryegrass and white clover (*Trifolium repens*). A total of 34 studies were used in the analyses, sixteen from raw data sets and eighteen from the literature (Appendix A). Half of the studies were conducted as agronomic evaluations of the forages, and the other half investigated the effect of forage treatment on livestock production. Only four international studies (from Japan, Sweden and Australia) met the criteria for inclusion. The studies reported chemical composition of the pastures determined by either wet chemistry techniques or estimation by calibrated near infra-red spectroscopy (NIRS).

Studies were grouped into four according to the pasture treatments that were used: (1) Ryegrass monoculture, RyMo; (2) Plantain monoculture, PlMo; (3) Ryegrass in mixed sward, RyWc; (4) Plantain in mixed sward, PlX.

**Calculations and statistical analyses**

Due to the range of techniques employed across the studies to quantify the concentration of readily fermentable carbohydrates, an estimation of non-structural carbohydrate (NSC) was made where possible using Equation 1[1]:

*NSC = 100 – (Crude protein + Neutral detergent Fibre + Ash + Lipid)*

[*Equation* 1]

To quantify the size of the effect between ryegrass and plantain pastures, the correlation coefficient $r$ was calculated as per Field (2005). Effect size was calculated for each study, year the study was conducted, and season in which the data were collected. Seasons were defined as per the DairyNZ Forage Value Index (FVI): winter, early spring, late spring, summer, and autumn (Chapman et al. 2017). For the raw data sets, a one-way analysis of variance (ANOVA; SAS Institute Inc., NC, USA) was performed to evaluate the effect of pasture treatment. The resulting F-ratio and residual degrees of freedom ($df_R$) were used to calculate $r$ according to Equation 2.

$$r = \sqrt{\frac{F(1,-)}{F(1,-) + df_R}}$$

[*Equation* 2]

For published data, unless the standard error of the mean (SEM) was reported, it was calculated using reported standard deviation (SD), standard error of the difference (SED), least significant difference (LSD) or P-values. T-value and residual *df* were calculated based on difference between ryegrass and plantain pastures, SEM, and sample size, and used to derive $r$ by Equation 3:

$$r = \sqrt{\frac{t^2}{t^2 + df}}$$

[*Equation* 3]

Where the result for plantain pastures was less than the ryegrass pastures $r$ is multiplied by -1 to obtain directional effects.

A meta analysis of the effect sizes was conducted to generate weighted effect sizes (LSM) using a random effects model (Proc Mixed, SAS/STAT 14.3) with a) intercept only or b) treatment as fixed effect, study as a random effect and $n$ as weighting variable.

Means from the raw data sets and literature data were used in a meta-analysis to generate weighted means (LSM) for the original variables using the same random effects model with pasture treatment, season, and their interaction as fixed effects, study as a random effect, and $n$ as weighting variable. Mean effect size and treatment least square means, with 95% confidence interval and associated P-value are reported.

The strength of the relationship is defined as strong when $r \geq 0.7$, moderate for $r$=0.50 – 0.69, weak for $r$=0.30 – 0.49, and very weak when $r$<0.30 (Schober et al. 2018).

## Results and Discussion

### Herbage dry matter content

Herbage dry matter (DM) content was significantly less in plantain monocultures or mixtures containing plantain than in ryegrass pastures (LSM 13.4 vs. 19.4%; P<0.01; Table 1). The size of the effect was strongly negative and consistent across all pasture comparisons ($r \geq$-0.68, P<0.001). Monocultures of plantain were on average 30% less in DM than ryegrass monocultures, and the effect was consistent across seasons (P pasture × season=0.665).

Lower urinary N concentration from livestock consuming plantain containing diets, compared to livestock consuming ryegrass diets, has been reported in several studies (Minneé et al. 2017; Box et al. 2017; Bryant et al. 2017; Bryant et al. 2018). One possible mechanism for this difference is the large water content (low DM content) of herbage containing plantain (O'Connell et al. 2016). The increased consumption of water from such a diet induces diuresis, which results in diluted urine (Atherton et al. 1968). In a recent study, it was demonstrated that cattle fed 45% of the daily DM intake in the form of plantain consumed ~ 60% more water in the feed than cattle fed diets without plantain (Minnée et al. unpublished data).

