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Fermentation Quality of Silages Produced from Wilted Sown Tropical Perennial Grass Pastures with or without a Bacterial Inoculant

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Abstract: High growth rates and rapid reproductive development and associated decline in feed quality of sown tropical perennial grass pastures present management challenges for livestock producers. Conservation of surplus forage as silage could be an effective management tool. Experiments were conducted to evaluate the fermentation quality of silages produced from tropical grasses. Five species (*Chloris gayana*, *Megathyrsus maximus*, *Panicum coloratum*, *Digitaria eriantha* and *Cenchrus clandestinus*) were ensiled without additives after a short, effective wilt at dry matter (DM) contents ranging from 302.4 to 650.1 g kg^{-1}. The fermentation profile of all silages in 2019 was typical for high DM silages, but in 2020 ammonia (% of total nitrogen: NH_3-N), acetic acid and pH levels were higher. In 2020 *M. maximus* (302.4 g kg^{-1} DM) was poorly preserved with 20.2% NH_3-N. The DM content of all other silages exceeded 350 g kg^{-1} and fermentation quality was generally good. In a second experiment, *M. maximus* was ensiled at 365 g kg^{-1} chopped and 447 g kg^{-1} DM chopped and unchopped, either without or with Pioneer 1171® (*Lactobacillus plantarum* and *Enterococcus faecium*) or Lallemand Magniva Classic® (*L. plantarum* and *Pediococcus pentasaceus*) bacterial inoculant. Inoculants increased lactic acid production, reduced pH and improved fermentation compared to Control, but D-lactate, L-lactate and acetic acid production differed between inoculants. Unchopped silages had higher pH and NH_3-N and better preserved protein fraction than chopped silages at the same DM content. In both experiments, wilting increased water soluble carbohydrates by 0.5–31.5 g kg^{-1} DM and ensiling increased degradation of the protein fraction. We concluded that a rapid and effective wilt combined with a bacterial additive resulted in well preserved tropical grass silages.

Keywords: tropical grass; silage; bacterial inoculant; forage conservation; protein degradation; lactic acid; fermentation

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1. Introduction

Tropical perennial grass pastures are an important forage resource for livestock production in regions across North and South America, Africa, Asia and Australia [1–3]. Production is typified by periods of rapid growth and high yields during the wet season, followed by deficits in quality and quantity during the dry season [2,4]. Traditionally in Australia, tropical pastures were sown across summer dominant rainfall environments of northern Australia; but, over recent decades, there has been expansion of tropical pastures into less traditional areas of south west Western Australia and northern New South Wales [5–8]. This expansion into more southerly locations with temperate environments is predicted to continue because of higher ambient temperatures caused by climate change [9]. However, compared to temperate species, the high growth rates and rapid onset of plant reproductive development (maturity) of tropical grasses present management challenges

for livestock producers. Increasing grazing pressure (stocking rate) to utilize rapid forage growth will maintain tropical grasses in a vegetative stage with higher forage quality for longer: but, in developed agricultural systems livestock numbers are typically based on the number of animals that can be grazed throughout the year. Consequently, they are below the stocking rate required to provide adequate grazing pressure during periods of high rates of pasture growth [10].

Harvesting and conserving surplus forage as silage effectively applies grazing pressure and returns the pasture to a vegetative growth stage, while also providing a stored forage reserve which can be used to offset periods of pasture deficit [4]. The importance of silages produced from tropical grass species is predicted to increase in order to meet the growing world demand for livestock products [11]. However, tropical grasses are considered difficult to ensile, principally because of the low concentration of plant sugars (water soluble carbohydrates: WSC) available for fermentation [12–16]. Silage is produced when WSC are fermented to acids, principally lactic acid in a well-fermented silage, under anaerobic conditions, which preserves the silage [17]. The recommended WSC content for a successful fermentation is between 50 and 86 g kg^{-1} dry matter (DM), depending on forage DM content when ensiled. This is based on previous recommendations of 25 to 30 g kg^{-1} (fresh basis) in the unwilted forage [12]. However, reports of tropical grass WSC content in the range of 10 to 70 g kg^{-1} DM are common [1,14,18]. Bacterial inoculants increase the efficiency and rate at which WSC are fermented to lactic acid, making better use of limited WSC, and have improved the fermentation characteristics of low WSC forages, including tropical grasses, at a range of DM contents [18–20]. The addition of other WSC sources, such as molasses, has improved fermentation, but the results were variable [15]. Moreover, it represents an additional cost and time input which is not profitable for large scale silage making operations.

A rapid and effective wilt concentrates WSC levels and is a management strategy that favors a rapid and effective lactic acid fermentation. Wilting of low DM forages prior to ensiling is recommended to reduce the growth of undesirable *Clostridium* species and the likelihood of a poor fermentation. Studies have shown that clostridial fermentations are not present in grass silages when forage DM contents exceeds 250 g kg^{-1} [21]. Furthermore, wilting to a DM over 300 g kg^{-1} is recommended to prevent silage effluent and the associated loss of soluble DM [22]. Generally, the target DM content of forage for ensiling in Australia is between 350 and 500 g kg^{-1}, being at the lower end for chopped and the higher end for baled silages [17]. Also, increasing forage DM content reduced the extent of fermentation because of reduced water activity in the silage [23]. Limited research with *Medicago sativa* L. demonstrated that chopping increases the rate and extent of fermentation compared to unchopped forage, presumably because WSC are more available to bacteria [24,25]. Consequently, less WSC is fermented when silages are produced at higher DM content in large bales from unchopped forage.

Research on best practice for ensiling of tropical grasses and legumes is limited compared to temperate species [4]. Research into ensiling tropical species has been conducted in Queensland, Australia; and, more recently, in areas of North and South America (principally Brazil), and Asia. However, much of this research was conducted on unwilted or lightly wilted forages, with DM contents < 250 g kg^{-1}, and therefore at risk of a poor fermentation. Consequently, farmer recommendations on producing silages from tropical species in Australia are based on imperfect data.

In this paper, we report on two experiments investigating the fermentation quality of silages produced from wilted tropical grasses. In the first experiment, we assessed the fermentation quality of forages produced from five tropical perennial grass species. In a second experiment, we assessed the effect of bacterial inoculants on silage quality for a single perennial grass species that was ensiled at two DM contents and, for the higher DM content, chopped or unchopped. The research was conducted to test the hypotheses that (1) good quality, well fermented silages can be produced from chopped or unchopped tropical grasses if adequately wilted; and (2) addition of a silage bacterial inoculant improves the fermentation quality of wilted tropical grass silage.

2. Materials and Methods

Two experiments were conducted at the New South Wales (NSW) Department of Primary Industries' Tamworth Research Institute, Tamworth (31.145917° S, 150.968021° E) to assess the quality and fermentation characteristics of silages produced from tropical grass species. The experiments were conducted in the same location in adjoining areas approximately 100 m apart on a brown chromosol soil which differed in pH_{Ca} between sites. In Experiment 1, pH_{Ca} was 8.2 at 0–0.1 m depth, increasing to 8.8–9.1 at 0.9–1.9 m [26]. In Experiment 2 pH_{Ca} was 6.9 at 0–0.1 m depth, increasing to 8.0–8.2 at 0.9–1.9 m [27]. The pastures were grown under dryland conditions with supplementary irrigation provided to assist establishment and achieve a sward for harvesting.

