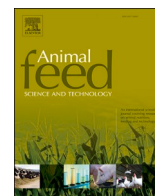




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Review article

## Forage plantain (*Plantago lanceolata* L.): Meta-analysis quantifying the decrease in nitrogen excretion, the increase in milk production, and the changes in milk composition of dairy cows grazing pastures containing plantain

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### ABSTRACT

Plantain (*Plantago lanceolata* L.) has been increasingly used as a forage component in dairy grazing systems due to its capacity to reduce nitrogen (N) losses, while improving or maintaining milk production. A meta-analysis was conducted to quantify the effect of plantain on milk production and urinary nitrogen (UN) excretion by dairy cows. The main outcomes of this analysis included the yield, solids (fat + protein), protein and fat components of milk, and N concentration in urine, daily urine volume, and total UN excretion by dairy cows. Overall, grazing pastures containing plantain significantly increased milk yield (weighted mean difference (WMD) = 1.02 kg/cow/day, 95% confidence interval (CI) = 0.55–1.46), milk solids yield (WMD = 0.07 kg/cow/day, 95% CI = 0.02–0.12), and milk protein yield (WMD = 23.4 g/cow/day, 95% CI = 11.3–35.5), maintained milk protein concentration and milk fat yield, but reduced milk fat concentration (WMD = –0.24%, 95% CI = –0.31 to –0.17). Feeding pastures containing plantain reduced total UN excretion by 22% (95% CI = 15–28), which was associated with a decrease of 30% in UN concentration (95% CI = 20–38) and an increase of 17% in daily urine volume (95% CI = 7–29). Subgroup analysis showed that cows grazing pastures containing plantain had a significantly higher milk yield in late lactation (WMD = 1.4 kg/cow/day, 95% CI = 0.8–1.9), but a similar milk yield in early lactation, compared to grazing control pastures. In addition, meta-regression analysis found statistical associations between the content of plantain in the diet and N concentration in urine ( $P < 0.001$ ), daily urine volume ( $P < 0.001$ ), and total UN excretion ( $P = 0.036$ ). The results suggest that incorporating plantain into grazing pastures is a potential strategy for improving farm productivity, while reducing the environmental impact of dairy farms.

**Abbreviations:** ADF, acid detergent fibre; CP, crude protein; CI, confidence interval; DM, dry matter; ME, metabolisable energy; N, nitrogen; NDF, neutral detergent fibre; WSC, water-soluble carbohydrate; ROM, the ratio of the mean; UN, urinary nitrogen; WMD, weighted mean difference; SD, standard deviation; SEM, standard error of the mean; SED, standard error of difference; MD, mean difference; DIM, day in milk.

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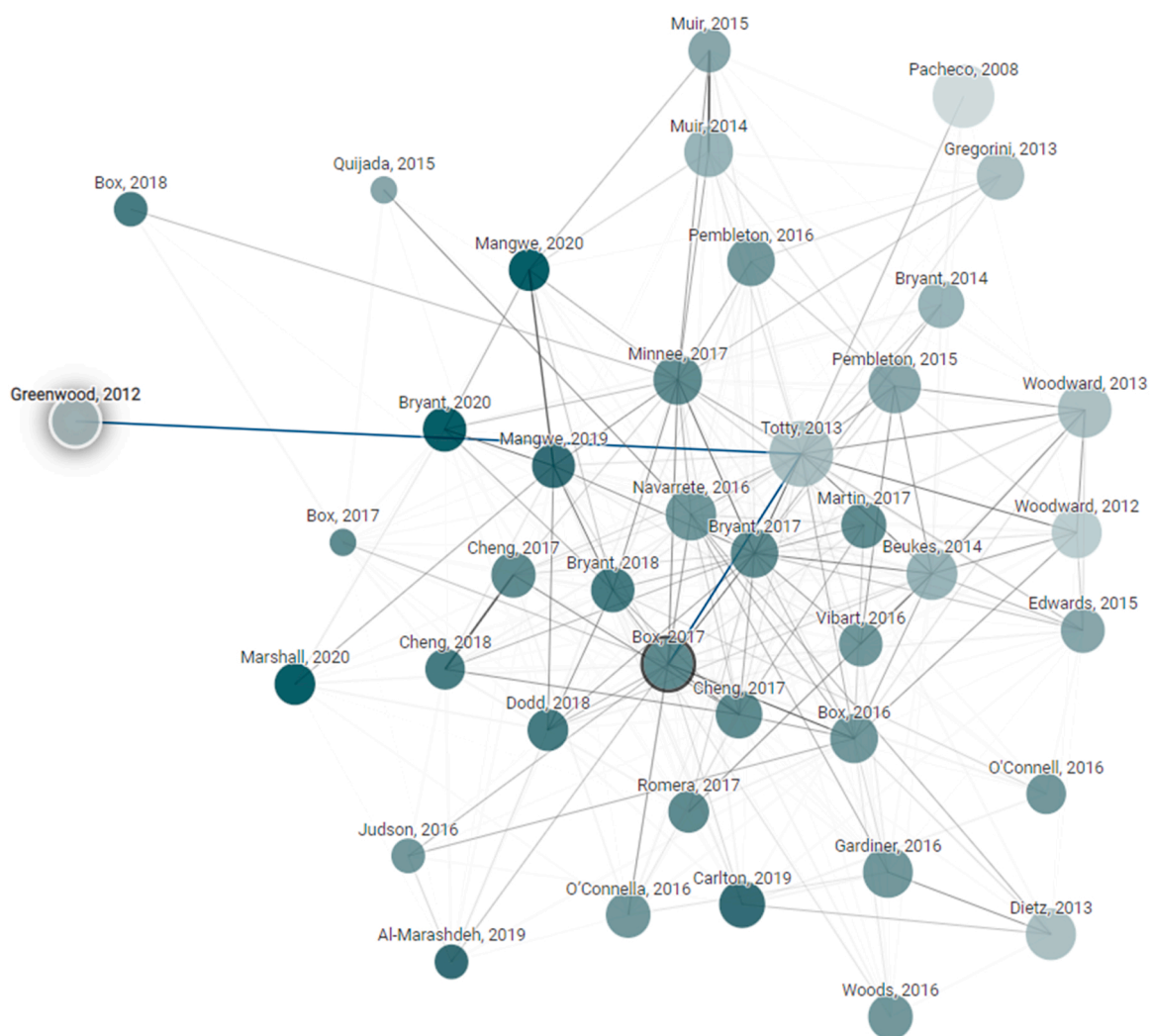
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## 1. Introduction