1 Equivalent to Equation 47 in Tylutki et al. (2008).

### Herbage nitrogen and nitrogen fractions

Generally, the herbage total N concentration of herbage of monocultures of plantain and ryegrass was similar (mean 3.1 g/100 g DM; $r$=0.03; P>0.05) (Table 2). Differences in pasture total N concentration appear influenced by the presence of other species, likely to be sown legumes, as the analyses showed that monoculture pastures of ryegrass or plantain had less total N than traditional ryegrass/white clover pastures or plantain in diverse swards with legumes. Legumes, such as white clover and lucerne (*Medicago sativa*) are common in diverse mixtures and typically have total N contents ~ 4.3 g/100 g DM (Martin et al. 2017), thus their presence in a sward will elevate total pasture N content.

**Table 1** Correlation coefficient (r) of the weighted effect size for effects of pasture type on herbage dry matter content, with least squares means with upper and lower confidence limits for pasture type.

| | | *r* (weighted effect) | | | | Perennial ryegrass | | | Plantain | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Comparison** | **Season** | **LSM** | **SE** | **df** | **P value** | **LSM** | **Lower** | **Upper** | **LSM** | **Lower** | **Upper** | **P value** |
| RyMo vs PlMo | All | -0.79 | 0.021 | 31 | < 0.001 | 21.1 | 19.2 | 23.0 | 14.8 | 12.9 | 16.7 | < 0.001 |
| RyMo vs PlX | All | -0.80 | 0.048 | 8 | < 0.001 | 20.6 | 17.0 | 24.2 | 13.3 | 9.7 | 16.9 | 0.0016 |
| RyWc vs PlMo | All | -0.83 | 0.030 | 23 | < 0.001 | 17.4 | 15.4 | 19.5 | 11.8 | 9.8 | 13.9 | < 0.001 |
| RyWc vs PlX | All | -0.68 | 0.057 | 36 | < 0.001 | 18.4 | 17.0 | 19.8 | 13.7 | 12.3 | 15.1 | < 0.001 |
| *Seasonal effect* | | | | | | | | | | | | |
| RyMo vs PlMo | Winter | -0.87 | 0.090 | 16 | < 0.001 | 18.3 | 12.9 | 23.7 | 12.3 | 6.9 | 17.7 | 0.1073 |
| RyMo vs PlMo | Early Spring | -0.78 | 0.079 | 16 | < 0.001 | 25.5 | 20.8 | 30.3 | 19.4 | 14.6 | 24.2 | 0.0592 |
| RyMo vs PlMo | Late Spring | -0.78 | 0.034 | 16 | < 0.001 | 20.6 | 18.4 | 22.9 | 14.3 | 12.1 | 16.6 | < 0.001 |
| RyMo vs PlMo | Summer | -0.82 | 0.032 | 16 | < 0.001 | 22.3 | 20.2 | 24.4 | 14.3 | 12.2 | 16.4 | < 0.001 |
| RyMo vs PlMo | Autumn | -0.68 | 0.037 | 16 | < 0.001 | 18.6 | 16.2 | 21.0 | 13.5 | 11.1 | 15.8 | 0.0009 |

LSM, Least squares means; SE, standard error of the mean; df, residual degrees of freedom (= number of data points contributing to the analyses minus 2); Lower, lower 95% confidence limit; Upper, upper 95% confidence limit.