In Experiment 1, five tropical grasses were sown as pure swards into plots 12 × 6.5 m on 20 November 2018 at commercial sowing rates: Rhodes grass (*Chloris gayana* Kunth cv. Katambora), two panics (*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs syn *Panicum maximum* Jacq. cv. Gatton and *P. coloratum* L. cv. Bambatsi), digit grass (*Digitaria eriantha* Steud. cv. Premier) and kikuyu (*Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone syn *Pennisetum clandestinum* Hochst. ex Chiov. cv. Whittet). Irrigation (34 mm) was applied over a 5 day period following sowing to assist establishment. All grasses established well despite receiving below average rainfall throughout summer, except for Bambatsi panic which had a lower population. Silages were produced from the plots on two occasions: November 2019 and March 2020. In October 2019, the experimental area was mown to 0.1 m and the cut material removed. Fertilizer was applied on 22 October (11 kg/ha phosphorus [P], 13.8 kg/ha sulphur [S] and 46 kg nitrogen [N]) and 57 mm irrigation applied over 2 days. On 5 November, the experimental area was mown again, the material removed, and irrigation (100 mm) applied. All grasses were cut for ensiling on 27 November, after 22 days regrowth. In 2020 the plots were mown, and material removed on 29 January and 24 February. Fertiliser was applied on 31 January (65 kg/ha N) with 50 mm irrigation, and 17 February (11 kg/ha P, 13.8 kg/ha S and 100 kg/ha N) with 25 mm rainfall. All forages were cut for ensiling on 11 March 2020 (16 days regrowth) except kikuyu which was cut on 19 March. Bambatsi panic was not ensiled in 2020 due to low yield.

Forages were cut to 0.1 m, except Whittet kikuyu which was cut to 0.05 m, using an Allan scythe and allowed to wilt undisturbed on the cut area for 3 to 5 h in 2019 and 18 to 24 h in 2020 prior to ensiling. Random samples of fresh and wilted forage were collected at cutting and at the time of ensiling (n = 2 per sample type) and stored frozen (−18 °C). The wilted forage was manually collected and chopped into approximately 45 mm lengths using a Ryobi® 2400W electric mulching shredder (Ryobi Limited, Fuchu, Hiroshima, Japan) prior to ensiling in plastic bag mini-silos (n = 3 per species) made of 100 µm polyethylene [28]. Each bag contained 3–6 kg of fresh forage when ensiled. The chopped forage was physically compacted, air evacuated from the bag with a household vacuum cleaner and the bag opening tied securely to obtain an airtight seal. Then, each bag was placed inside a second bag of the same type and the vacuuming and tying process repeated. The bags were packed into 200 L drums surrounded by damp sand with a layer of damp sand on the top to maintain weight on the bags. All silages were opened on 22 July 2020, the silage was thoroughly mixed, and a subsample was taken to determine dry matter content and chemical composition.

In Experiment 2, a 6 year old sward of Premier digit grass was prepared from October 2020 as a source of forage for conservation. Fertiliser (100 kg N ha^{-1}) was applied on 13 January 2021 and the sward was mown and the cut material removed on the 28 January 2021. On 16 February 2021, after 19 days regrowth, average plant growth stage was 3.5 leaves, with some tillers on approximately 30% of plants commencing stem elongation. A section of the pasture sufficient to make all silage treatments was cut to 0.1 m using an Allan scythe at approximately 9.50 am and allowed to wilt. A relatively low yield (675 kg DM ha^{-1}) combined with high temperatures and intense sun resulted in a rapid wilt. Random samples of wilted forage were collected manually when DM content reached approximately 350 g kg^{-1} (11.35 am) and 450 g kg^{-1} (2.00 pm) on the same day. Hereafter

these samples are referred to as W1 and W2 with the unwilted forage referred to as W0. Random samples of fresh and wilted forage were collected at cutting and at the time of ensiling (n = 2 per sample type) and stored frozen as in Experiment 1.

The forage was ensiled either chopped (both W1 and W2) using a garden mulcher as in Experiment 1, or unchopped (only W2). Hence there were three forage treatments (chopped at 350 g kg^{-1} DM, chopped at 450 g kg^{-1} DM or unchopped at 450 g kg^{-1} DM). Additionally, there were three additive treatments: nil (Control), Pioneer® 1174 and Lallemand® Magniva Classic bacterial inoculant; hereafter referred to as Control, 1174 and Classic respectively. The 1174 contained selected strains of *Lactobacillus plantarum* and *Enterococcus faecium* and was applied at the rate of 250,000 colony forming units (CFU) g^{-1} fresh forage. Classic contained selected strains of *L. plantarum* and *Pediococcus pentosaceus* in a ratio of 9:1 and was applied at the rate of 200,000 CFU g^{-1} fresh forage. These were applied at 100 mL of inoculant per 25 kg of fresh forage, adjusted so that each provided the recommended rate of bacteria application per tonne of fresh forage. The Control silages had the same rate of water applied. Forages were ensiled in plastic bag mini-silos (n = 3 per treatment) and stored as described in Experiment 1. Silages were opened on 17 July 2021 and the silage was thoroughly mixed and subsampled as in Experiment 1.

Thawed forage samples were dried at 80 °C in a fan-forced oven to determine DM content [29], and ground through a Perten® 3100 laboratory mill fitted with a 1 mm screen prior to laboratory analyses. Water soluble carbohydrates content was determined using AFIA method 1.11A and nitrogen (N) was determined using the Dumas combustion method with a Leco CNS 2000® analyser (Leco, St. Joseph, MI, USA) (AFIA Method—1.6R) [30,31]. Crude protein (CP) content was calculated as N × 6.25 [30]. Organic matter (OM) was determined by heating samples in a muffle furnace at 550 °C for six hours. Starch was determined by flow injection analysis after preparation with alkaline ferricyanide according to AOAC 996.11 [32]. Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined sequentially using the filter bag method (Ankom® 200/220 fibre analyser, ANKOM technology, Macedon, NY, USA) [33]. Predicted in vivo digestibility (digestible organic matter (DM basis): IVDOMD) was determined by the pepsin/cellulase technique (AFIA Method—1.7R) and used to calculate metabolizable energy (MJ kg^{-1} DM: ME) content [30].

Silage samples were dried at 80 °C in a fan-forced oven to determine DM content. Silage pH was determined using an Activon Model 210 pH meter (Activon Scientific Products Co Pty Ltd., Sydney, NSW, Australia). Nitrogen was determined on fresh silage and WSC content on dry silage using the same methods as for forage [30,31]. Ammonia nitrogen (NH_3-N) content was determined by Kjeldahl titration of an acid extract prepared from fresh silages (AFIA Method—1.6R) [30]. Protein fractions were determined by the method of Licitra et al. [30,34]. Volatile fatty acids were determined by gas chromatography (Agilent Model 6890N with Agilent Chemstations software; Agilent Technologies, Inc., Santa Clara, CA, USA) using a modification of the method of Porter and Murray (2001) [35]. Briefly, silage extracts were prepared by weighing 20 g silage and adding 100 mL of extracting solution composed of 1% orthophosphoric acid and 1% formic acid and containing 184 ppm 4-methyl valeric acid as an internal standard.

