Plantain silage quality under variable management practices

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Abstract
Two studies investigated the effect of regrowth and additives on preservation and quality of plantain ensiled in spring using a micro-silage technique. Study 1 compared the effect of regrowth at four (4L), five (5L) or six leaf (6L) appearance. Study 2 compared fertilisers: 20 kg N/ha (20N), 20N plus potassium and phosphorus (20NPK) or 40 kg N/ha with P and K (40NPK) and additives: cellulose enzyme (ENZ), molasses (MOL), Biosil (BIO) or no additives (CON). After 180 days, wet chemistry was performed on all silages. Silages were dark brown and had a sweet smell, though fermentation was limited with an average pH of 5.2 and 5.8 in Study 1 and 2, respectively. However, pH declined ($P<0.05$) with early harvest, and use of N fertiliser or additives. Harvesting plantain for silage following a long regrowth is not recommended as the high stem content at this time contributed to low crude protein and low digestibility.

Keywords: *Plantago lanceolata*, nitrogen, inoculant, lactic acid

Introduction
For New Zealand farming systems, harvesting pasture for ensiling is an effective means of managing feed surpluses. The ensiled pasture, whether stored in a pit or wrapped as baleage, provides a cost-effective feed source during periods of feed deficit. To successfully ensile forages so that they may be stored and safely fed to livestock months later requires rapid removal of air so that reduction in pH via the production of lactic acid can occur, and maintenance of an anaerobic environment. Low pH caused by lactic acid preserves the forage preventing accumulation of harmful bacteria (Bolsen *et al*. 1996). Management factors which have shown to influence silage preservation and quality include: use of additives (Chamberlain & Robertson 1992; Kleinmans *et al*. 2011), regrowth interval and N fertilisation (Jacobs *et al*. 1998). Consequently, the protocols for ensiling ryegrass-based pastures are well established.

However, less is understood about the ensiling properties of alternative pasture species. The increasing adoption of forage plantain (*Plantago lanceolata*) into conventional pasture systems for nitrogen (N) loss mitigation (Woods *et al*. 2016; Box *et al*. 2016), emphasises the need for information on its management and conservation. There is little information on plantain silage; only one report was found that suggested plantain can be ensiled, but compared with ryegrass and lucerne (*Medicago sativa*) silages, plantain silage has lower digestibility (Raeside *et al*. 2012). The current study investigated the effects of management practises that influence preservation and quality of plantain silage.

Methods
Experimental site and treatments
Two experiments investigating the effects of regrowth interval (Study 1), fertiliser and additives (Study 2) on plantain silage, were conducted under irrigation at the Lincoln University Research Dairy Farm, Canterbury, New Zealand (43º64'S, 172º46'E). The plantain pastures (cultivar 'Tonic') were established following cultivation in March 2014 and were rotationally grazed with dairy cows (Box *et al*. 2017). On 14th November 2016 the experimental area for both studies (0.60 ha) was mown to 6 cm.

Study 1
Study 1 was a completely randomised design with three stages of maturity of plantain and five silo replicates. The plant maturity stages were defined as four (4L), five (5L) and six (6L) leaves (L)/plantain tiller. After mowing, urea fertiliser was applied at 25 kg N/ha and the area left to regrow until silage harvest on 14th, 21st and 28th of December corresponding to the 4L, 5L and 6L growth stages, respectively. Mowing took place between 1300 and 1400 h and herbage was wilted for 24-48 h or when herbage passed the "squeeze test" as described by Moran (2005). Dry matter content (%) was determined by oven-drying a subsample of 50 g fresh weight (FW) for 48 h at 60°C. Approximately 500 g of wilted plantain (leaf and stem, weeds and legume were discarded) was pressed firmly into a 23 x 38 cm plastic bag to remove any air. The mini silo was inserted into a second plastic bag which also had the air removed and all bags were then stored in black plastic drums in a shed for 180 days.

Study 2

Study 2 utilised a split-plot design with two fertiliser and three additive treatments and four blocks (replicates). The main plot fertiliser treatments were: N only at 20 kg/ha as urea (46% N), (20N); N, P and K at 20:1:15 kg/ha (20NPK), and N, P and K at 40:1:15 kg/ha (40NPK). The sub-plot additive treatments were: no additives (CON), cellulose enzyme (ENZ), Biosil, (BIO) and molasses (MOL). After mowing on 14th November 2016, treatment plots (each 10 m^2) were marked and the fertiliser treatments were applied by hand to designated plots. On 27 December 2016, all plots were mown to 6 cm height at 1600 h and ensiled once the herbage passed the "squeeze test".