Plantain (*Plantago lanceolata* L.) is a perennial forage herb, which has been increasingly used in the last decade as a special purpose crop, or a component of pasture mixes for dairy cattle in temperate countries such as New Zealand, Australia, and Ireland. Forage plantain is tolerant to drought and heat (Stewart, 1996; Cranston et al., 2015), and produces more dry matter (DM) than perennial ryegrass – white clover in summer and autumn in dryland pastoral systems (Moorhead and Piggot, 2009). Sole plantain contains 110 g/kg DM higher non-structural fibre, 26 g/kg DM lower soluble nitrogen (N) in N content (Minnée et al., 2019), and 1.5 times greater macro minerals (Mangwe et al., 2019) than perennial ryegrass (*Lolium perenne* L.). Furthermore, it has a high concentration of secondary compounds, aucubin and acteoside (Navarrete et al., 2016) that are known to not exist in traditional perennial ryegrass and white clover (*Trifolium repens* L.).

Intensive studies have suggested that plantain as a pure sward (Box et al., 2017; Mangwe et al., 2019) or combined in a mixed pasture reduces urinary nitrogen (UN) excretion from cow urine, while maintaining or increasing milk production. Feeding pastures containing plantain reduces N concentration in cow urine, and increases urine volume and frequency (Mangwe et al., 2019; Minnée et al., 2020); therefore, potentially decreasing N leaching from dairy farms (Bryant et al., 2019). Other studies have shown the benefit of plantain forage in improving milk yield (McCarthy et al., 2020) and altering the fatty acid profile (Mangwe et al., 2018). However, the effects of plantain on N excretion and milk production are highly variable across studies. For example, pasture containing plantain can reduce UN excretion from 0 (Minnée et al., 2020) to 44% (Mangwe et al., 2019), while maintaining (Nkomboni et al., 2021) or increasing milk yield (Totty et al., 2013). This raises the questions “to what extent does incorporating plantain in grazing pastures affect N excretion and milk production of dairy cows?” and “how are the effects influenced by experimental conditions such as



**Fig. 1.** Papers published from 2012 to 2021 connected with Box et al. (2017) on milk production and N excretion of dairy cows grazing pastures containing plantain.

lactation stage and the content of plantain in the diet?”.

The present review paper used meta-analysis to compare milk production and UN excretion by dairy cows offered either pasture containing plantain (treatment pasture) or not (control pasture). Additionally, the effects of plantain at different lactation stages and the effect of varying plantain content in the diet were compared.

## 2. Materials and methods

This meta-analysis was conducted in compliance with the method described in “Cochrane Handbook for Systematic Reviews of Interventions” (Higgins et al., 2021) and the statistical analysis introduced in “Doing Meta-Analysis in R” (Harrer et al., 2021). The review questions were: (1) to what extent does the use of plantain in grazed pastures affect milk production and UN excretion by dairy cows? And (2) how are the effects of plantain different over stages of lactation and with varying contents of plantain in the diet of dairy cows?

### 2.1. Literature search

Online search engines, Web of Science (<http://webofknowledge.com>), and Google Scholar (<https://scholar.google.com>) were used in the literature search to obtain relevant studies. Keywords for searching were “plantain” AND “dairy cows”. The reference lists from publications were reviewed to find more studies. Moreover, a ‘connected papers graph’ was created and used <https://www.connectedpapers.com>, to obtain a visual overview to double-check against the list of studies produced by the literature search (Fig. 1).