Data were analyzed using the REML function within Genstat for both experiments [36]. In Experiment 1, species, year (2019 or 2020), sample type (at cutting or after wilting) and all interactions were the fixed effects for forages; species, year (2019 or 2020) and their interaction were the fixed effects for silages; and species, year (2019 or 2020), sample type (at cutting, after wilting or silage) and all interactions were the fixed effects for the analyses of protein fractions. In Experiment 2, wilt was the sole fixed effect for forages, while treatment, defined as the individual DM, inoculant and chop length combination, was the sole fixed effect for silages. Protein fractions were analyzed with each combination of sample type (forage or silage) and level of wilting as individual data points for fixed effects. There were no random effects fitted for either experiment.

3. Results

3.1. Experiment 1

Yields in November 2019 were not recorded but were observed to be lower than typical for these species. The average number of leaves per plant and the proportion of plants showing stem elongation for each species at cutting were: Katambora Rhodes, 5.5, < 5%; Gatton panic, 4, 0%; Bambatsi panic, 5.5, 20%; Premier digit grass, 4.5, 5% and Whittet kikuyu, 5.5, 0%. The Bambatsi panic plots also contained 5% of plants that were flowering. In 2020 yields were 2310, 1644, 564 and 805 kg DM ha^{-1} for Katambora, Premier digit, Whittet kikuyu and Bambatsi panic respectively. Gatton panic was not wilted and ensiled due to low yield (not recorded). The average number of leaves per plant and the proportion of plants showing stem elongation and flowering for each species at cutting were: Katambora Rhodes, 5.6, 5%, 0%; Gatton panic, 4.6, 2%, 0%; Bambatsi panic, 4.8, 70%, 3%; Premier digit, 4.6, 80%, 5% and Whittet kikuyu, 6.3, not applicable, 5%. Forage composition for both years is presented in Table 1.

Table 1. Composition [1] (g kg^{-1}) of tropical perennial grasses grown at Tamworth Agricultural Institute at the time of cutting (Cut) and ensiling (Ensile) in November 2019 and March 2020.

Species	DM [1]		WSC [2]		Starch [3]	ADF [3]	NDF [3]	IVDOMD [3]	CP [3]
	Cut	Ensile	Cut	Ensile					
				November 2019					
Katambora Rhodes grass	210.1	453.1	43.5 ^{b}	49.5 ^{d}	22.1 ^{ab}	283.6 ^{bcd}	590.2 ^{cd}	611.1 ^{ab}	184.2 ^{c}
Premier digit	262.8	650.1	34.5 ^{a}	50.5 ^{de}	27.4 ^{bcd}	299.3 ^{cd}	620.9 ^{e}	616.3 ^{bc}	170.6 ^{b}
Whittet kikuyu	267.9	615.4	58.5 ^{g}	63.5 ^{h}	29.0 ^{cd}	211.8 ^{a}	495.5 ^{a}	695.4 ^{e}	247.6 ^{g}
Gatton panic	242.7	515.6	42.0 ^{b}	69.5 ^{i}	40.1 ^{e}	240.6 ^{ab}	532.7 ^{b}	653.1 ^{d}	207.8 ^{e}
Bambatsi panic	238.3	464.6	48.5 ^{d}	62.5 ^{h}	32.8 ^{d}	265.7 ^{bc}	624.5 ^{e}	594.1 ^{a}	192.6 ^{cd}
				March 2020					
Katambora Rhodes grass	192.2	442.3	54.0 ^{ef}	54.5 ^{f}	25.1 ^{abc}	298.6 ^{cd}	582.7 ^{cd}	624.9 ^{bc}	167.5 ^{b}
Premier digit	180.9	376.6	47.5 ^{cd}	54 ^{ef}	20.5 ^{a}	314.8 ^{d}	626.3 ^{e}	625.4 ^{bc}	189.4 ^{cd}
Whittet kikuyu	220.2	415.3	69.0 ^{i}	77.0 ^{j}	22.4 ^{ab}	262.3 ^{bc}	573.0 ^{c}	630.8 ^{c}	152.4 ^{a}
Gatton panic	224.1	302.4	69.5 ^{i}	101.0 ^{k}	63.8 ^{f}	242.2 ^{ab}	512.6 ^{ab}	685.7 ^{e}	193.5 ^{d}
Bambatsi panic [4]	167.5	-	44.5 ^{bc}	-	24.9 ^{abc}	264.8 ^{bc}	603.0 ^{de}	625.6 ^{bc}	234.6 ^{f}
p value	not analyzed		0.003		0.009	<0.001	<0.001	<0.001	<0.001

[1] DM: oven dry matter content; WSC: water soluble carbohydrates; ADF: acid detergent fibre; NDF: neutral detergent fibre; IVDOMD: digestible organic matter (dry matter basis); CP: crude protein. Expressed as g kg^{-1} DM unless otherwise indicated. [2] Significant species x year x sample type interaction. [3] Significant species x year interaction. Data averaged over sample type. Values within a parameter with different superscript letters are significantly different ($p < 0.05$). [4] Not ensiled.

Forage WSC content ranged from 34.5 to 101.0 g kg^{-1} DM, and varied with the interaction between species, year and sample type ($p = 0.003$). Water soluble carbohydrates content of Katambora Rhodes grass, Premier digit, Whittet kikuyu and Bambatsi panic at cutting was lower ($p < 0.05$) in 2019 compared to 2020, while that of Gatton panic was higher ($p < 0.05$). Wilting increased ($p < 0.05$) WSC content for all species in 2019 and 2020 but the increase was not consistent between species. The proportional increase ranged from 0.9% for Katambora Rhodes grass in 2020 to 65.5% for Gatton panic in 2019. Whittet kikuyu had the highest ($p < 0.05$; 2019) or equal highest ($p < 0.05$; 2020) WSC content at cutting, but not after wilting. In contrast, Premier digit had the lowest or equal lowest ($p < 0.05$) WSC content at cutting in both years. Starch, ADF and NDF content varied with the interaction between species and year ($p < 0.001$). Starch content declined between cutting and ensiling ($p < 0.05$), but the decline was greater in 2020 (42.1 vs. 20.6 g kg^{-1} DM) than observed in 2019 (35.3 vs. 25.2 g kg^{-1} DM), while the starch content at ensiling did not vary between years. In contrast, ADF and NDF content were unaffected by wilting.

Crude protein content at cutting ranged from 152.4 to 247.6 g kg^{-1} DM and the interaction between species and year was significant ($p < 0.001$). Highest CP content was observed for Whittet kikuyu in 2019 and Bambatsi panic in 2020, with both exceeding

200 g kg^{-1} DM, as did Gatton panic in 2019. The CP content of Katambora Rhodes grass, Whittet kikuyu and Gatton panic was higher ($p < 0.05$) in 2019 compared to 2020; while the reverse was observed for Premier digit and Bambatsi panic. Crude protein content increased ($p < 0.05$) between cutting (184.1 g kg^{-1} DM) and ensiling (190.9 g kg^{-1} DM) in 2020, with no change in 2019 (201.5 vs. 199.7 g kg^{-1} DM). Crude protein content of the fresh forage and silages is presented in Table 2. Digestibility (IVDOMD) ranged from 594.1 to 695.4 g kg^{-1} DM and there was a significant interaction ($p < 0.001$) between species and year. Highest IVDOMD was observed on Whittet kikuyu in November 2019 which was not different to Gatton panic in March 2020. Digestibility of Whittet kikuyu was higher ($p < 0.05$) and Gatton panic and Bambatsi panic lower ($p < 0.05$) for 2019 compared to 2020.

