Effects of Citrus species pulp on the silage fermentation of *Cenchrus purpureus – Calopogonium mucunoides* mixtures

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Abstract
The effects of *Citrus sinensis* (CS) – sweet orange and *Citrus maxima* (CMA) – Shaddock pulps on silage fermentative properties and microbial counts from a blend of *Cenchrus purpureus* (CP) and *Calopogonium mucunoides* (CM) in varying amounts during 60 days of ensiling were evaluated. The silage treatments consisted of T1-100% CP, T2-60% CP + 10% CMA + 30% CM and T3-60% CP + 10% CS + 30% CM were subjected to a completely randomized design. The dry matter content, pH, Ammonia-N and Fleig score of the silages were not significantly different ($P < 0.05$) in all the treatments. Higher values were recorded for lactic (T2 (5.03%) and T3 (5.19%)), acetic (T2 (3.77%) and T3 (3.89%)), propionic (T2 (0.25%) and T3 (0.26%)) and butyric acid (T2 (0.0377) and T3 (0.0389%)) and total VFA (T2 (5.66%) and T3 (5.84%)) for silages with citrus inclusion above sole grass silage. Sole grass silage consistently recorded lower microbial contents while T3 had higher yeast counts above other silages. In conclusion, ensiling citrus spp pulp alongside grass and legume, produced silages of better fermentative characteristics and microbial contents. And as a result, inclusion of citrus spp pulp into silage will offer both quantitative and qualitative fodder for animals most especially throughout the dry season.

Keywords: Citrus spp. pulp, *Cenchrus purpureus*, *Calopogonium mucunoides*, silage fermentative characteristics, microbial stability

Description of the Problem

Forage resources, which ruminants primarily rely on, are affordable and economic feed sources particularly during the rainy season. The use of conventional feed resources and the preservation of forages as hay or silage when they are plentiful and of high quality are crucial for mitigating issues like feed intake, poor digestibility, and animal performance brought about by the low quality of the limited supply of forage (**1**). When fully grown, most tropical grasses, such as *Cenchrus purpureus*, formally referred to as *Pennisetum purpureus*, have a relatively low protein content. The tropics' high temperatures also encourage the growth of more stem than leaf, resulting in lignified and fibrous grasses. Legumes, which are rich in protein, can be augmented with grasses to provide animals with high-quality feed.

Legumes and grasses combined will enhance fermentable qualities, resulting in silage of sufficient quality. The incorporation of citrus by-products into diets is suitable because of the ability of ruminants to ferment high fibre feeds in the rumen (**2**), and have also been shown to reduce foodborne pathogens because they contain essential oils with antimicrobial properties that can break down the cell wall of gram-negative bacteria. This study aims to evaluate the effects of citrus species pulps on the silage fermentation of grass legume mixtures.

Materials and Methods

The study was carried out at the Directorate of University Farms, Federal University of Agriculture Abeokuta, Nigeria. Forages such as *C. purpureus*, *C. mucunoides* and two *Citrus* species, *Citrus sinensis* (sweet orange) and *Citrus maxima* (shaddock or pomelo) were mixed at different proportions to make silage. The CP was harvested at 2 months after regrow from 20 cm above ground level with CM that were harvested from an established plot at vegetative stage. The *Citrus* spp. pulps were gotten from GulpDrinkz Nigeria Limited, Abeokuta, Ogun State after juice extraction. The grass, legume and the pulps were wilted individually to reduce moisture contents. They were chopped separately to 2-3cm long and ensiled by compressing very well into laboratory silos of 960 ml at different proportions by thoroughly mixing them together into different treatments of T1 - (100% CP), T2 - (60% CP + 10% CMA + 30% CM) and T3 - (60% CP + 10% CS + 30% CM) respectively. The silos were properly covered and sealed with duct tape.

At the end of 60 days ensiling duration, the silos were opened and fermentative characteristics of the silage such as pH, ammonia nitrogen and organic acids were determined according to (**3**) procedure while microbial counts were determined according to the procedures of (**4**). Flieg score was computed as follows: Flieg point = 220 + (2 × %DM-15) - 40 × pH, and silage quality was indexed as poor (0–20), low (21–40), medium (41–60), good (61–80) and very good (>81) according to (**5**).

Result and Discussion

The dry matter content, pH, Ammonia-N and Fleig score of the silages were not significantly different ($P < 0.05$) in all the treatments whereas there was significant ($P < 0.05$) difference for other parameters. Higher ($P < 0.05$) values were recorded for lactic, acetic, propionic and butyric acids and total VFA for silages with citrus inclusion above sole grass silage.

