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ASSOCIATIVE EFFECTS OF A MIXED BRACHIARIA DECUMBENS AND PENNISETUM PURPUREUM GRASS FEED ON THE ENSILING PROPERTIES

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Abstract: This study aimed to evaluate the effects of the association between two tropical grasses, Signal grass (*Brachiaria decumbens*) and Napier grass (*Pennisetum purpureum*) on the proximate composition, in vitro digestibility, and fermentation characteristics of ensiled material. A complete randomized design was used on three treatments namely Signal grass, Napier grass, and Signal-Napier grass combination. Silage was made using molasses applied in proportions of 1:2 with water and mixed with silage at the rate of 5% for a 5kg bag of 2.5cm cut grass. Signal-Napier grass combination silage was superior in almost all parameters to the two sole crop silages. After ensiling, a significant difference ($p<0.05$) in most parameters was recorded. A significant difference ($p=0.0004$) in pH was determined where pH was lower in the sole Napier grass silage than that of the Signal-Napier combination and Signal grass. A similar outcome ($P<0.05$) was recorded for crude protein, ash, ether extracts, nitrogen-free extracts, and neutral detergent fiber. However, no significant difference ($p>0.05$) was obtained in dry matter ($p=0.1524$), crude fiber ($p=0.5924$), and ADF ($p=0.1168$). Although having poor digestibility values in all treatments, Signal grass proved to be better than the rest. Organoleptic characteristics were promising, with normal color, smell, and texture changes observed. These results indicated that the association of grasses had an impressive positive effect on the nutritional value and quality of silages. Therefore, the use of mixed grass silages is encouraged.

Key words: Napier grass, Signal grass, molasses, Signal-Napier grass, silage, forage

Introduction

Climate change has led to higher-than-normal temperatures, inconsistent rainfalls, and shorter rainfall seasons (*Rama et al., 2022*). Because of poor plant productivity arising from these extreme weather conditions and a shift in seasonality, good-quality pastures are rapidly declining as productivity is inversely related to consumption (*FAO, 2015*). Such is the case of Napier grass which livestock heavily depend upon. Against this background, it is therefore important to consider alternative feed sources like silages for the animals.

Silages are any crop residues, agricultural or organic industrial by-products, preserved in the absence of air by artificial or natural acidification and used for animal feed, especially in winter (*Moran, 2005*). Fermentation improves the quality characteristics of the plant which includes acceptability and palatability (*FAO, 2012*). Silage is an important food source for farm animals especially during the dry season contributing over 50% of nutrition on the farm (*Khan et al., 2021*). Its use is spurred by the significant negative impacts caused by climate change on plant productivity worldwide (*Lone et al., 2017*).

In modern animal husbandry, harvesting of forage crops is done when they are at maximal yield and nutritional value. Grasses are also used in the manufacture of silages. Signal grass (*Brachiaria decumbens*) and Napier grass *(Pennisetum purpureum)* are perennial grasses found in most semi-arid and humid areas and are readily available resources that can be used for the provision of livestock feeds *(Cook et al., 2005)*. Furthermore, these grasses are easy to establish, making them beneficial in smallholder communities.

Napier grass is a high-yielding green fodder providing an excellent nutrition for cattle. Besides yield, Napier has crude protein ranging from 17 to 18 percent (*Orodho, 2006*). It is very palatable because the stalks are tender. The leaves are smooth and hairless and animals enjoy their sweet juice (*Randa et al., 2017*). On the other hand, Signal grass is aggressive and has the ability to grow well under varying soil and moisture conditions (*Muniandy et al., 2019*). It is a low-growing leafy grass with an erect or trailing habit. Despite the excellent productivity of Signal grass, it contains chemicals that can damage the liver and cause skin photosensitization in cattle and sheep if consumed in excess (*Awad et al., 2012*). Due to its low crude protein of around 8% (DM), it becomes imperative to improve the nutritional status of Signal grass through association with highly nutritious grasses or legumes (*Fisher and Kerridge, 1996; Muniandy et al., 2019*).