Additives were made up to solutions and mixed with the forage immediately before ensiling as described in Study 1. Cellulose enzyme (Sigma Aldrich) was added at 125 mg/500 g wilted plantain (Tengerdy *et al*. 1991). The commercial product Biosil (combination of cellulase, hemicellulase, amylase and beta glucanase) was made as 1 mg/ml and 2 ml applied to 500 g of wilted plantain. Molasses was added to wilted plantain at 10 ml/500 g as recommended by Bolsen *et al*. (1996).

Silage analysis

After 180 days storage the mini-silos were visually assessed and the proportion of mould on the external surface area estimated. Upon opening the bags the aroma of the silage was noted as being either sweet (fermented) or sour (spoiled). Subsamples (25 g) were sorted into leaf and stem and oven-dried to determine botanical composition. The pH, buffering capacity (BC), lactic acid concentration and NH_3-N were all determined on fresh silage; BC (meq/100 g DM) was measured using the titration method of Playne & McDonald (1966). Briefly, 20 g of chopped fresh silage was macerated in 250 ml of distilled water and a pH meter (Thermo scientific, USA) was inserted to record the pH before titration to pH 3 and 6 using 0.1 N-HCl and 0.1 N-NaOH, respectively. Lactic acid and NH_3-N were measured using Randox (Rx Daytona, UK).

Dry matter percentage was determined by weighing a 50 g FW sample and oven-drying it at 60°C for 48 h. Nutritive characteristics were determined by wet chemistry using freeze-dried and ground herbage. Crude protein (CP), neutral (NDF) and acid detergent fibre (ADF) were measured using the method of Van Soest *et al.* (1991). Dry matter digestibility (DMD) was determined by the *in vitro* pepsin-cellulase method, modified from Clark *et al*. (1982).

Statistical analysis

To compare the effect of regrowth interval on preservation and quality variables, one-way analysis of variance (ANOVA) was performed using GenStat version 18 (VSN International, 2015), where regrowth was the fixed term and silo was the random term. For Study 2, the variables were compared using the split-plot design model with N fertiliser as the whole plot term and additive as the subplot term and block the random term.

Table 1 Effects of regrowth stage on fermentation characteristic of plantain silage.

Parameter	4L	5L	6L	SEM	P value
pH	5.13 ^{a}	5.00 ^{a}	5.52 ^{b}	0.06	<0.001
BC (meq/100 g DM)	146 ^{a}	210 ^{b}	156 ^{a}	7.19	<0.001
Lactic acid (% of DM)	0.25 ^{a}	0.59 ^{b}	0.18 ^{a}	0.04	<0.001
NH_3-N (%)	0.78	0.86	0.74	0.05	0.143

Means with different superscripts within rows are significantly different (P<0.05).
Where 4L is emergence of 4 leaves/plant, 5L is emergence of five leaves and 6L is emergence of six leaves.

Table 2 Effects of regrowth stage (leaf appearance) on nutritive value (% of DM) of silage plantain.

Parameter	4L	5L	6L	SEM	P Value
Dry matter[1]	38.7 ^{b}	28.7 ^{a}	32.5 ^{ab}	1.33	0.002
Organic matter	85.7 ^{a}	88 ^{b}	88.5 ^{b}	0.53	0.012
Crude protein	13.4	13.0	11.8	0.45.	0.082
Acid detergent fibre	33.0 ^{a}	36.0 ^{b}	39.6^c	0.35	<0.001
Neutral detergent fibre	45.6 ^{a}	48.7 ^{a}	52.8 ^{b}	0.69	<0.001
Dry matter digestibility	62.2 ^{b}	60.2 ^{b}	54.4 ^{a}	0.87	<0.001

Means with different superscripts within rows are significantly different (P<0.05). SEM is the standard error of the mean. [1] Dry matter includes volatile fatty acids and ammonia, and where 4L is the emergence of four leaves/plant, 5L is emergence of five leaves and 6L is emergence of six leaves.

Results

All silages were dark brown in colour and had a pleasant sweet smell. There was a large percentage of stem which accounted for 50.4, 59.5 and 73.6 ± 0.04% of DM for 4L, 5L and 6L silages, respectively, in Study 1. In Study 2, the percentage of stem was 72-90 ± 0.35% of DM. Mould was estimated to be 20, 34 and 47% of the external surface area for the 4L, 5L and 6L respectively, and 45-50% of the surface area,

in Study 2, although silos with the MOL treatment had the least mould (43%). The presence of mould was not visually evident in the middle of the bag as signs of mould had disappeared within 2 cm from the surface.