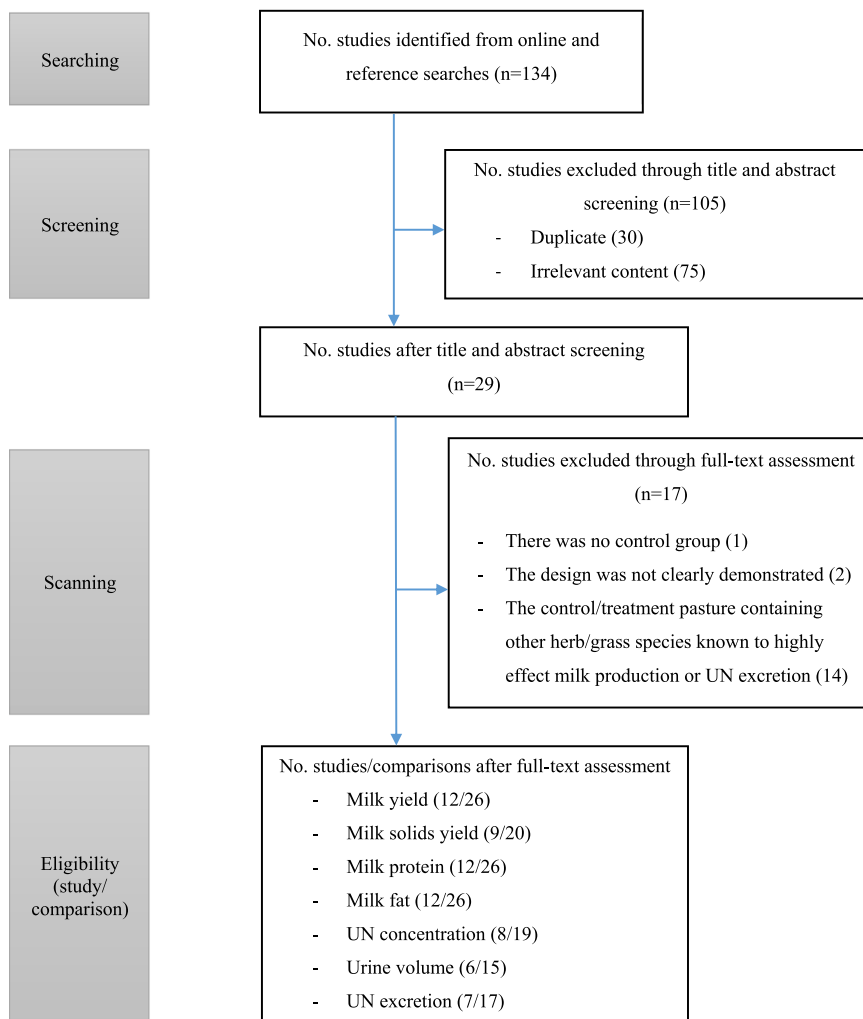


Fig. 2. The process of literature search and study selection, and the number of studies within each step.

## 2.2. Publication selection and selection criteria

The literature search and study selection processes are presented in Fig. 2. A total of 134 studies was obtained from the literature search. In which, 122 were excluded by title and abstract screening (105) and full manuscript scanning (17). A total of 12 studies from New Zealand (11) and Australia (1) were selected for this review. All selected studies (i) contained a full manuscript in English, (ii) were conducted on lactating dairy cows using at least a treatment pasture and a control pasture, (iii) demonstrated a clear experimental design, (iv) had at least one comparison for milk production or UN excretion, and (v) presented at least one measure of the statistical variance of the comparisons (standard deviation, standard error, or P-value). Studies with pastures containing other herbs or grasses, with known potential to greatly affect milk production or N excretion, such as chicory (*Cichorium intybus* L.), were excluded in this meta-analysis.

A total of 26 comparisons from 12 studies was included in this meta-analysis. This provided a sample size of 766 dairy cows. Control pastures were mainly perennial ryegrass and white clover, but three contained tall fescue (*Festuca arundinacea* L.) and five included lucerne (*Medicago sativa* L.). Treatment swards were pure plantain or mixed pastures containing plantain with an average content of 434 g/kg DM. The 26 comparisons included 18 in the late, seven in the early lactation period, and one combination of all lactation seasons (Table 1). The number of comparisons is enough to conduct a meta-analysis and subgroup analyses for milk yield, milk solids, milk protein concentration, milk protein yield, milk fat concentration, milk fat yield, UN concentration, daily urine volume and UN excretion (Higgins et al., 2021).

Eggers' test for the funnel plot asymmetry found no significant publication bias for any comparisons with  $P = 0.072, 0.920, 0.226, 0.803, 0.796, 0.707, 0.743, 0.146, 0.262$  for milk yield, milk solids yield, milk protein concentration, milk protein yield, milk fat concentration, milk fat yield, UN concentration, daily urine volume and UN excretion, respectively (Fig. 3).

## 2.3. Data extraction

Relevant data were extracted from all selected studies into a database in Microsoft Excel. The database included study characteristics, research design, outcome comparison of milk production, urinary excretion, and herbage nutritive values. Outcome data for each comparison were presented with a mean value and a standard deviation.

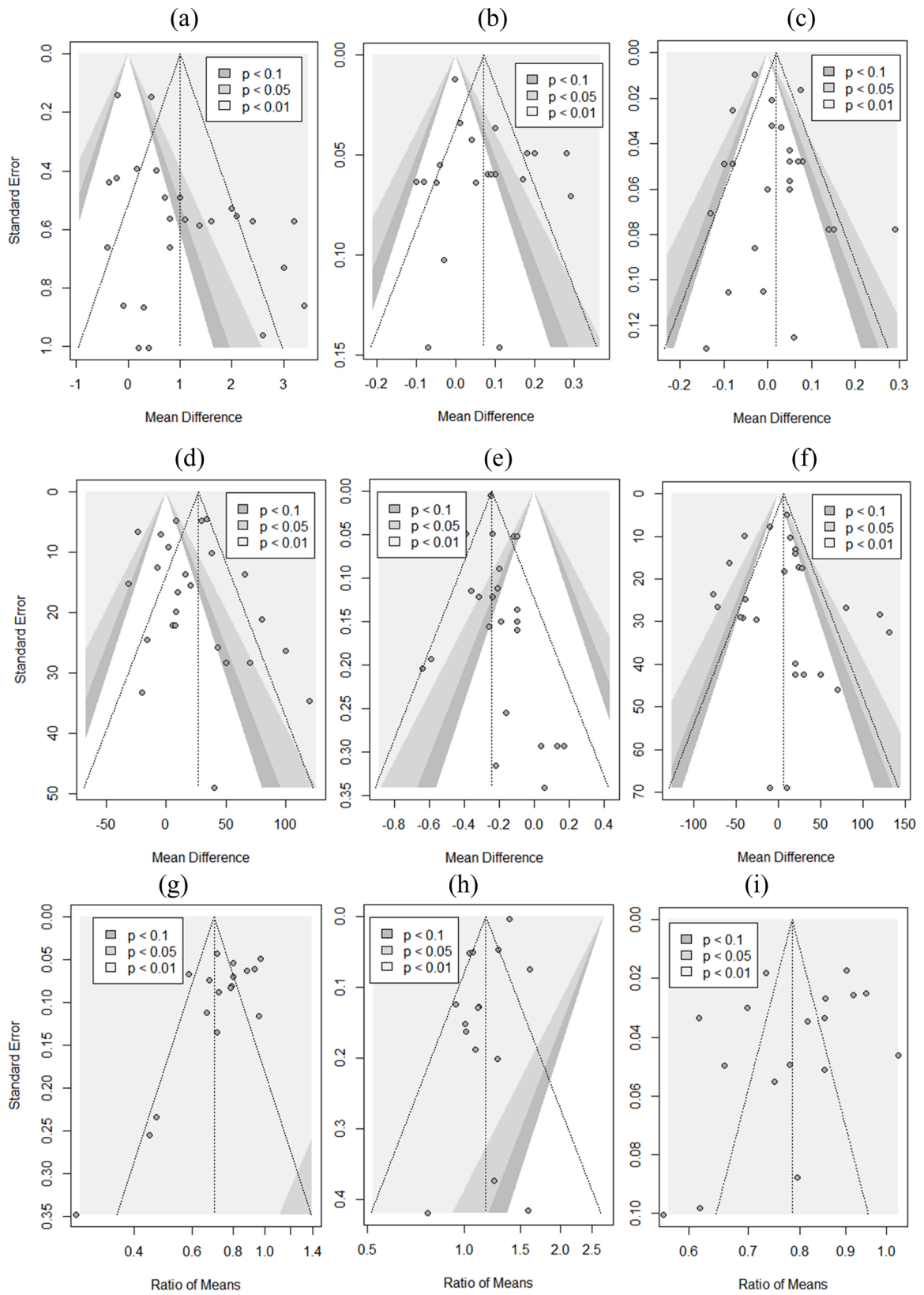
New variables were created from the extracted data for use as analysed outcomes or independent factors in sub-group analyses. The lactation stage was grouped by days in milk (DIM), in which early and late lactations were denoted as 0–150 and  $\geq 151$  DIM, respectively. If the data were not reported in studies, metabolisable energy (ME) was converted from the digestibility of organic matter in DM (DOMD), using the equation  $ME \text{ (MJ/kg DM)} = \text{DOMD (g/kg DM)} \times 0.0194 - 2.577$  (Freer et al., 2007). Crude protein (CP) was obtained from N concentration by the equation  $CP \text{ (g/kg DM)} = N \text{ (g/kg DM)} \times 6.25$  (Jiang et al., 2014). The quantity of UN excretion was estimated from milk urea nitrogen (MUN), using the equation  $UN_{\text{excretion}} \text{ (g/cow/day)} = 15.1 \times MUN \text{ (mg/dL)} + 27.8$  (Kohn