In 2019, the ensiled forages were drier than preferred for chopped silage. The DM of Katambora Rhodes grass, Gatton panic and Bambatsi panic were in the target range for baled silages, while that of Premier digit and Whittet kikuyu was too high. In 2020, Premier digit and Whittet kikuyu were within the normal range for fine chop silage while Katambora was slightly higher and closer to that of baled silage. When opened the silages generally had a typical odour associated with well fermented silages. Composition of the silages are presented in Table 3.

Silage pH values in 2019 were typical for silages having undergone a restricted fermentation with limited acid production, consistent with the DM contents; while the 2020 silages were all above pH 6, implying that essentially no fermentation had occurred. There was insufficient sample available to analyse samples from either year for lactic acid content to confirm the lack of fermentation. Consequently, the dried silage samples were analysed to determine the levels of residual WSC (data not presented) and the reduction in WSC calculated. The loss in WSC ranged from 25.2 to 86.3 (average 38.9) g glucose kg^{-1} DM, equivalent to 0.039 to 0.252 (average 0.114) mol glucose kg^{-1} DM (data not analysed). In 2019 silage fermentation quality, as indicated by NH_3-N content, were either excellent (<5%) or good (5–10%), whereas in 2020 the NH_3-N content of Premier digit was moderate (10–15%) and Gatton panic poor (>15%).

The level of volatile fatty acids (VFA) was low for all silages, with a significant interaction between species and year for several acids: acetic, propionic, butyric, valeric, iso-valeric, hexanoic and total VFA. Total VFA content was higher ($p < 0.05$) for most species in 2020 than 2019 and acetic acid was the major acid, averaging 91.6% of total VFA across all silages. Acetic acid content was highest ($p < 0.05$) for Gatton panic followed by Premier digit in 2020, and lowest ($p < 0.05$) for Premier digit and Whittet kikuyu in 2019. Acetic acid content ranged from 1.55 to 16.79 (average 6.36) g kg^{-1} DM equivalent to 0.026 to 0.280 (average 0.106) mol kg^{-1} DM. Highest ($p < 0.05$) levels of propionic acid, butyric acid and valeric acid were observed with Gatton panic, iso-valeric acid for Premier digit and Whittet kikuyu and hexanoic acid for Premier digit, all in 2020. Gatton panic and Premier digit in 2020 had the highest ($p < 0.05$) ethanol content, while Katambora (2019 and 2020) and Premier digit (2019) had the lowest ($p < 0.05$) or equal lowest ethanol content. Ethanol was higher ($p < 0.05$) in 2020 compared to 2019 for Gatton panic and Premier digit.

The proportion of CP present as NPN, true protein (TP), insoluble protein (IP) and neutral detergent insoluble crude protein (NDICP) varied with the interaction between species, year, and sample type (all $p < 0.001$). The level of NPN declined ($p < 0.05$) between cutting and wilting for all species except Premier digit in 2019, but only for Katambora Rhodes grass in 2020. The proportion of N present as NPN was higher ($p < 0.05$) and the proportion of N present as TP, IP or NDICP was lower ($p < 0.05$) in the silages compared to the forages at both cutting and after wilting. Premier digit in 2019 had the lowest ($p < 0.05$) level of NPN, and the highest ($p < 0.05$) level of TP, IP and NDICP of all forages and silages. Furthermore, the increase in NPN (15.3%) for Premier digit in 2019 was less than other species and years, which ranged from 35.6 to 94.6%. In contrast, Katambora Rhodes and Bambatsi panic 2019 forage had the highest ($p < 0.05$) NPN and lowest ($p < 0.05$) TP and IP. Lowest ($p < 0.05$) NDICP was for Katambora Rhodes in 2019 and 2020 and Whittet kikuyu in 2019. Forage acid detergent insoluble crude protein (ADICP) was unaffected by species, year, or sample type, averaging 56.6 g CP kg^{-1} CP.

Table 2. Protein fraction components (g CP kg^{-1} CP) of fresh (Fresh) and wilted (Wilt) forages and silages (Silage) produced from tropical perennial grasses grown at Tamworth Agricultural Institute and harvested and ensiled in November 2019 and March 2020.

Pasture Species	Non-Protein Nitrogen			True Protein			Insoluble Protein			Neutral Detergent Insoluble		
	Fresh	Wilt	Silage	Fresh	Wilt	Silage	Fresh	Wilt	Silage	Fresh	Wilt	Silage
						November 2019						
Katambora Rhodes grass	423.5 $^{d}_{b}$	363.3 $^{f}_{a}$	607.0 $^{e}_{c}$	576.5 $^{a}_{b}$	636.7 $^{a}_{c}$	393.0 $^{b}_{a}$	587.3 $^{a}_{b}$	634.0 $^{a}_{c}$	382.2 $^{b}_{a}$	300.9 $^{ab}_{b}$	335.7 $^{b}_{c}$	191.0 $^{c}_{a}$
Premier digit	260.2 $^{a}_{a}$	242.6 $^{a}_{a}$	299.9 $^{a}_{b}$	739.7 $^{d}_{a}$	757.5 $^{f}_{a}$	700.1 $^{f}_{b}$	736.4 $^{e}_{b}$	748.0 $^{d}_{b}$	693.5 $^{f}_{a}$	520.2 $^{f}_{b}$	467.6 $^{e}_{a}$	484.6 $^{f}_{a}$
Whittet kikuyu	313.4 $^{b}_{b}$	246.1 $^{a}_{a}$	432.2 $^{b}_{c}$	686.6 $^{c}_{b}$	753.9 $^{f}_{c}$	567.8 $^{e}_{a}$	675.7 $^{cd}_{b}$	751.4 $^{d}_{c}$	557.7 $^{e}_{a}$	288.5 $^{a}_{b}$	302.2 $^{a}_{b}$	243.8 $^{d}_{a}$
Gatton panic	327.5 $^{b}_{b}$	278.2 $^{b}_{a}$	511.7 $^{c}_{c}$	672.5 $^{c}_{b}$	721.8 $^{e}_{c}$	488.3 $^{d}_{a}$	675.4 $^{cd}_{b}$	714.7 $^{c}_{c}$	473.6 $^{d}_{a}$	383.8 $^{cd}_{b}$	404.9 $^{d}_{b}$	250.3 $^{d}_{a}$
Bambatsi panic	402.0 $^{d}_{b}$	352.3 $^{ef}_{a}$	553.8 $^{d}_{c}$	598.0 $^{a}_{b}$	647.7 $^{ab}_{c}$	446.2 $^{c}_{a}$	593.6 $^{a}_{b}$	649.5 $^{ab}_{c}$	438.0 $^{c}_{a}$	408.4 $^{d}_{b}$	429.8 $^{d}_{b}$	246.0 $^{d}_{a}$
						March 2020						
Katambora Rhodes grass	368.0 $^{c}_{b}$	329.3 $^{de}_{a}$	519.4 $^{c}_{c}$	632.0 $^{b}_{b}$	670.7 $^{bc}_{c}$	480.6 $^{d}_{a}$	624.4 $^{b}_{b}$	657.3 $^{ab}_{c}$	460.1 $^{cd}_{a}$	311.3 $^{ab}_{b}$	369.3 $^{c}_{c}$	228.2 $^{d}_{a}$
Premier digit	301.4 $^{b}_{a}$	294.9 $^{bc}_{a}$	408.6 $^{b}_{b}$	698.6 $^{c}_{b}$	705.1 $^{de}_{b}$	591.4 $^{e}_{a}$	693.5 $^{d}_{b}$	697.9 $^{c}_{b}$	579.4 $^{e}_{a}$	476.4 $^{e}_{b}$	482.0 $^{e}_{b}$	362.0 $^{e}_{a}$
Whittet kikuyu	324.7 $^{b}_{a}$	307.6 $^{cd}_{a}$	597.3 $^{e}_{b}$	675.3 $^{c}_{b}$	692.4 $^{cd}_{b}$	402.7 $^{b}_{a}$	656.7 $^{c}_{b}$	671.3 $^{b}_{b}$	383.2 $^{b}_{a}$	327.9 $^{b}_{b}$	352.3 $^{bc}_{b}$	156.7 $^{b}_{a}$
Gatton panic	326.3 $^{b}_{a}$	320.0 $^{cd}_{a}$	634.9 $^{f}_{b}$	673.7 $^{c}_{b}$	680.0 $^{cd}_{b}$	365.1 $^{a}_{a}$	667.2 $^{c}_{b}$	671.7 $^{b}_{b}$	343.2 $^{a}_{a}$	373.4 $^{c}_{b}$	375.2 $^{c}_{b}$	125.9 $^{a}_{a}$
p value [1]		<0.001			<0.001			<0.001			<0.001	