This study, therefore, tested the hypothesis that mixing Signal grass and Napier grass leads to better proximate composition, in vitro organic matter digestibility, and fermentation characteristics of ensiled material than fermenting the same species individually.

Materials and Methods

Study area

The research was carried out at the Grasslands research station in Marondera. The Research station is located in the Highveld and its latitude is 18° 11', longitude 31°28' E, and altitude 1630m. The mean day length is around 13 hours during the summer down to 11 hours during winter. The area receives an average annual rainfall of 873 mm and the hot summer days stand between September and December with October identified as the hottest month of the year having maximum temperatures above 30°C. Soils are mostly acidic (pH 4.5) deep brown, fine-loamy kaolinitic thermic derived from granite (*Mapiye et al., 2006*). Large and small ruminant production is practiced with cattle, goats, and sheep being the most dominant.

Experimental design and layout

For this study, Signal grass and Napier were left to grow under natural pasture rangeland conditions. To ensure optimal nutritional quality, the grasses were harvested during their vegetative growth stage (2 months from establishment), while still juicy and prime (Table 1) as recommended by *Johansson (2010)*. The grasses were harvested at random across the field, pre-wilted for 24 hours, cut into 2.5 cm particles using a machete, and then put into micro-silos for ensiling.

Table 1. Chemical composition of the different forage grasses before ensiling

Variable	Treatments			SED	Contrasts Anova (P- value)
	Signal grass	Napier grass	Signal-Napier combination		Trt[2]
Dry matter	86.40	87.00	87.33	0.7694	0.5096
Crude protein	8.10[b]	7.40[c]	9.90[a]	0.7462	0.0017
Crude fiber	29.07[b]	31.85[a]	33.52[a]	0.8824	0.0066
Ash	7.62	6.82	7.69	0.3272	0.0676
Ether Extracts	1.25[b]	2.32[a]	2.55[a]	0.0577	0.0001
pH	6.30	6.10	6.10	0.0667	0.0787
NFE	40.30[a]	45.20[a]	46.60[a]	0.9549	0.9322
NDF	65.50	67.90	67.20	0.7126	0.9929
ADF	23.60	24.50	24.70	0.3382	0.9983

Means within a row followed by a different superscript are significantly different ($P < 0.05$)
[1]SED=Standard Error of Difference.[2]Trt = Treatment

Three treatments were under study: Napier grass, Signal grass, and a Signal-Napier grass combination. For each treatment, 5kg of forage was used, and molasses was added at a rate of 5%, diluted with water at a rate of 1 part molasses to 2 parts water, and mixed thoroughly with the forage. To make micro-silos, the forage mixed with molasses was then placed in polythene bags, carefully avoiding any holes, and gently squeezed to let the air out before tightly sealing the bags with strong duct tape to create an anaerobic environment. All bags containing each treatment were stored in a storeroom at room temperature for 8 weeks. After 8 weeks, the grass in the bags was premixed independently, and silage samples were collected for silage quality and proximate analysis.

Sampling procedures

The silage bags were opened 62 days after ensiling. Two samples weighing 200g were taken from each ensiling bag for each replicate. The first sample was used for pH analysis, and the second was for dry matter, crude protein, crude fibre, and ash.

Data collection

Proximate analysis was done on the samples from each treatment before ensiling and after 8 weeks of ensiling to obtain the chemical properties of the feed. The samples were analysed for dry matter (DM). Ash, crude protein (CP), and crude fibre (CF) according to *AOAC (2016)* procedure. ADF and NDF were obtained through the Van Soest nutrient determination (*Van Soest and Wine, 1967; Van Soest, 1990*). The pH of the silage was measured using a digital pH meter. Other qualities which include, smell, texture, and colour were obtained through the organoleptic test (*Randa et al., 2017*). Percentage spoilage was recorded upon opening the silage bags.