**Table 1**

Summary of studies investigating the effect of pastures containing plantain on milk production and urinary nitrogen excretion of dairy cows.

Studies	N <sup>a</sup>	Control pasture <sup>a</sup>	Treatment pasture <sup>a</sup>	Farm system	Lactation stage	Plantain content (g/kg DM)
Box et al. (2017)	24	RGWC	RGWC-PL	Irrigated	late	499
Box et al. (2017)	24	RGWC	PL	Irrigated	late	896
Box et al. (2017)	24	RGWC	RGWC-PL	Irrigated	early	334
Box et al. (2017)	24	RGWC	PL	Irrigated	early	678
Dodd et al. (2018)	30	TF-LC	TF-LC-PL	Rain-fed	late	510
Dodd et al. (2018)	30	TF-LC	TF-LC-PL	Rain-fed	late	390
Dodd et al. (2018)	30	RG-LC	RG-LC-PL	Rain-fed	early	510
Dodd et al. (2018)	30	RG-LC	RG-LC-PL	Rain-fed	early	370
Mangwe et al. (2018)	36	RGWC	WC-PL	NA	late	510
Mangwe et al. (2019)	36	RGWC	WC-PL	Irrigated	late	780
Marshall et al. (2020)	48	RGWC	RGWC-PL	NA	early	210
Marshall et al. (2020)	48	RGWC	RGWC-PL	NA	late	210
Marshall et al. (2021)	16	RG	PL	NA	late	900
Minnee et al. (2017)	15	RG	RG-PL	NA	late	196
Minnee et al. (2017)	15	RG	RG-PL	NA	late	392
Pembleton et al. (2016)	24	RG	RGWC-PL	NA	early	140
Pembleton et al. (2016)	24	RG	RGWC-PL	NA	late	360
Pembleton et al. (2016)	24	RG	RGWC-PL	NA	early	430
Waghorn et al. (2019)	10	TF-LC	TF-LC-PL	Rain-fed	late	179
Minnee et al. (2020)	8	RG	RG-PL	NA	late	170
Minnee et al. (2020)	8	RG	RG-PL	NA	late	330
Minnee et al. (2020)	8	RG	RG-PL	NA	late	450
Nkomboni et al. (2021)	24	RGWC	RGWC-PL	Irrigated	late	210
Nkomboni et al. (2021)	24	RGWC	RGWC-PL	Irrigated	late	390
Nkomboni et al. (2021)	24	RGWC	RGWC-PL	Irrigated	late	680
Al-Marashdeh et al. (2021)	158	RGWC	RGWC-PL	Irrigated	All seasons	765

<sup>a</sup> N = no. cows; RGWC = perennial ryegrass-white clover; PL = plantain; TF = tall fescue, LC = lucerne, NA = data is not available or not applicable.



(caption on next page)

**Fig. 3.** Funnel plots; (a) milk yield, (b) milk solids yield, (c) milk protein concentration, (d) milk protein yield, (e) milk fat concentration, (f) milk fat yield, (g) nitrogen concentration in urine, (h) daily urine volume, (i) urinary nitrogen excretion.

et al., 2002).

Continuous variables of outcomes were extracted with the values of mean and standard deviation (SD). If SD was not reported in studies, it was obtained from standard error of the mean (SEM), standard error of difference (SED), or P-value. Specifically, SD was converted from SEM according to the formula  $SD = SEM \times \sqrt{N}$ , in which N is the sample size; and from SED using the formula  $SD = \frac{SED}{\sqrt{\frac{1}{N_c} + \frac{1}{N_e}}}$ , where  $N_c$  and  $N_e$  refer to the number of cows in the control and treatment groups, respectively. For studies reporting P-value between groups, SD was assumed to be the same for all groups, and it was calculated using mean difference (MD) between two groups and the P-value. In this calculation, the t-value was obtained from the P-value according to the function  $[TINV(probability, degree\ of\ freedom)]$  in Microsoft Excel, where TINV is the t-value (two-tailed), the probability is P-value, and degree of freedom equalled "N-1". The SEM was then calculated via the equation  $SEM = MD/t$  for converting to SD by the above formula. In studies that

**Table 2**

Lactation stage, number of comparisons (N), weighted mean difference (WMD), 95% confidence interval (CI), within studies heterogeneity ( $I^2$ ), P-value of meta-analysis and subgroup analyses.