[1] *p* value for the species × year × sample type (fresh, wilt or silage) interaction. Values in the same column with different superscript letters are significantly different ($p < 0.05$). Values for the same parameter in the same row with different subscript letters are significantly different ($p < 0.05$).

Table 3. Composition of silages produced from tropical perennial grasses grown at Tamworth Agricultural Institute and harvested and ensiled in November 2019 and March 2020.

Pasture Species	DM [1] (g kg^{-1})	CP [1] (g kg^{-1} DM)	pH	NH_3-N [1] (% of Total N)	WSC [2] (g kg^{-1} Fresh)	Volatile Fatty Acid (g kg^{-1} DM)								Ethanol (g kg^{-1} DM)
						Acetic	Propionic	Butyric	Iso-Butyric	Valeric	Iso-Valeric	Hexanoic	Total	
November 2019														
Katambora Rhodes grass	426.6 ^{cd}	184.3 ^{d}	4.9 ^{a}	5.1 ^{b}	22.4 ^{ab}	5.81 ^{c}	0.18 ^{b}	0.009 ^{a}	0.18	0.017 ^{b}	0.039 ^{ab}	0.004 ^{a}	6.29 ^{c}	0.147 ^{a}
Premier digit	666.1 ^{g}	177.5 ^{c}	5.9 ^{bc}	2.5 ^{a}	32.8 ^{e}	1.55 ^{a}	0.07 ^{a}	0.010 ^{a}	0.12	0.008 ^{a}	0.031 ^{a}	0.004 ^{a}	1.82 ^{a}	0.114 ^{a}
Whittet kikuyu	579.3 ^{f}	264.0 ^{h}	5.8 ^{b}	2.3 ^{a}	39.1 ^{g}	1.55 ^{a}	0.05 ^{a}	0.007 ^{a}	0.12	0.005 ^{a}	0.027 ^{a}	0.002 ^{a}	1.78 ^{a}	0.182 ^{b}
Gatton panic	502.3 ^{e}	207.9 ^{g}	5.6 ^{b}	6.7 ^{c}	35.8 ^{f}	3.67 ^{b}	0.04 ^{a}	0.018 ^{ab}	0.21	0.003 ^{a}	0.031 ^{a}	0.008 ^{a}	4.03 ^{b}	0.185 ^{b}
Bambatsi panic	420.6 ^{cd}	196.7 ^{f}	4.9 ^{a}	6.4 ^{c}	29.0 ^{c}	5.65 ^{c}	0.17 ^{b}	0.027 ^{b}	0.11	0.020 ^{b}	0.049 ^{bc}	0.010 ^{a}	6.09 ^{c}	0.177 ^{b}
March 2020														
Katambora Rhodes grass	444.5 ^{d}	167.3 ^{b}	6.7 ^{d}	6.9 ^{c}	24.1 ^{b}	6.93 ^{c}	0.03 ^{a}	0.010 ^{a}	0.13	0.003 ^{a}	0.032 ^{a}	0.006 ^{a}	7.19 ^{c}	0.144 ^{ab}
Premier digit	354.4 ^{b}	186.8 ^{de}	6.2 ^{c}	10.5 ^{d}	20.3 ^{a}	11.71 ^{d}	0.10 ^{ab}	0.011 ^{a}	0.41	0.006 ^{a}	0.069 ^{d}	0.105 ^{c}	12.46 ^{d}	0.276 ^{c}
Whittet kikuyu	404.0 ^{c}	157.4 ^{a}	6.2 ^{c}	6.5 ^{c}	32.0 ^{de}	3.59 ^{b}	0.05 ^{a}	0.012 ^{a}	0.19	0.005 ^{a}	0.057 ^{cd}	0.009 ^{a}	3.96 ^{b}	0.181 ^{b}
Gatton panic	263.7 ^{a}	189.6 ^{e}	6.2 ^{c}	20.2 ^{e}	30.5 ^{cd}	16.79 ^{e}	0.38 ^{c}	0.047 ^{c}	0.21	0.029 ^{c}	0.048 ^{bc}	0.042 ^{b}	17.61 ^{e}	0.301 ^{c}
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	0.088	<0.001	0.003	<0.001	<0.001	<0.001

[1] DM: oven dry matter; CP: crude protein; NH_3-N: ammonia nitrogen as a % of total N. [2] WSC: water soluble carbohydrates content of the forage at the time of ensiling. Values in the same column with different superscript letters are significantly different ($p < 0.05$).

3.2. Experiment 2

Composition of the pasture was determined at the time of cutting and both times of ensiling. Digestibility, fibre, starch, CP, NPN and TP content did not change during wilting (Table 4). However, WSC increased ($p < 0.05$) with wilting periods, consistent with previous observations with summer growing species (Table 5). Water soluble carbohydrate content of the forage at the time of ensiling was 21.2 and 29.1 g kg^{-1} of fresh forage for the W1 and W2 treatments respectively. Both neutral and acid detergent insoluble CP content also declined by approximately 15% following the longer wilt (Table 5).

Table 4. Composition of Premier digit grass at mowing and ensiling.

Digestible organic matter (DM basis) (g kg^{-1})	606.7
Neutral detergent fibre (g kg^{-1} DM)	645.0
Acid detergent fibre (g kg^{-1} DM)	315.0
Starch (g kg^{-1} DM)	21.7
Crude protein (g kg^{-1} DM)	138.7
Non-protein nitrogen (g CP kg^{-1} DM)	138.7
True protein (g CP kg^{-1} DM)	117.0

No effect of wilting on these parameters.

Table 5. Composition of Premier digit grass at Tamworth Agricultural Institute at the time of cutting (W0) and after two wilting periods (W1 and W2).

Component	Length of Wilt			*p* Value
	W0	W1	W2	
Dry matter content (g kg^{-1})	223	365	447	NA
Water soluble carbohydrate (g kg^{-1} DM)	43.5 ^{a}	58.0 ^{b}	65.0 ^{c}	0.003
Neutral detergent insoluble protein (g CP kg^{-1} DM)	81.5 ^{b}	81.0 ^{b}	69.5 ^{a}	0.046
Acid detergent insoluble protein (g CP kg^{-1} DM)	11.0 ^{b}	10.5 ^{b}	9.0 ^{a}	0.033

Values in the same row with different superscript letters are significantly different ($p < 0.05$). NA: not analyzed.