Chemical Analysis

The pre-dried original samples and ensiled samples were analyzed for dry matter content, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ether extracts (EE). Dry matter (DM) determination samples were oven dried for 72 hours at 65°C until constant weight. Ash determination samples were burned at 600°C until constant weight using a muffle furnace. Burnt samples were then used to determine individual minerals. The Kjeldahl method was used to analyze crude protein (CP). Samples were digested in hot concentrated sulphuric acid and N was liberated as ammonia. Neutral detergent fiber (NDF) was tested by boiling the forage in a neutral detergent solution and measuring the insoluble residue (cell wall contents). Acid detergent fiber (ADF) determination samples were digested in an acid detergent solution hemicellulose was dissolved leaving cellulose and lignin. Ether extracts (EE) were determined by passing hot petroleum ether through a feed sample, dissolving crude fat, the solvent evaporated, and the remainder being Ether Extracts.

Statistical analysis

The data for a randomized complete design was analysed using the one-way ANOVA incorporating the multiple comparisons tests on GraphPad Prism software (2020 version). Treatment means were compared to the Signal-Napier combination and the means were separated using Tukey's studentized Range at a 0.05 significance level (P<0.05).

Results

Chemical composition after ensiling

Statistical analysis of the dry matter showed that there was no significant difference among the three treatments, Napier grass, Signal grass, and Signal-Napier grass combination. However, the result (Table 2) shows that the Signal-Napier grass combination had 1.01% and 0.35% higher dry matter than both Napier and Signal grass respectively. CP content of both sole silages (Napier and Signal grass) was lower than that of the Signal-Napier grass combination.

Table 2. Chemical composition of the different silages after ensiling

Variable	Treatments			SED	Contrasts Anova (P- value)
	Signal grass	Napier grass	Signal-Napier combination		Trt[2]
Dry matter	83.69	83.03	84.04	0.4452	0.1524
Crude protein	9.47[b]	8.51[c]	10.40[a]	0.1834	0.0001
Crude fiber	28.24	29.96	28.09	0.8709	0.5924
Ash	9.67 [b]	12.00[a]	12.00[a]	0.3175	0.0005
Ether Extracts	1.21[c]	1.91[b]	2.14[a]	0.0361	0.0001
pH	4.30[a]	3.80[b]	3.90[b]	0.0882	0.0004
NFE	33.65[c]	35.52[b]	37.73[a]	0.6206	0.0018
NDF	61.33[b]	63.62[a]	64.19[a]	0.8557	0.0342
ADF	22.78	23.62	23.88	0.4590	0.1168

Means within a row followed by a different superscript are significantly different (P < 0.05)
[1]SED=Standard Error of Difference.[2]Trt = Treatment

Table 2 shows that the Signal-Napier grass combination had a CP that is 0.93% higher than Signal grass and 1.89% higher than Napier grass. The statistical analysis shows that there was a significant difference among the three treatments ($p<0.05$).

No significant difference ($p>0.05$) was observed in the CF content of the three treatments with differences of 1.72%, 1.87%, and 0.15% between Signal and Napier grass, Napier and Signal-Napier combination and Signal and Signal-Napier combination respectively. Ash content also showed that there was a significant difference in the composition of the three treatments ($p<0.05$). Tabular values of ash composition show that more content was obtained in both the Napier and Signal-Napier grass combination which is 2.33% higher than Signal grass (Table 2). The pH from the three treatments also showed that there was a significant difference ($p<0.05$). Table 2 indicates that Napier grass silage had a lower pH value difference of 0.1 when compared to the Signal-Napier combination and a greater difference of 0.5 when compared to Signal grass.

The nitrogen-free extract content of Napier grass and Signal grass differed by 2.21 and 4.08, respectively, when compared to the Signal-Napier grass combination. The study found a significant difference ($p<0.05$) among the three treatments. Signal grass had a significantly higher nitrogen-free extract content than the other silage grasses, with both Napier and Signal-Napier grass combinations having a difference of 2.29% and 3.29%, respectively, when compared to Signal grass. However, there was no significant difference in the ADF content of the three treatments, with an average of 23.42% as shown in Table 2.