**Table 2** Correlation coefficient (r) of the weighted effect size for effects of pasture type on herbage nitrogen (N) content, and proportions of soluble and degradable N within total N, with least squares means with upper and lower confidence limits for pasture type.

| | | r (weighted effect) | | | | Perennial ryegrass | | | Plantain | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Comparison** | **Season** | **LSM** | **SE** | **df** | **P value** | **LSM** | **Lower 1/4** | **Upper 1/4** | **LSM** | **Lower 1/4** | **Upper 1/4** | **P value** |
| *Total Nitrogen (% dry matter)* | | | | | | | | | | | | |
| RyMo vs PlMo | All | 0.03 | 0.070 | 39 | 0.6768 | 3.1 | 2.7 | 3.4 | 3.2 | 2.8 | 3.5 | 0.5207 |
| RyMo vs PlX | All | 0.24 | 0.100 | 11 | 0.0381 | 3.0 | 2.6 | 3.4 | 3.4 | 3.0 | 3.8 | 0.0979 |
| RyWc vs PlMo | All | -0.47 | 0.078 | 32 | < 0.001 | 3.4 | 3.1 | 3.6 | 3.1 | 2.8 | 3.3 | 0.0050 |
| RyWc vs PlX | All | 0.09 | 0.080 | 43 | 0.2871 | 3.1 | 2.8 | 3.4 | 3.2 | 3.0 | 3.5 | 0.0707 |
| *Soluble N (% of total N content)* | | | | | | | | | | | | |
| RyMo vs PlMo | All | -0.97 | 0.012 | 3 | < 0.001 | 38.4 | 31.8 | 45.0 | 12.0 | 5.3 | 18.6 | 0.0012 |
| RyMo vs PlX | All | -0.81 | 0.105 | 3 | 0.0046 | 38.4 | 27.3 | 44.3 | 27.3 | 21.4 | 33.3 | 0.0171 |
| RyWc vs PlMo | All | -0.97 | 0.014 | 3 | < 0.001 | 38.4 | 29.3 | 47.2 | 12.0 | 2.9 | 21.0 | 0.0015 |
| RyWc vs PlX | All | -0.78 | 0.127 | 3 | 0.0088 | 38.4 | 29.8 | 46.9 | 27.4 | 18.8 | 36.0 | 0.0363 |
| *Degradable N (% of total N content)* | | | | | | | | | | | | |
| RyMo vs PlMo | All | -0.97 | 0.012 | 3 | < 0.001 | 69.2 | 65.9 | 72.5 | 56.0 | 52.7 | 59.3 | 0.0011 |
| RyMo vs PlX | All | -0.81 | 0.104 | 3 | 0.0044 | 69.2 | 66.3 | 72.2 | 63.7 | 60.7 | 66.6 | 0.0167 |
| RyWc vs PlMo | All | -0.97 | 0.014 | 3 | < 0.001 | 69.2 | 64.7 | 73.7 | 56.0 | 51.5 | 60.5 | 0.0015 |
| RyWc vs PlX | All | -0.78 | 0.127 | 3 | 0.0089 | 69.2 | 64.9 | 73.5 | 63.7 | 59.4 | 68.01 | 0.0363 |

While total N content for plantain and ryegrass monocultures was similar, there were strong negative effect sizes for the proportions of total N that are soluble and degradable (*r*=-0.97, P<0.001; Table 2). Soluble N content in plantain and ryegrass monocultures was ~ 12 vs 38% of total N, respectively (P<0.001), and degradable N content in plantain and ryegrass monocultures 56% and 69% of total N, respectively (P=0.001). Reductions in the amount of soluble and degradable N may explain reduced rumen ammonia concentrations of livestock fed plantain compared to ryegrass (Minneé et al. 2017, Swainson & Hoskin 2006). It might also explain observed differences in N partitioning (Totty et al. 2013), since increased undegradable N can result in more N being partitioned to faeces (Bryant et al. 2012) which is then less susceptible to leaching and conversion to nitrous oxide (a potent greenhouse gas) (Lockyer & Whitehead 1990).

**Carbohydrate**

Plantain contained less structural carbohydrate (SC) (measured as neutral detergent fibre, NDF) than ryegrass (LSM 29 and 43 g/100g DM from PlMo and RyMo pastures respectively, P<0.001, Table 3). Therefore, the presence of plantain in a pasture with ryegrass will influence the SC content of the whole pasture. An interaction between pasture type and season was observed for NDF content, where the effect size was smaller in winter (*r*=-0.74) compared with other seasons (*r*>-0.80), which may reflect a vegetative state with lesser dead matter content of ryegrass in winter compared with other seasons.