Study 1

The mean pH of plantain silage was 5.2 and was highest for 6L (Table 1). Buffering capacity (BC) and lactic acid were highest for 5L ($P<0.001$) silage. Across all regrowth stages the average CP content was less than 14% and DMD was less than 65%. However, DMD was highest at earlier stages of regrowth (4L and 5L) (Table 2). Increasing the regrowth interval increased ADF% of plantain silage and the NDF levels at 4L and 5L were lower than for 6L. There were no differences in CP contents across treatments.

Study 2

There was no interaction between fertiliser and additives for silage characteristics (Table 3). However, additives reduced pH compared to CON. The higher N fertiliser also resulted in lower pH. Average lactic acid was 0.54% and was greater by increasing fertiliser and by using MOL or BIO. By comparison, NH_3-N was low at an average of 0.57% and was increased by extra fertiliser and reduced by MOL.

The average CP content was 11.6% and DMD averaged 50.7% (Table 4). There was no interaction between additive and fertiliser on nutritive value of plantain silage (Table 4). Increasing N fertiliser reduced DM content. Adding molasses reduced NDF content while adding enzymes increased ADF content.

Table 3 Effect of additives and fertiliser on plantain silage pH, BC (meq/100gDM), lactic acid concentration (% of DM) and NH_3-N (%).

Fertiliser	Additive	pH	BC	Lactic acid	NH_3-N
20N	CON	6.30	256	0.23 ^{d}	0.52 ^{ab}
	BIO	5.63	201	0.65 ^{abc}	0.56 ^{ab}
	ENZ	6.18	270	0.52 ^{abcd}	0.45 ^{b}
	MOL	5.39	212	0.48 ^{abcd}	0.65 ^{ab}
20NPK	CON	6.43	297	0.45 ^{bcd}	0.50 ^{ab}
	BIO	5.71	282	0.79 ^{ab}	0.43 ^{b}
	ENZ	5.86	322	0.23 ^{d}	0.57 ^{ab}
	MOL	5.75	267	0.82 ^{a}	0.74 ^{a}
40NPK	CON	5.89	304	0.41 ^{cd}	0.61 ^{ab}
	BIO	5.31	298	0.56 ^{abcd}	0.58 ^{ab}
	ENZ	5.58	351	0.68 ^{abc}	0.71 ^{a}
	MOL	5.40	217	0.62 ^{abc}	0.52 ^{ab}
SEM		0.87	36.3	0.11	0.08
	Fertiliser	0.02	0.04	0.33	0.30
P value	Additive	<0.001	0.003	0.004	0.06
	A x F	0.66	0.57	0.035	0.009

Means with different superscripts within columns are significantly different ($P<0.05$). Where 20N is Nitrogen applied at 20 kg N/ha; 20NPK is N P K (20:1:15) and 40NPK is NPK (40:1:15)
Where CON is Control, BIO is Biosil, ENZ is Enzyme, MOL is molasses.

Table 4 Effect of additive and fertiliser on nutritive value characteristics (% of DM) for plantain silage.

Fertiliser	Additive	DM	OM	CP	ADF	NDF	DMD
20N	CON	27.3	90.8	11.1	40.8	56.5	50.7
	BIO	27.9	90.6	10.9	41.1	56.7	50.2
	ENZ	29.1	90.2	11.4	42.4	57.0	50.5
	MOL	28.4	90.3	11.1	41.3	54.5	52.8
20NPK	CON	25.6	90.1	11.7	43.0	57.4	50.8
	BIO	25.1	89.9	12.2	41.9	56.7	52.1
	ENZ	24.8	90.0	11.9	43.9	58.1	49.7
	MOL	25.5	90.7	11.5	40.6	55.4	49.5
40NPK	CON	23.9	90.3	11.4	41.6	58.1	48.3
	BIO	24.6	89.7	12.6	41.1	54.5	51.9
	ENZ	23.9	90.0	11.7	43.8	57.4	48.7
	MOL	26.5	90.4	11.4	40.9	54.4	53.8
SEM		1.19	0.34	0.44	0.87	0.98	1.29
	Fertiliser	<0.001	0.24	0.08	0.31	0.49	0.84
P value	Additive	0.65	0.32	0.44	0.01	0.008	0.09
	Fe x Ad	0.81	0.71	0.77	0.72	0.63	0.15

Where: DM is dry matter; OM organic matter; CP crude protein; ADF acid detergent fibre, NDF neutral detergent fibre and DMD *in vitro* dry matter digestibility; 20N is nitrogen applied at 20 kg DM/ha. 20NPK, N P K (20:1:15) and 40NPK, NPK (40:1:15). CON is Control, BIO is Biosil, ENZ is Enzyme and MOL is molasses.