Physical Evaluation

No spoilage was recorded in all treatments at 8 weeks. Color change ranged from light green to moderately yellow in all treatments before and after ensiling. There was no significant difference in color amongst the three treatments as they all were moderately yellow after ensiling. Differences in smell were observed amongst ensiled Napier grass, Signal grass, and Signal-Napier combination. A slightly sour smell was the final result for ensiled Napier whilst both Signal grass and Signal-Napier combination smelt moderately sour. All treatments smelt slightly sweet before ensiling. Change in texture was observed in all three treatments as they shifted from dry and coarse before ensiling to slightly moist and coarse after ensiling. This is highlighted in Table 3.

Table 3. Physical evaluation of Silage quality characteristics

Silage	Before ensiling			After ensiling		
	Color	Smell	Texture	Color	Smell	Texture
Napier grass	Bluish-green	Slightly sweet	Dry and course	Moderately yellow	Slightly sour	Slightly moist and course
Signal grass	Light green	Slightly sweet	Dry and course	Light green/yellow	Moderately sour	Slightly moist and course
Signal-Napier grass Combination	Bluish-green	Slightly sweet	Dry and course	Moderately yellow	Moderately sour	Slightly moist and course

Discussion

The research focussed on the investigation of the associative effects of mixing *Brachiaria* and *Pennisetum* grass species on the ensiling properties. No spoilage was observed in any of the treatment samples after ensiling. This is mainly an indicator of the presence of desirable species of bacteria in the silage. Desirable species such as Lactic acid bacteria (LAB) only proliferate in the absence of oxygen which shows that the bags were tightly sealed (*Mugoti et al., 2022*). This is in agreement with *Kiczorowski et al. (2022)*, who stated that LAB are known to produce various natural compounds such as diacetyl, ethanol, hydrogen peroxide, reuterin, acetaldehyde, acetoin, carbon dioxide, and bacteriocins. These compounds act as bio-preservatives and help in inhibiting the growth of different types of microorganisms, including pathogenic, non-pathogenic, and spoilage microorganisms.

There were no significant differences in the dry matter (DM) content of the three treatments. However, the tabulated values mean otherwise as they show a higher DM content in the combined Signal-Napier grass combination than all the other treatments. The high DM content in the Signal-Napier grass combination could be a result of the differences in the dry matter content of the two grass species in which Napier grass provides more dry matter than Signal grass making the combination slightly superior. This is supported by *Cook et al. (2005)* who noted that Napier grass provided more DM than Signal grass as it is a leafier plant than Signal grass. However, the values tend to range above the normal range of DM content and this may be a result of human error when preparing the samples for analysis. A simple error in weighing the samples can also affect the data obtained.

Crude protein results after ensiling indicated that there was a significant difference among the three treatments. Values from the results of the three treatments show that the two sole types of grass namely Napier and Signal grass had a lower crude protein composition than the Signal-Napier grass combination. This can also be explained by the fact that associating the grasses was more advantageous as the differences in crude protein content of the two feeds, when combined, give a higher value than the sole silage treatments. These results were in disagreement with work done by *Mtengeti et al. (2013)* in which crude protein content of different grass silage feeds had no significant difference, with Signal grass having a slightly higher CP value than Napier.

The crude fibre (CF) content of the three grass treatments (Napier, Signal, and Signal-Napier combination) had no significant difference after ensiling. This may be a result of the low variances exhibited by the grasses before ensiling and also the microbial action to slightly lower the CF values. This is in agreement with work done by *Desta et al. (2016)* who attested that presence of lactic acid bacteria during fermentation has led to a slight reduction in the crude fibre content of the feed. They also, indicated that the low decline of CF could also be a result of the inhibition of undesirable bacteria during fermentation due to molasses availability which enhances the formation of formic acid and rapid development of Lactic acid bacteria which ultimately lowers DM loss in silage.