Non-structural carbohydrates (NSC) are the primary source of energy for rumen microbes (Kebreab et al. 2009). Plantain was shown to have consistently greater NSC content than ryegrass (Table 3), with a trend for greater effect size and difference in autumn (*r*=0.91, P<0.001; 30 vs 16 g/100 g DM from PlMo and RyMo respectively, P<0.001). The NSC: N ratio of the diet has a substantial effect on N partitioning in ruminants, where the proportion of dietary N eaten partitioned to urine declines as NSC: N increases (Edwards et al. 2007; Belanche et al. 2013; Moorby 2014). Given that the N content of herbage from plantain pastures was similar to, or less than, herbage from ryegrass pastures (Table 2), the NSC: N ratio in plantain should consistently favour partitioning of dietary N from urine to other sinks, and may explain differences in urinary N excretion and production responses observed when livestock are fed diets containing plantain (Box et al. 2017; Hutton et al. 2011; Moorhead et al. 2002).

**Ash and lipid content**

Mineral (ash) content was greater in pastures containing plantain (*r*>0.6 for all pasture comparisons) compared with ryegrass. In monoculture, ash content was 12.8 and 10.2 g/100 g DM for PlMo and RyMo respectively (P<0.001), and the effect was consistent across seasons. A small (*r*=-0.19) but significant effect of reduced lipid content in plantain compared to ryegrass monocultures was observed. Greater effects were observed comparing plantain pastures with RyWc indicating legumes in pastures elevate herbage lipid content (*r*=-0.85; 2.5 vs. 3.5 g/100g for PlMo and RyWc respectively).

**Herbage digestibility**

The digestibility of plantain and ryegrass monoculture pastures was similar (*r*=-0.13; LSM 77.8 vs 77.2% for RyMo and PlMo respectively, P=0.339). However, there was a trend (P=0.053) for an interaction between pasture and season where digestibility was reduced to 74% in plantain in late spring and summer compared

**Table 3** Correlation coefficient (r) of the weighted effect size for effects of pasture type on herbage neutral fibre, acid detergent fibre and non-structural carbohydrate content, with least squares means (% of dry matter) with upper and lower confidence limits for pasture type.

| | | r (weighted effect) | | | | Perennial ryegrass | | | Plantain | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Comparison | Season | LSM | SE | df | P value | LSM | Lower 1/4 | Upper 1/4 | LSM | Lower 1/4 | Upper 1/4 | P value |
| *Neutral detergent fibre* | | | | | | | | | | | | RyMo vs |
| PlMo | All | -0.90 | 0.020 | 33 | < 0.001 | 43.1 | 40.0 | 46.2 | 28.7 | 25.5 | 31.8 | < 0.001 |
| *Acid detergent fibre* | | | | | | | | | | | | |
| RyMo vs PlMo | All | -0.65 | 0.074 | 32 | < 0.001 | 24.6 | 22.7 | 26.6 | 20.8 | 18.8 | 22.7 | < 0.001 |
| *Non-structural carbohydrate* | | | | | | | | | | | | |
| RyMo vs PlMo | All | 0.77 | 0.049 | 7 | < 0.001 | 21.1 | 19.6 | 22.6 | 29.9 | 28.4 | 31.4 | < 0.001 |

to 78% in ryegrass. This may reflect the reproductive stage of plantain at that time of year when the plant flowers profusely (Lee et al. 2015), or elevation in concentration of secondary compounds in plantain herbage (aucubin and acteoside) in these seasons (Box et al. 2019).

## Conclusions

Perennial ryegrass and plantain did not differ in two commonly measured, and important, attributes for livestock nitrogen metabolism: total herbage nitrogen content and herbage digestibility. However, there were clear and consistent differences in DM and carbohydrate content, the partitioning of total herbage N between degradable and non-degradable forms, indicating that this may be more important than total N for influencing urinary N excretion.

This study highlighted compositional differences between ryegrass and plantain that are not routinely measured. Considering the likely importance of the alternative indicators for N partitioning and urine N excretion, we suggest future field research or simulation modelling addressing the effect of forage species on N leaching risk in grazing livestock production systems will need to include these attributes.

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