Upon opening, all silages appeared well fermented, with no adverse fermentation products apparent based on visual and olfactory appraisal. Composition of the silages are shown in Table 6. Addition of an inoculant reduced ($p < 0.05$) both pH and NH_3-N compared to the Control at both DM contents (W1 and W2) and physical forms (chopped and unchopped), clearly showing the effect of improved fermentation. However, despite the high pH observed in the Control silages, fermentation quality was still excellent (<5% NH_3-N) or good (5–10% NH_3-N) for all silages.

Dry matter content of the W1 chopped and W2 unchopped Control silages was less ($p < 0.05$) than for the inoculated silages, whereas the DM content of W2 chopped 1174 was lower ($p < 0.05$) than observed for the other two W2 chopped silages. Chopping increased ($p < 0.05$) DM content of the Control but reduced ($p < 0.05$) DM content of the 1174 silages at W2. A lower DM determined by oven drying can indicate a more extensive fermentation because more volatile compounds are lost during the drying process.

The level of VFA for all silages (Table 6) was consistent with well-preserved, wilted silages, and similar to values observed for well-preserved silages produced in Experiment 1. Acetic acid represented 94.2–98.4% of the total VFA measured. Acetic acid levels were higher ($p < 0.05$) in the W1 compared to the W2 silages, and higher for the 1174 compared to the Control and Classic silages. Propionic and butyric acid content did not vary with treatment, averaging 0.017 and 0.016 g kg^{-1} DM respectively.

Table 6. Effect of dry matter content (Wilt), physical form (Form) and inoculant on the composition[1] of silages produced from Premier digit grass.

Wilt [1]	Form	Inoculant	DM [3] (g kg^{-1})	CP (g kg^{-1} DM)	pH	NH_3-N [2] (% of Total N)	Lactic Acid (g kg^{-1} DM)			Volatile Fatty Acid (g kg^{-1} DM)					
							D-Lactate	L-Lactate	Total	Acetic	Iso-Butyric	Valeric	Iso-Valeric	Hexanoic	Total
W1	Chopped	Nil	317.3 ^{a}	145.1 ^{c}	5.4 ^{d}	8.3 ^{f}	8.8 ^{ab}	8.8 ^{b}	17.5 ^{bc}	6.1 ^{f}	0.016 ^{d}	0.003 ^{b}	0.070 ^{e}	0.021 ^{d}	6.3 ^{d}
W1	Chopped	1174	341.7 ^{b}	140.8 ^{bc}	4.4 ^{ab}	5.0 ^{d}	34.8 ^{e}	16.0 ^{c}	50.6 ^{f}	10.0 ^{g}	0.009 ^{c}	0.002 ^{ab}	0.034 ^{bcd}	0.015 ^{c}	10.2 ^{f}
W1	Chopped	Classic	339.3 ^{b}	141.9 ^{bc}	4.3 ^{a}	4.3 ^{b}	29.0 ^{de}	24.4 ^{d}	53.3 ^{f}	7.1 ^{f}	0.003 ^{a}	0.003 ^{b}	0.021 ^{a}	0.006 ^{a}	7.9 ^{e}
W2	Chopped	Nil	450.3 ^{de}	136.3 ^{ab}	5.9 ^{e}	5.0 ^{d}	2.9 ^{a}	2.3 ^{a}	5.1 ^{ab}	1.3 ^{a}	0.008 ^{bc}	0.002 ^{ab}	0.044 ^{d}	0.022 ^{d}	1.4 ^{a}
W2	Chopped	1174	409.7 ^{c}	132.8 ^{a}	4.3 ^{a}	4.0 ^{a}	35.3 ^{e}	9.8 ^{b}	45.1 ^{ef}	5.7 ^{e}	0.004 ^{ab}	0.002 ^{ab}	0.033 ^{bc}	0.013 ^{b}	5.2 ^{c}
W2	Chopped	Classic	433.7 ^{d}	136.0 ^{ab}	4.3 ^{a}	4.0 ^{a}	22.5 ^{cd}	28.0 ^{d}	50.5 ^{f}	3.0 ^{c}	0.004 ^{ab}	0.003 ^{b}	0.034 ^{bcd}	0.006 ^{a}	3.1 ^{b}
W2	Unchopped	Nil	411.7 ^{c}	130.5 ^{a}	6.0 ^{e}	5.7 ^{e}	1.5 ^{a}	1.0 ^{a}	2.5 ^{a}	1.7 ^{ab}	0.010 ^{c}	0.002 ^{ab}	0.042 ^{cd}	0.021 ^{d}	1.8 ^{a}
W2	Unchopped	1174	455.5 ^{e}	134.4 ^{a}	4.5 ^{bc}	4.0 ^{a}	23.8 ^{cd}	6.8 ^{b}	30.6 ^{cd}	4.3 ^{d}	0.003 ^{a}	0.001 ^{a}	0.029 ^{ab}	0.012 ^{b}	4.5 ^{c}
W2	Unchopped	Classic	442.0 ^{d}	130.4 ^{a}	4.6 ^{c}	4.7 ^{c}	17.4 ^{bc}	19.3 ^{c}	36.7 ^{de}	2.6 ^{bc}	0.016 ^{d}	0.003 ^{b}	0.070 ^{e}	0.021 ^{d}	2.7 ^{b}
	p value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	<0.001	<0.001	<0.001

[1] Wilted to a nominal dry matter (DM) content of 350 (W1) or 500 (W2) g kg^{-1}. [2] DM: oven dry matter; NH_3-N: ammonia nitrogen as a % of total N. Values in the same column with different superscript letters are significantly different ($p < 0.05$).

Levels of D-lactate, L-lactate and total lactic acid varied ($p < 0.001$) between silages (Table 6). Both inoculants increased ($p < 0.05$) total lactic acid compared to Control silages. Chopping increased ($p < 0.05$) lactic acid content of the inoculated W2 silages compared to unchopped silages with the same inoculant. Despite no differences in total lactic acid, silages inoculated with Classic had higher ($p < 0.05$) L-lactate levels at each wilt by chopping combination. Conversely, D-lactate levels were higher ($p < 0.05$) for the 1174 W2 chopped silages.

The protein fraction components of the fresh and wilted forages and silages are presented in Table 7. Non-protein nitrogen levels were lower ($p < 0.05$) in forages compared to silages. Forage and silage NPN levels were unaffected by wilting. Inoculants reduced silage NPN, though the difference between Control and 1174 was not significant for the W2 chopped silages. Non-protein N levels were lower ($p < 0.05$) for inoculated chopped W1 silage compared inoculated unchopped W2 silage. Conversely, TP and IP levels were higher ($p < 0.05$) in forages compared to silages, and inoculants increased TP and IP content except for between the Control and 1174 chopped W2 silages; and were higher ($p < 0.05$) for inoculated chopped W1 silage compared inoculated unchopped W2 silage.

Table 7. Protein fraction components [1] of Premier digit grass forage at cutting (W0) and silages produced from forage wilted to a nominal dry matter content of 350 g kg^{-1} (W1) or 500 g kg^{-1} (W2) grown at Tamworth Agricultural Institute and harvested and ensiled in 2021.