Results on the ensiling properties of mixed grasses showed that associating different grasses had a significant impact on the ash content, with a statistical difference observed compared to using independent grasses. Interestingly, the combined Signal-Napier grass treatment had the highest ash content compared to the other two treatments. This finding is consistent with previous research by *Rambau et al. (2022)* and *Santos et al. (2016)* that have shown that Napier grass tends to have more ash than Signal grass, indicating that it is slightly richer in minerals, even though the data is from separate studies with varying environmental conditions. By associating these two types of grass, their unique properties were also combined, resulting in a superior mix that is rich in minerals. These findings are consistent with previous studies conducted by *Mtengeti et al. (2013)* and *Kebede et al. (2016)*, which have also demonstrated the benefits of associating different grasses for improved ensiling properties.

The significant difference obtained in ether extract (EE) content of the three treatments after ensiling is ultimately a result of the higher values obtained in Napier grass before ensiling. The association of Napier and Signal grass made this combination superior in EE content which outpaced signal grass. This may be because of the heat that is produced during fermentation which affects the state of EE. This was highlighted by *Baer et al. (1998)* who stated that high temperatures building off during fermentation affected EE composition by affecting membrane fluidity. In addition, the production of ethanol, a product of fermentation affects EE as ethanol can dissolve some of the freely available fatty compounds (*Baer et al., 1998; Han and Zhou, 2013*). *Han and Zhou (2013)* suggested that the reduction in the fat content of silage is due to the oxidation of unsaturated fatty acids during the early stages of ensiling.

Nitrogen Free Extract (NFE) is the estimate of water-soluble polysaccharides within the grasses. An improvement in NFE after ensiling with the highest quantities obtained in the Signal-Napier grass combination shows that fermentation can assist in breaking down fibre and making it available as NFE. This is in agreement with *Miksusanti et al. (2019)* who state that presence of moisture and microbial populations during fermentation can improve nutrient availability and increase the organic matter rations in a diet.

Neutral Detergent Fibre (NDF) results showed that there was a significant difference in the treatments, with both Napier and Signal-Napier grass combination possessing a higher value as compared to Signal grass. This signifies that Signal grass has higher digestibility. However, all of the grasses had a percentage greater than 50% indicating low digestibility. Ensiling helped reduce the NDF levels to show that microbial action can improve feed digestibility (*Dehghani et al., 2012*). *Halik et al. (2014),* also indicated that microbial and enzymatic actions help to improve the digestibility of the feed as these act on the cell walls and other cell components making them available as water solubles. In this case, associating the grasses diminished the feed digestibility as Napier possesses a higher NDF content. Similar sentiments by *Kebede et al. (2016)* indicated an overall high NDF content of the Napier grass in their assessment of digestibility values in the different genera of Napier grasses.

Acid detergent Fibre (ADF) results indicated no significant differences amongst the three ensiled treatments. Since ADF value is inversely related to digestibility, the low ADF values indicate a much more desirable grass feed. Ensiling further lowered the ADF content though it was not statistically significant. This reduction may be a result of fermentation, microbial action, and enzymatic action on the non-digestible components of the grasses. The results favour Signal grass to indicate its low lignin content as compared to Napier grass. The association of types of grass grasses resulted in a higher ADF value which is not desirable, even though the value is not significant. These results are however in disagreement with *Solvita et al. (2015)* who indicated that grasses tend to have a higher ADF when compared to crop residues like that of maize with an average of 26% ADF in grasses.

After ensiling, the pH of the three treatments had a significant difference having the lowest pH from the Napier grass silage and the highest from Signal grass. Lower pH is an indication of good quality silage as it is a representative of lactic acid, the requirement in silage to assure its preservation *(Moran, 2005)*. The low pH status could be a result of a high energy status of the silage which is readily available to facilitate the formation of lactic acid responsible for lowering the pH (*Sarwatt et al., 1992*).