Variable*	Lactation stage	N	WMD	95% CI	$I^2$ (%)	P
<b>Milk production</b>						
Milk yield (kg/cow/day)	Overall	26	1.02	0.55–1.46	79	< 0.001
	Early lactation	7	0.36	-0.33–1.05	50	0.407
	Late lactation	18	1.38	0.83–1.94	55	< 0.001
Milk solids (kg/cow/day)	Overall	20	0.07	0.02–0.12	68	0.012
	Early lactation	5	0.02	-0.09–0.05	0.0	0.545
	Late lactation	14	0.11	0.05–0.17	56	0.002
Milk protein (g/100 g milk)	Overall	26	0.02	-0.02–0.06	71	0.274
	Early lactation	7	-0.01	-0.06–0.04	57	0.575
	Late lactation	18	0.03	-0.02–0.08	58	0.162
Milk protein (g/cow/day)	Overall	26	23.4	11.3–35.5	68	< 0.001
	Early lactation	7	12.2	-22.7–47.0	70	0.410
	Late lactation	18	31.1	14.7–47.6	60	0.003
Milk fat (g/100 g milk)	Overall	26	-0.24	-0.31 to -0.17	57	< 0.001
	Early lactation	7	-0.13	-0.17 to -0.09	0	< 0.001
	Late lactation	18	-0.29	-0.38 to -0.19	55	< 0.001
Milk fat (g/cow/day)	Overall	26	6.3	-14.8–27.4	79	0.554
	Early lactation	7	-21.9	-53.9–10.1	70	0.146
	Late lactation	18	15.4	-3.5–34.2	35	0.101
<b>UN concentration and excretion</b>						
UN concentration (ROM)	Overall	19	0.70	0.62–0.80	91	< 0.001
	Early lactation	4	0.72	0.52–0.98	40	0.044
	Late lactation	15	0.70	0.59–0.82	93	< 0.001
Urine volume (ROM)	Overall	15	1.17	1.07–1.29	85	0.003
	Early lactation	5	10.6	0.94–1.20	0	0.244
	Late lactation	10	1.19	1.05–1.36	77	0.010
UN excretion (ROM)	Overall	16	0.78	0.72–0.85	95	< 0.001
	Early lactation	4	0.73	0.58–0.92	88	0.023
	Late lactation	13	0.80	0.72–0.89	96	< 0.001
<b>Nutritive characteristics</b>						
DM intake (kg/cow/day)	Overall	22	1.03	0.56–1.50	74	< 0.001
	Early lactation	6	0.34	-0.83–1.51	72	0.487
	Late lactation	16	1.32	0.83–1.81	46	< 0.001
CP (g/kg DM)	Overall	26	-12.8	-20.4 to - 5.1	60	0.002
	Early lactation	7	-6.9	-17.6–3.8	0	0.164
	Late lactation	18	-16.0	-26.7 to - 5.1	0	0.006
ME (MJ/kg DM)	Overall	26	-0.01	-0.12–0.09	88	0.754
	Early lactation	7	0.03	-0.15–0.22	74	0.662
	Late lactation	18	-0.02	-0.18–0.14	86	0.774
ADF (g/kg DM)	Overall	20	-18.7	-29.2 to - 8.4	87	0.001
	Early lactation	5	-7.8	-21.8–6.3	73	0.200
	Late lactation	14	-23.7	-38.1 to - 9.2	88	0.004
NDF (g/kg DM)	Overall	24	-80.4	-101.9 to - 58.9	95	< 0.001
	Early lactation	7	-61.1	-87.3 to - 35.0	57	0.001
	Late lactation	16	-88.0	-120.2 to - 47.8	82	< 0.001
WSC (g/kg DM)	Overall	17	8.8	-15.0–32.7	92	0.442
	Early lactation	3	-39.9	-129.5–49.8	88	0.196
	Late lactation	14	19.3	-04.9–43.5	92	0.109

\* ADF = acid detergent fibre; CP = crude protein; DM = dry matter, ME = metabolisable energy; NDF = non-acid detergent fibre, UN = urinary nitrogen concentration; WSC = water-soluble carbohydrate.