Ensiling is a complex bacterial fermentation process that causes a buildup of organic acids and lowers pH (**6**). Low pH values (<5.0) were recorded in this study, which are indicators of silage stability, adequate preservation quality and good silage according to (**7**). This fact implies that the activity of undesirable microorganisms was slowed down by low pH. The amount of ammonia-N in silage remains the effective indicator of protein degradation (**8**). The ammonia nitrogen concentration in this study, fall within the range mentioned by (**9**), since a concentration of more than 100 – 150 g/kg of ammonia nitrogen suggests that significant protein breakdown took place during ensiling.

Good preservation is evident from the silages' high lactic acid contents. Higher concentration of lactic acid bacteria results in a lower pH recorded in this study. This was consistent with reports by (**9**) that lactic acid is the primary factor lowering the pH of ensiled forage and that a lack of lactic acid bacteria in silage may cause the pH drop. Lower acetic acid content than lactic acid was recorded in this study which supports the finding of (**9**) that silage with a moderate acetic acid content (30–40 g/kg DM) can be advantageous because it inhibits yeasts, improving stability when exposed to air. Conversion of water-soluble carbohydrates to organic acids is by anaerobic bacteria, the low total VFAs found in this study may be related to the lower total anaerobic bacterial numbers. The quality of fermentation is indicated by the Flieg score. According to findings from (**5**) and (**10**), the treatments in this study exhibited good fermentation quality, as indicated by their flieg scores, which ranged from 69.80 to 91.40.

The results presented in the Table 2 revealed a significant ($P < 0.05$) difference among the treatments. Treatments T2 and T3 recorded higher ($P < 0.05$) contents of total anaerobic bacteria counts and total lactic acid count compared to T1 having lower ($P < 0.05$) total aerobic count and total lactic acid count values. Silage T3 had higher ($P < 0.05$) yeast counts above other silages. The kinds and quantities of bacteria that are involved in the bacterially driven process of ensiling are crucial to the quality of the fermentation (**11**). The higher LAB content in this study above, sole grass is consistent with (**12**) report, that a high abundance of lactobacillus is commonly found in silage due to their high acid-resistant nature. As a result, these bacteria are essential for most of the lactic acid production and pH decline in silage. T3 silage has a low amount of yeast, which supports (**13**) results that silage with low levels of fungus and yeast is safe for animal nutrition.

Table 1: Effects of Citrus spp. pulps on *Cenchrus purpureus* and *Calopogonium mucunoides* silage mixtures at varying proportions on fermentative characteristics

Treatments	Dry Matter %	pH	Ammonia-N %	Lactic acid %	Acetic acid %	Propionic acid %	Butyric acid %	Total VFA %	Fleig score
T1	25.33	4.42	8.93	4.47^{b}	3.35^{b}	0.22^{b}	0.0335^{b}	5.03^{b}	78.86
T2	29.00	4.83	9.36	5.03^{a}	3.77^{a}	0.25^{a}	0.0377^{a}	5.66^{a}	69.80
T3	25.00	4.09	8.51	5.19^{a}	3.89^{a}	0.26^{a}	0.0389^{a}	5.84^{a}	91.40
SEM	0.86	0.31	3.47	0.11	0.08	0.01	0.00	0.13	11.64
P-value	0.090	0.680	0.670	0.000	0.000	0.000	0.000	0.000	0.798

$^{a,\ b}$ means on the same column with different superscripts are significantly different ($p < 0.05$), SEM = standard error of means. T1-100% CP, T2-60% CP + 10% CMA + 30% CM and T3-60% CP + 10% CS + 30% CM.

Treatments	TABC X10^{6}/g	TLC X10^{6}/g	TYC X10^{6}/g	TFC X10^{6}/g
T1	0.8^{b}	1.0^{b}	0.00^{b}	0.00
T2	3.7^{a}	4.3^{a}	0.00^{b}	0.00
T3	2.5^{a}	4.0^{a}	0.40^{a}	0.00
SEM	0.48	0.58	0.68	0.00
P-value	0.014	0.005	0.000	0.000

Table 2: Effects of Citrus spp. pulps on *Cenchrus purpureus* and *Calopogonium mucunoides* silage mixtures at varying proportions on microbial counts

$^{a,\ b}$ means on the same column with different superscripts are significantly different ($p < 0.05$), SEM = standard error of means. T1-100% CP, T2-60% CP + 10% CMA + 30% CM and T3-60% CP + 10% CS + 30% CM., TABC: Total anaerobic bacteria count, TLC: Total lactobacilli count, TYC: Total yeast count, TFC: Total fungi count.

Conclusion and Application

Silage fermentation is a complex process that involves interactions between numerous variables.

In conclusion, ensiling citrus spp pulp alongside grass and legume, produced silages of better fermentative characteristics and microbial contents. And as a result, inclusion of citrus spp pulp into silage will offer both quantitative and qualitative fodder for animals most especially throughout the dry season.

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