	Wilt	Form	Inoculant	Protein Fractions (g CP kg^{-1} CP) [1]				
				NPN	TP	IP	NDICP	ADICP
Forage	W0	Unchopped	-	159.7 ^{a}	839.6 ^{e}	807.8 ^{e}	585.3 ^{g}	81.0 ^{f}
	W1	Unchopped	-	137.4 ^{a}	861.5 ^{e}	830.6 ^{e}	577.7 ^{g}	74.4 ^{cdef}
	W2	Unchopped	-	167.8 ^{a}	832.2 ^{e}	810.0 ^{e}	513.3 ^{f}	67.3 ^{abcde}
Silages	W1	Chopped	Nil	379.9 ^{e}	620.1 ^{a}	575.3 ^{a}	344.0 ^{a}	76.8 ^{def}
	W1	Chopped	1174	289.4 ^{b}	710.6 ^{d}	677.0 ^{cd}	412.3 ^{de}	65.2 ^{abcd}
	W1	Chopped	Classic	283.9 ^{b}	716.1 ^{d}	686.9 ^{d}	417.3 ^{e}	69.9 ^{abcdef}
	W2	Chopped	Nil	347.9 ^{de}	652.1 ^{ab}	618.0 ^{b}	384.2 ^{bcd}	73.6 ^{bcdef}
	W2	Chopped	1174	315.2 ^{bcd}	684.8 ^{bcd}	647.8 ^{bc}	397.2 ^{cde}	61.4 ^{abc}
	W2	Chopped	Classic	304.5 ^{bc}	695.5 ^{cd}	662.9 ^{cd}	401.6 ^{cde}	59.8 ^{a}
	W2	Unchopped	Nil	373.6 ^{e}	626.4 ^{a}	587.4 ^{ab}	369.0 ^{abc}	79.7 ^{ef}
	W2	Unchopped	1174	336.2 ^{cd}	663.8 ^{bc}	622.4 ^{b}	346.8 ^{a}	60.9 ^{ab}
	W2	Unchopped	Classic	336.8 ^{cd}	663.2 ^{bc}	628.2 ^{b}	363.5 ^{ab}	68.2 ^{abcdef}
	p value			<0.001	<0.001	<0.001	<0.001	0.029

[1] CP: crude protein; NPN: Non-protein nitrogen; TP: True protein; IP: Insoluble protein; NDICP: Neutral detergent insoluble crude protein; ADICP: Acid detergent insoluble crude protein. Values in the same column with different superscript letters are significantly different ($p < 0.05$).

Similarly, NDICP levels were higher ($p < 0.05$) in forages compared to silages. Forages with the longer wilting period (W2) also had lower NDICP levels ($p < 0.05$) compared to W0 and W1. Inoculants increased ($p < 0.05$) NDICP level for the W1 silage compared to the Control W1 silage. Inoculated chopped W1 silage had higher ($p < 0.05$) NDICP levels than inoculated unchopped W2 silage. Forage ADICP declined ($p < 0.05$) as wilting period increased (W0 to W2). The level of ADICP was lower ($p < 0.05$) for chopped W2 Classic silage compared to chopped W2 Control silage; and for unchopped W2 1174 silage compared to unchopped W2 Control silage.

4. Discussion

These experiments were conducted to test the hypotheses that (1) good quality, well fermented silages can be produced from chopped or unchopped tropical grasses if adequately wilted; and (2) addition of a silage bacterial inoculant improves the fermentation quality of wilted tropical grass silage. The silages in both experiments were generally well preserved based on the NH_3-N content, low levels of VFAs and olfactory assessment, while

Experiment 2 clearly showed that bacterial inoculants improved fermentation characteristics. Therefore, we have proven both hypotheses to be true. However, we also acknowledge the likelihood that fermentation quality of silages produced in both experiments was positively influenced by the rapid and effective wilts achieved, which we attributed to the low yields and ideal drying conditions. However, these conditions also made management of the wilting process difficult in Experiment 1 and caused inconsistent forage DM content between species and years.

The WSC content of all the grasses was within the expected range for tropical grass species; and generally, less than for temperate grasses [14,37–40]. The recommended level of WSC was achieved for most forages following wilting. The exceptions being Katambora Rhodes grass in both years and Premier digit grass in March 2020 of Experiment 1 and W1 in Experiment 2. Interestingly, the WSC content of most forages increased during wilting. Previous research reported that while photosynthesis can still occur and plants produce WSC during the initial period of wilting, production is less than WSC loss by enzymic respiration [41]. Therefore, we discount continued respiration as an explanation for the increase we observed. Instead, we speculate that during wilting plant enzymes converted starch (and possibly other carbohydrates) to WSC to meet cellular needs. Starch is an important substrate in tropical grasses and acts as a reserve to store photosynthate and meet plant demands for sucrose [38]. Also, our results are consistent with previous research showing the starch content of kikuyu declined while WSC remained constant during wilting [30]. We did not observe a decline in starch content, but we suspect that other carbohydrates were converted directly to WSC or to WSC via starch. We recommend that further research is needed to investigate changes in various carbohydrate fractions during wilting. Conversion of starch or other carbohydrates to WSC would be beneficial by offsetting WSC decline due to plant enzymic activity and provide additional WSC for fermentation. This process would help to ameliorate the negative effects of low WSC of tropical grasses at the time of cutting.

Water soluble carbohydrates are the primary substrate for silage fermentation by lactic acid bacteria. However, evidence exists that some species, including propionic acid bacteria, can ferment starch and possibly pectin and hemicellulose [40]. More recent research has identified several new species of importance in tropical grass silages [42,43], while Ross (unpublished data) identified previously unreported *Lactobacillus dulbruekii* and *Lactobacillus leichmannii* in Australian silages over 30 years ago [17]. In a review of silage microbiology literature, Muck reported that several new species had been identified, and that populations of individual species changed over time during the ensiling period [44]. The latter was associated with changes in the content of different fermentation products. Furthermore, differences in epiphytic species and strains between different regions, forage moisture content and temperature are known to influence the dominant bacterial species in silages [43,45–47]. We speculate that higher temperatures, relatively dry conditions and consequently different dominant bacteria resulted in a fermentation profile that would be considered atypical when compared to silages produced from temperate species in cooler environments.

However, as previously stated, the silages produced in these studies were satisfactorily preserved based on NH_3-N level and olfactory assessment. The exception was Gatton panic ensiled in 2020, which contained 20.2% NH_3-N. We attribute this result to a lower DM content at ensiling (302.4 g kg^{-1}) compared to the other forages. In the absence of additives e.g., bacterial inoculants, additional WSC sources, we recommend wilting to a minimum DM content of 350 g kg^{-1} for tropical grasses, which is higher than 300 g kg^{-1} which was previously recommended [16]. We recommend that further research is needed to assess optimum forage DM content for ensiling key tropical grass species. This should encompass DM contents ranging from 250 to 500 g kg^{-1}, in 50 g kg^{-1} increments, to mimic current industry practices. Additionally, this research should investigate the effect of wilting rate *per se* in addition to final DM content, as both have been shown to affect intake by livestock [48].