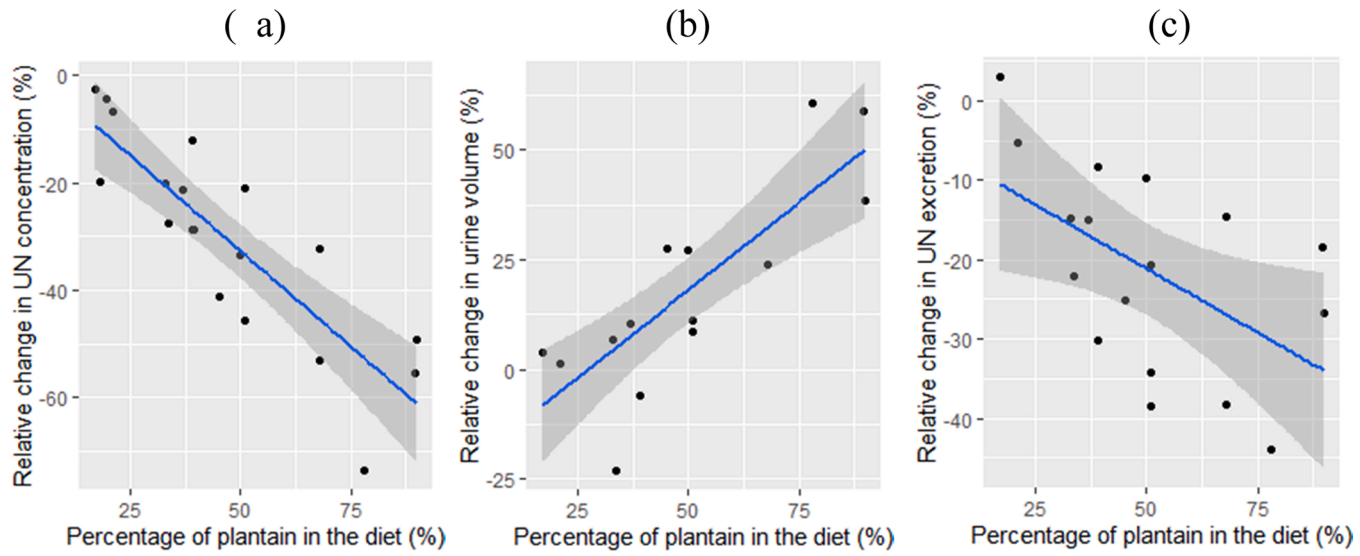


Fig. 4. The relationship between plantain percentage in the pasture diet and the relative changes in urinary nitrogen (UN) concentration (a), daily urine volume (b), UN excretion (c).

reported  $P < 0.01$  or  $< 0.05$ ,  $P = 0.01$  or  $0.05$  were used for the calculation (Higgins et al., 2021).

#### 2.4. Data analysis

The meta-analysis was conducted using Rstudio, as described by Harrer et al. (2021). A random-effect model was applied for the meta-analysis, using the Meta and Metafor packages (Core Team, 2021). The population was experimental lactation cows, and the intervention and comparator were pastures either containing plantain or not. The primary outcomes were assessed, including milk yield, milk solids, milk protein (concentration and yield), milk fat (concentration and yield), N concentration in urine, daily urine volume, and total UN excretion. In addition, nutritive values, including DM intake (DMI), CP, ME, acid detergent fibre (ADF), neutral detergent fibre (NDF), and water-soluble carbohydrate (WSC) were evaluated for support for the effect of treatment pasture on the main outcomes.

In the meta-analysis, weighted mean differences (WMD) were estimated from the MD or ratio of the mean (ROM) and the weight of individual studies. The MD ( $MD = M_{\text{treatment}} - M_{\text{control}}$ ) was used for most of the comparisons, except UN concentration, daily urine volume and UN excretion that were presented by ROM ( $ROM = M_{\text{treatment}}/M_{\text{control}}$ ). Relative change (RC) for UN concentration and UN excretion was achieved from ROM via  $RC (\%) = (1 - ROM) \times 100$ . A significant difference was declared when the P-value was less than 0.05, or the 95% CI range did not include a zero value.

Sub-group analysis was conducted to identify the effect of different lactation stages (early: 0 – 150 DIM and late:  $\geq 151$  DIM). Moreover, meta-regression was implemented to investigate the impact of the content of plantain in the diet on the outcomes. Regression models were used to estimate the weighted effect size of the relationship when statistical effects were identified. The model was formed by the equation:  $\theta_k = \theta + \beta x_k + \epsilon_k + \zeta_k$ , where  $\theta_k$  refers to the effect size of the study  $k$ ,  $\beta x_k$  is the coefficient of the predictor,  $\epsilon_k$  and  $\zeta_k$  denote sampling errors and an overarching distribution of effect size (Higgins et al., 2021).

Heterogeneity among studies was identified for meta-analysis by a chi-squared ( $\chi^2$ ) test with the significance level at  $P \leq 0.01$ . The  $I^2$  among studies refers to low heterogeneity at 25%, moderate heterogeneity at 50%, and substantial heterogeneity at 75% (Harrer et al., 2021). Also, publication bias within studies was evaluated by funnel plots to identify if low effect size studies were missed in the meta-analysis. The Eggers's test was regarded as significant at  $P \leq 0.01$  (Higgins et al., 2021).

### 3. Results

#### 3.1. Increased milk production

The mean differences of milk yield and composition for lactating cows grazing treatment pastures compared with control pastures are presented in Table 2. Overall, milk yield (WMD = 1.02 kg/cow/day, 95% CI = 0.55–1.46), milk solids yield (WMD = 0.07 kg/cow/day, 95% CI = 0.02–0.12), and milk protein yield (WMD = 23.4 g/cow/day, 95% CI = 11.3–35.5) were significantly increased by grazing pastures containing plantain. In contrast, feeding treatment pastures decreased fat concentration in milk (WMD =  $-0.24$  g/100 g milk, 95% CI =  $-0.31$  to  $-0.17$ ), while milk fat yield and milk protein concentration were maintained. Heterogeneity within studies was substantial for milk fat concentration ( $I^2 = 57\%$ ), milk solids yield ( $I^2 = 68\%$ ), milk protein concentration ( $I^2 = 71\%$ ), milk protein yield ( $I^2 = 68\%$ ), and high for milk yield ( $I^2 = 79\%$ ) and milk fat yield ( $I^2 = 79\%$ ).

Sub-group analysis by lactation stages showed that most milk production parameters were increased in late lactation, but were similar in early lactation. In late lactation, cows grazing pastures containing plantain had higher milk yield (WMD = 1.4 kg/cow/day, 95% CI = 0.8–1.9), milk solids yield (WMD = 0.11, 95% CI = 0.05–0.17), and milk protein yield (WMD = 31.1 g/cow/day, 95% CI = 14.7–47.6). There were no differences in milk yield, milk solids, protein concentration, and protein yield in early lactation ( $P > 0.05$ ). Milk fat concentration of cows grazing treatment pastures was lower than grazing control pastures in both early lactation (WMD =  $-0.13$  g/100 g milk, 95% CI =  $-0.17$  to  $-0.09$ ) and late lactation (WMD =  $-0.29$  g/100 g milk, 95% CI =  $-0.40$  to  $-0.17$ ). The variation between studies was low or moderate for all sub-group comparisons, except for milk protein yield and milk fat yield in early lactation, where it was substantial with  $I^2 = 70\%$ . Meta-regression analysis conducted to determine the effect of plantain content on milk production, showed no significant correlation with milk yield, milk solids yield, milk protein concentration, milk protein yield, milk fat concentration or milk fat yield.