Discussion

Preservation characteristics

Well fermented ryegrass silages should have a pH of 3.5-4.5, however, a survey by Howse *et al*. (1996) of pasture silages revealed the typical pH tended to be between 4.5 and 5.0. Plantain silage in this study, had a higher pH ranging from 5.0 to 6.4. The high initial pH may have inactivated plant proteases, reduced the extent of protein degradation maintaining a higher pH. Restriction of proteolysis caused by anti-microbial compounds such as aucubin or acteoside (Isselstein 1993a,b; cited by Stewart 1996) may have limited microbial activity and/or the slow degradability of plant proteins. Evidence of slow protein degradation by rumen bacteria has also been demonstrated for *in vitro* fermentation studies (Naverette *et al*. 2016). However, the production of NH_3-N in this study was low compared with many ryegrass-based silages (Howse *et al*. 1996). Low concentrations of ammonia are indicative of good quality silage as less N has been lost. Also, increasing the N fertiliser rate appeared to improve fermentation characteristics, as pH was lowest when 40 compared with 20 kg N/ha was applied. This response may be due to the increased N available for LAB (Lactic Acid Bacteria) or increased moisture content with the higher N fertiliser reducing DM%, making fermentation conditions more favourable.

Achieving low pH in silage requires rapid growth of LAB and the subsequent production of lactic acid. Lactic acid is needed to decrease pH (Muck 2004) and BC (Pahlow *et al*. 2003). Low pH and BC improves the aerobic stability of silage when exposed to air (Muck 2004). In this study, the high pH corresponds with the low lactic acid concentrations of less than 1% of DM. Growth of LAB may have been inhibited either by the lack of supply of either sugars or protein or both. Readily fermentable carbohydrates are the main energy source for LAB. Previous studies have reported low water soluble carbohydrates in plantain (Navarette *et al*. 2016). A deficiency in fermentable sugars was indicated by the positive response to the addition of molasses in the current study, which resulted in an increase in lactic acid with a drop in pH.

The availability of substrates for LAB is also influenced by cell rupture during the ensiling process. In this study, plantain was not macerated which may have slowed the availability of substrates. In preliminary investigations (3 months prior), comparing chopping lengths, showed little difference in looks or smell of mini-silos after 90 days. Because most commercial mowers do not chop forage this research replicated silage making as occurs at a commercial level. Although our results suggest there was little activity from LAB, the sweet smell of the silage indicated it was not spoiled. Anecdotal observations after feeding the silage to stock confirmed acceptable palatability.

Nutritive value of plantain silage

Overall, the quality of plantain silage was poor with CP content less than 14% and DMD under 65%, even for the youngest plantain (4L). However, the DMD of the silages in this study at the early growth stage (4L and 5L, Table 2) are similar to published values for DMD of ryegrass (62% DMD) and lucerne (61% DMD) silage stored for 42 days (Raeside *et al*. 2012). The low digestibility of the plantain silage in this study is likely explained by the high content of NDF which in both Study 1 and 2 exceeded 40% of DM. For the current study, herbage was harvested in December when seed head was prevalent, particularly at the later leaf stages and this likely contributed to high NDF and poor DMD. The negative effect of flowering on silage quality was also noted by Howse *et al*. (1996) in a survey of ryegrass-based silages, reporting that harvests occurring after November were of poorer quality as seed head started to develop.

The reduction in DMD with advancing maturity, as leaf appearance increased, was not unexpected as Lee *et al*. (2015) observed that younger plantain leaf and stem are higher in digestibility than that of older material. With regards to additives, there was little difference in DMD among treatments, but a marginal improvement in DMD when molasses was added might be explained by a dilution effect of sugars over fibre as NDF was reduced by MOL.

The implications of low CP under all management regimes trialled here may present some advantages in terms of a late-season low-N feed to reduce N intake and subsequent urinary N losses. From an environmental point of view, plantain silage may offer benefits as a low N supplement in autumn which is likely to be cheaper than alternative bought-in feeds. Furthermore, plantain silage has fewer health risks compared to high sugar crops such fodder beet (*Beta vulgaris*). Future research should focus on improving the digestibility of plantain silage.

Conclusions

The fermentation characteristics of plantain mini-silos did not follow conventional rules for adequate fermentation yet the odour characteristics indicated that the silage was not 'spoiled'. Additives and fertilisers, which improved conditions for lactic acid bacteria, did improve the preservation characteristics. Delaying harvest from four to the six leaf growth stage reduced silage quality because of a lower CP and DMD; this reduction in quality was not offset by using additives or fertiliser once seed head was prevalent at the sixth leaf stage. The poor quality of plantain silage demonstrated

in this study suggests it would not be suitable as a supplement in early lactation, but it could be fed to cows in autumn to reduce N intake and to aid drying-off.

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