Silage pH, NH_3-N and VFA content in 2019 were typical of a restricted fermentation observed in silages produced from high DM content forages [12]. However, the very high pH values of all silages in 2020 indicate that negligible fermentation occurred; the major fermentation products were non-acidifying or less acidifying than lactic acid; there was a breakdown of fermentation acids to other non-acidifying products; or a combination of these factors. Our interpretation is limited by the difficulty in achieving uniform DM content between years and species. However, the results for Katambora Rhodes grass provided a clear indication of a year effect with higher pH in 2020 despite similar forage DM and WSC content. This would further support our speculation that different bacteria dominated fermentation in 2020 compared to 2019.

The weather between November 2019 and March 2020 was hot with average minimum and maximum temperatures of 13.2 and 32.2 °C respectively, and with temperatures exceeding 40 °C on 10 occasions during December and January. We consider it probable that high temperatures and solar radiation levels provided relatively hostile conditions for epiphytic bacteria and, as a result, altered the bacterial species composition and numbers between the 2019 and 2020 harvests. Further research is warranted to identify dominant epiphytic bacteria on tropical grass species. We recommend that this research should consider temporal changes in dominant bacteria and the influence of temperature, solar radiation, rainfall, and humidity.

We considered a fermentation dominated by yeasts as a possible explanation for the high pH of all silages in 2020 but discounted this as the levels of ethanol (0.114 to 0.301 g kg^{-1}) were low relative to the loss of WSC (25.5 to 86.3 g kg^{-1} DM) during fermentation. We also compared the quantity of acetic acid produced against the reduction in WSC to further investigate differences in fermentation products between years given the lack of lactic acid data. McDonald et al. reported that enterobacteria can ferment 1 unit of glucose to 1 unit of acetate plus 1 unit of ethanol, with a 41.4% loss of DM [12]. Based on the number of moles (mol) of WSC (as glucose equivalents), we calculated that in 2020 the average loss in WSC during the period of ensiling was equivalent to 0.144 mol kg^{-1} DM, while the average level of acetic acid present in the silages was 0.162 mol kg^{-1} DM. Therefore, we consider it probable the fermentation was dominated by enterobacteria or another species, with acetic acid the primary fermentation product and very little lactic acid was produced. Also, we think that the low level of acetic acid and other products is evidence that there was little fermentation and reduction in pH. This is consistent with Li et al. who reported that under tropical climate conditions, high DM silages showed greater acetate production and limited fermentation [4]. However, the extent of fermentation seems extremely limited on this occasion, further emphasizing the importance of research into fermentation of tropical forages produced after experiencing prolonged hot and relatively dry conditions. In contrast, the production of acetic acid in 2019 (0.061 g kg^{-1} DM) was less than the loss of WSC (0.089 mol kg^{-1} DM). Further evidence that the bacterial species present were different.

The NPN content of the fresh forages in Experiment 1 was within the range of 205.9 to 560.1 g CP kg^{-1} CP previously reported for a number of tropical grass species [49,50]; but higher than reported for temperate species [41,51,52] and well-managed kikuyu and paspalum (*Paspalum dilatatum*) grown in Australia [52]. As expected, the proportion of NPN increased during ensiling for all forages [53]. The relatively high NPN content of Katambora Rhodes grass contrasted with Premier digit grass forage and silage which had relatively low NPN and high TP [53]. High levels of degraded protein in silage are associated with reduced silage intake and less efficient utilization of the protein fraction by ruminants [54], and the ability to select superior species based on protein quality would benefit livestock production. We recommend further research to confirm if consistent species differences in forage protein fractions exist, and additionally, this research should investigate any potential flow-on effects to silage protein composition.

Protein quality remained unchanged or improved during wilting based on NPN content, in contrast to expectations as previous reports reported that proteolysis caused by

plant enzymes normally leads to an increase in NPN [41]. However, degradation during wilting declines as DM content increases, and is more extensive when wilting is slow and inefficient [41]. We speculate that the relatively high DM content of several grasses at cutting, combined with a rapid and efficient wilt in both experiments, effectively prevented proteolysis. However, we cannot explain the mechanism that reduced NPN content of five forages in Experiment 1. Further research is required to understand changes to the protein fraction of forages immediately after cutting.

In Experiment 2, the fermentation quality of the W1 chopped Control silage was considered good, with a lactic acid to acetic acid ratio of 2.9:1 and an NH_3-N content of 8.3%. This is despite lactic acid content being lower and pH higher than normally observed in silages with a similar DM content [39]. However, we note that good fermentation quality was expected for all silages, as the DM content of W1 and W2 forage (365 and 447 g kg^{-1} respectively) would have disadvantaged undesirable *Clostridia* spp. and favoured desirable bacteria. The low level of propionic acid and butyric acid of the W1 unchopped Control silage was consistent with the DM contents.

Increasing forage DM content reduced lactic acid level and increased silage pH for the Control but not the inoculated chopped silages. We speculate that the selected strains in the commercial inoculants were more efficient at higher DM than the epiphytic bacteria. Furthermore, an increase in DM content reduced NH_3-N, but changes to protein fractions were equivocal with both IP and NDICP content increasing but NPN, TP and ADICP unchanged. However, overall levels of NPN were low for all silages, which again we attribute to the rapid and effective wilt reducing proteolysis by plant enzymes [53]. Additionally, we speculate that limited proteolysis may have reduced total degradation and even masked the impacts of fermentation. Unchopped W2 silages similarly had higher pH and NH_3-N and lower IP and NDICP than chopped W2 silages which was expected as faster and more extensive fermentation was reported for chopped compared to unchopped lucerne [24]. Nevertheless, these data are extremely relevant because unchopped, baled silages are common in Australian farming systems and the potential negative effects of not chopping need to be understood.

Inoculants increased lactic acid and reduced pH and NH_3-N, regardless of DM content or chop length as expected, and consistent with other reports [18,42,55,56]. The higher DM content of all silages in our study may have even enhanced the benefits of inoculants by restricting competition from *Clostridia* spp., in contrast to other studies where bacterial inoculants were less effective in low DM tropical grass silages [45]. Differences in fermentation products due to inoculant treatment can be attributed to differences in species and strains present and their efficacy when used for tropical grass. Both inoculants contained *L. plantarum*, but 1174 contained *E. faecium* while the Classic contained *P. pentosaceus*. D-lactate was higher in silages inoculated with 1174 compared to Classic, while L-lactate was higher for Classic, however, total lactic acid levels were similar for both ensiles forages. Pioneer 1174 inoculated silages had higher acetic acid than Control and Classic silages, and commensurately higher total VFA levels. These variations could be due to differences in primary fermentation products or a shift from lactic acid to acetic acid during later stages of storage [44], but neither can be confirmed. Both inoculants were equally effective in our study.

5. Conclusions

Our major conclusion was that production of well fermented silages from tropical perennial grass can be achieved with good management. Thus providing a strategy for farmers to better utilise tropical grass pastures as both a grazing and conserved forage option. For effective ensiling, we recommend that forages are wilted to a DM content of 350 g kg^{-1} DM content or higher and a bacterial inoculant used to more efficiently utilise available WSC. Furthermore, we conclude that, while fermentation quality of baled silage will be poorer than chopped silage, the practical implications for livestock will be minor, provided the wilt is rapid and effective, and a bacterial inoculant is used.

Additionally, we recommend that additional research is needed to understand the mechanism(s) behind changes in carbohydrate fractions, particularly WSC content, during wilting and potential implications for ensiling. Knowledge of dominant epiphytic bacteria and the effect of seasonal conditions on temporal changes are not well understood. We think that further research should investigate epiphytic species and strains with potential as inoculants.

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