#### 3.2. Reduced UN concentration and excretion

The meta-analysis showed a significant reduction in UN concentration and excretion for cows grazing treatment pastures, compared to control pastures (Table 2). There was an overall reduction of 30% in UN concentration (95% CI = 20–38) and 22% in UN excretion (95% CI = 15–28), and an increase of 17% in daily urine volume (95% CI = 7–29). The heterogeneity within studies in these analyses was high for UN concentration ( $I^2 = 91\%$ ), daily urine volume ( $I^2 = 85\%$ ), and UN excretion ( $I^2 = 95\%$ ). Sub-group analysis showed grazing pastures containing plantain reduced UN concentration, and UN excretion in both early and late lactation stages. However, the effect of plantain on increased daily urine volume was observed only in late lactation.

Meta-regression analysis for the effect of the content of plantain in treatment pastures indicated that increased content of plantain was statistically associated with a reduction in UN concentration ( $F(df1 = 1, df2 = 17) = 33.8, P < 0.001, R^2 = 69\%$ ), a decrease in UN excretion ( $F(df1 = 1, df2 = 15) = 5.3, P = 0.036, R^2 = 22\%$ ), and an increase in urine volume ( $F(df1 = 1, df2 = 13) = 28.3, P < 0.001, R^2 = 64\%$ ) (Fig. 4). The estimated curves for these changes are below:

- (a) Relative change of UN concentration (%):  $y = -0.0645 \times \text{plantain content (g/kg DM)} + 0.61$   
 (b) Relative change of daily urine volume (%):  $y = 0.0659 \times \text{plantain content (g/kg DM)} - 12.73$   
 (c) Relative change of UN excretion (%):  $y = -0.0297 \times \text{plantain content (g/kg DM)} - 5.86$

## 4. Discussion

### 4.1. Milk yield

Our analysis showed an increase of 1.02 kg/cow/day in milk yield for cows grazing pastures containing an average of 434 g plantain per kg DM versus control pastures over the full lactation across all studies. The change was greater in late lactation than in early lactation, but the increased milk yield was not significantly associated with the content of plantain in the diet. In late lactation, a decline in the nutritive value of perennial ryegrass – white clover is often a limit to milk production (Kemp et al., 2000). As a drought and heat tolerant herb, plantain can maintain its nutritive value during summer and autumn, especially in dryland systems. In contrast, the good growing conditions in spring and early summer result in the difference in nutritive value between plantain and perennial ryegrass – white clover not being large enough for a measurable effect of plantain on milk production. The effect of the content of plantain on milk production may be lower than the seasonal effect, and insufficient to provide a linear regression across studies in the present analysis with different experimental conditions. The recorded content of plantain resulting in a significant increase in milk yield ranged from 300 g/kg DM (Minneé et al., 2017) to 900 g/kg DM (Box et al., 2017). However, some studies showed a similar milk yield within this range, either in early lactation (Pembleton et al., 2016) or late lactation (Minneé et al., 2020). This lack of variation in milk yield in response to varying contents of plantain in the pastures is probably because the nutritive value of plantain varied according to its age, development stage and farm condition (Moorhead and Piggot, 2009); therefore, an advantage of pastures containing plantain for milk production was not observed in a number of studies. For example, due to its heat and drought tolerance, plantain can provide a greater quality and quantity of forage feed than ryegrass under rain-fed farm systems and lower rainfall conditions in summer and autumn (Al-Marashdeh et al., 2021). As a result, plantain increases DMI and milk yield in most of the studies conducted under dryland farm systems (Waghorn et al., 2019; Minneé et al., 2020), but maintains DMI and milk yield in studies with irrigated pastures (Marshall et al., 2020; Al-Marashdeh et al., 2021). The differences in experimental conditions among studies could be the reasons causing the high heterogeneity of other outcomes such as milk fat yield and milk protein yield in the present analysis. However, the limited data sources do not allow us to conduct analysis to determine the effect of these factors.

Increased milk production due to plantain is associated with a higher DMI from pastures containing plantain and a change in herbage quality (Minneé et al., 2020; Wilson et al., 2020). However, DMI often varies greatly in grazing studies, so it is difficult to conclude differences within a single study. Previous studies showed different results for DMI from pastures containing plantain compared to traditional pastures, ranging from higher DMI (Box et al., 2017; Bryant et al., 2018) to similar DMI (Pembleton et al., 2016; Minneé et al., 2017). The present analysis of 22 comparisons from nine studies provides clear evidence that offering plantain increased DMI by 1.03 kg/cow/day. Pastures containing plantain have lower concentrations of ADF (–19 g/kg DM) and NDF (–80 g/kg DM), compared to control pastures, requiring less time for rumination and digestion than control pastures (Mangwe et al., 2019). This lower fibre concentration in pastures means that cows can consume and digest more pasture in the same period. In addition, adding plantain into a mixed sward could provide more opportunities for diet selection, assisting cows in having a higher DMI (Pembleton et al., 2016). A higher DMI from pastures containing plantain can result in a higher ME intake by cows, leading to greater milk production. The CP concentration in the plantain diet was slightly lower than in grass-based pastures, by 13 g/kg DM, in the present analysis. However, this is not expected to affect milk production because the mean CP in pastures containing plantain (198 g/kg DM) is adequate for lactating dairy cows (National Research Council, 2001).