No significant differences were observed in the organoleptic parameters which may be a result that proves that all the grasses had an almost similar fermentation environment and not too many differences in the outward appearance, smell, and texture before ensiling. This is in agreement with *Randa et al. (2017)* who had almost similar results in the Napier silage. Signal grass had, however, the slowest change in colour which may be a result of the slightly lower temperature (room temperature of 25^0C). This was explained by *Zhu et al. (2022)* who stated that colour change during fermentation is mainly due to the moderately high temperatures of about 35^0C which can lead to a complete colour change in leaves from green to yellow. Interestingly, the experiment showed progress toward a yellow colour in all treatments.

From all these observations, it is highly crucial to implore the new practice of combining feeds as it seemed a little more beneficial than the old-fashioned way of using sole forages in silage making as proven by the statistical differences in most outcomes. This however gives evidence that there will be positive effects on implementing this new initiative and it is advantageous to the animals as they benefit from the associative effects on a nutritional basis.

Conclusion

Associating silages had a beneficial output as compared to the sole independent grass silages as indicated by the good physical characteristics and improved nutrient composition of the silage. The study, therefore, aligns with the alternative hypothesis which states that there is a significant difference in the ensiling properties of the grasses associated with the test.

Recommendations

The adoption of association of the grasses when making silages is highly recommended as a few negative effects were observed and the nutritive value of the feed would not be compromised. Also, further studies need to be undertaken to know the keeping quality of the mixed feeds and further carrying this research to the fodder crops such as maize. Analysis of the anti-nutritional factors needs to be done to determine if there are any effects with this method when silage-making. Tests to determine acceptability and palatability need to be done to have an all-inclusive conclusion to the use of mixed grass silages in livestock diets.

Asocijativni efekti mešanja trave *Brachiaria decumbens* i *Pennisetum Purpureum* na svojstva siliranja

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Rezime

Cilj ovog istraživanja je bio da se procene asocijativni efekti dve vrste trave, tropske (*Brachiaria decumbens*) i peraste oštre trave (*Pennisetum purpureum*) na približni sastav, in vitro svarljivost i karakteristike fermentacije siliranog materijala. Kompletan randomizovani dizajn je korišćen za tri tretmana, a to su tropska trava, perasta oštra trava i njihova kombinacija. Silaža je napravljena korišćenjem melase nanešene u proporcijama 1:2 sa vodom i pomešane sa silažom u količini od 5% za vreću od 5 kg pokošene trave od 2,5 cm. Kombinovana silaža tropske i peraste oštre trave bila je superiornija u skoro svim parametrima u odnosu na dve silaže pojedinačnih useva. Nakon siliranja zabeležena je značajna razlika (p<0,05) u većini parametara. Utvrđena je značajna razlika (p=0,0004) u pH, gde je pH bio niži u silaži peraste oštre trave, u poređenju sa silažom od kombinacije trava, kao i samo tropske trave. Sličan ishod (P<0,05) je zabeležen za sirove proteine, pepeo, etarske ekstrakte, ekstrakte bez azota i NDF. Međutim, nije dobijena značajna razlika (p>0,05) u suvoj materiji (p=0,1524), sirovim vlaknima (p=0,5924) i ADF-u (p=0,1168). Iako ima slabu svarljivost u svim tretmanima, tropska trava se pokazala boljom od ostalih. Organoleptičke karakteristike su bile obećavajuće, uz normalne promene boje, mirisa i teksture. Ovi rezultati su pokazali da je udruživanje trava imalo impresivan pozitivan efekat na nutritivnu vrednost i kvalitet silaže. Zbog toga se podstiče upotreba mešanih travnatih silaža.

Ključne reči: perasta oštra trava, tropska trava, melasa, kombinacija tropske i peraste oštre trave, silaža, krma

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Declaration of Interests

The authors declare that there is no conflict of interest surrounding the publication of this research.

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