### 4.2. Milk composition

The inclusion of plantain in the diet of dairy cows maintained milk protein concentration and milk fat yield, but increased protein yield, and reduced fat concentration. The increase in protein yield has been reported in previous studies as the main contributor to higher milk solids in cows grazing pastures containing plantain (Box et al., 2017; Wilson et al., 2020). To date, the only potentially negative impact of feeding plantain has been reported to be a depression in milk fat concentration (Dodd et al., 2018; Minneé et al., 2020), although other studies have also reported no effect of plantain on milk fat (Bryant et al., 2018; Nkomboni et al., 2021). In the present analysis, the milk fat concentration of cows grazing treatment pastures was 2.4 g/100 g milk lower than for control pastures. Key drivers for reducing fat concentration in milk may be related to differences in NDF content, the rumen pH and the ruminal passages of plantain and ryegrass. Lower NDF content in plantain could lead to an insufficient supply of acetate for de novo fat production, therefore, depressing milk fat concentration (Palmquist et al., 1993). Moreover, Minneé et al. (2017) indicated a lower pH in the rumen of cows fed plantain than ryegrass and white clover, which could be caused by a higher content of readily-degradable carbohydrates in plantain forage. This higher content of readily-degradable carbohydrates can restrict lipolysis and inhibit milk fat secretion in dairy cows (Chouinard et al., 1999). In addition, the fast ruminal passage of plantain reduces the exposure of forage lipids to lipolysis and biohydrogenation in the rumen (Dewhurst et al., 2003). If the reduction in milk fat concentration is associated with the change in carbohydrate content and the transformation of specific fatty acids in the rumen, then a supply of additional feed sources with plantain pastures could be an option to balance carbohydrate content. Further studies are required to investigate these effects of plantain and provide solutions to mitigate the decrease in milk fat concentration.

### 4.3. Nitrogen excretion

This meta-analysis provides strong evidence to support the hypothesis that incorporating plantain in the diet of dairy cows can reduce UN excretion and N-loading onto paddocks. The present study showed a reduction of 30% in urine-N concentration and 22% in total UN excretion, whilst increasing daily urine volume by 17% in cows grazing pastures containing an average of 434 g plantain per kg DM with the range from 140 to 900 g/kg DM. Linear regression curves (a), (b), and (c) allow reasonable estimations for the changes in N concentration, urine volume and UN excretion when the content of plantain in a mixed pasture is identified. These models can explain 69%, 64% and 22% of changes in UN concentration, urine volume and UN excretion, respectively, associated with the content of plantain within the range from 140 to 900 g/kg DM in the diet. More studies are required to improve the accuracy of estimation curves.

A reduction in UN excretion from dairy cows fed plantain has been extensively reported in previous studies (Mangwe et al., 2019; Minnée et al., 2020), and is associated with an increase in urine volume and a decrease in UN concentration in the urine. While the reduction in UN excretion is driven by the content of plantain in the diet, a certain proportion of plantain is required to achieve a significant effect. Most studies agree that diets containing 200 g plantain per kg DM or less do not affect UN concentration (Minnee et al., 2017), urine volume (Bryant et al., 2018), and total UN excretion (Minnee et al., 2020). The contents of plantain reported to have a significant effect were 300 g/kg DM for reducing UN concentration, 450 g/kg DM for increasing urine volume (Minnee et al., 2020), and 500 g/kg DM for decreasing UN excretion (Box et al., 2017). The regression models in the present study estimated a reduction of 15%, 21% and 27% in UN excretion when 300, 500 and 700 g plantain per kg DM were included in the diet of dairy cows, respectively. However, the effect of plantain on decreasing N losses from farms can be even greater than these figures due to the reduction of N loading at the paddock level. The dilution of N through increased urine volume can spread urine N further throughout the paddock, allowing pastures to uptake more N from the same amount of deposited urine N onto soils (Di and Cameron, 2007). The target content of plantain in swards is suggested to be at least 300 g/kg DM, especially during the critical period between late summer and autumn, to significantly reduce UN excretion onto paddocks. In practice though, at the farm level, it is difficult to maintain plantain content above 30% over an extended period, e.g. beyond three years (Dodd et al., 2019). Further studies are required to investigate the strategies to achieve and maintain this targeted content of plantain in mixed pastures.

A key mechanism for the lower UN concentration in cows eating plantain is the higher proportion of undegradable N (Minnee et al., 2020). The greater undegraded N content allows more N to pass through the rumen to be digested in the small intestine, where more N is partitioned to milk and faeces (Wilson et al., 2020), and less N is excreted into urine (Bryant et al., 2018). Minnee et al. (2020) reported an 11% reduction in N partitioned to urine, and a 14% increase in N partitioned to milk and faeces, when 450 g/kg DM plantain was included in the diet. Moreover, the higher water and mineral contents in plantain forage are potential causes of the increase in daily urine volume. Water content in plantain can be 5% higher than in ryegrass, resulting in a higher water intake in diets with more plantain (Minnee et al., 2020). The concentration of calcium, sodium (Na) and potassium (K) in plantain are higher than in perennial ryegrass (Sanderson et al., 2003; Box et al., 2018). High Na and K contents have been suggested as an explanation for increased urine volume from cows grazing plantain (Bannink et al., 1999; Spek et al., 2012). Mangwe et al. (2019) indicated that cows grazing plantain consumed 5.4 and 1.3 times more Na and K, and urinated 1.6 times more urine than cows grazing a perennial ryegrass-white clover pasture. In addition, the reduction in UN excretion might also be related to aucubin in plantain, which is associated with a reduction in ammonia production in the rumen fluid and causes diuresis (O'Connell et al., 2016).

## 5. Conclusion

The present review quantified the effect of plantain in pastures on increasing milk production and decreasing UN excretion of dairy cows. The analysis showed that grazing pastures containing an average of 43% plantain increased the yield of milk, milk solids, and milk protein, with a greater increase in late lactation. Fat concentration in milk was slightly reduced, but milk fat yield was maintained due to increased overall milk solids. Moreover, feeding plantain pastures reduced UN concentration and total UN excretion, while increasing the urine volume of dairy cows with the responses linear over the content of plantain within the range of 140–900 g/kg DM. These changes in N output can result in a lower N loading at paddock level, contributing to decreased N lost from dairy farms to the environment.

### CRedit authorship contribution statement

**Nguyen T. Thi:** Methodology, Data curation, Formal analysis, Writing – original draft. **S. Navarrete:** Supervision, Writing – review & editing. **D.J. Horne:** Supervision, Writing – review & editing. **D.J. Donaghy:** Supervision, Writing – review & editing. **P.D. Kemp:** Supervision, Writing – review & editing.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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