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Effect of adding graded levels of lablab forages on fermentation characteristics of Brachiaria silage

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ABSTRACT

Ensiling as a method of forage feed conservation is the most appropriate in conserving of crude protein (CP) enhanced forages for sustainable dairy production. This is attributed to the fact that with this method, protein dependent lactic acid bacteria (LAB) hydrolyze water soluble carbohydrates (WSC) into short chain volatile fatty acids (VFAs) which are precursors for milk synthesis while the proteins buffers the excessive organic acids to produce more acetate and propionate. The study therefore aimed at assessing the quality of Brachiaria silage which was protein supplemented with graded levels of lablab forage. To achieve this objective, wilted Brachiaria forage (*Brachiaria hybrid cv Mulato* II) was collected, wilted and ensiled with and Lablab purpureus forages at inclusion levels of 0%, 10%, 20%, 30% Lablab purpureus forage. The resultant 4 treatments were assessed in a completely randomized design in 3 replicates. All silages were prepared using plastic jar mini-silos to laboratory scale and then incubated at room temperature (±30°C) for 45 days. After the 45 days, chemical analysis for quantification of water soluble carbohydrate (WSC), none protein nitrogen (NH3-N), Acid detergent fiber (ADF), Neutral detergent fiber (NDF), pH, in-vitro organic matter digestibility (INVOMD), acetic acid, lactic acid and propionic acid composition were conducted. The results indicated that; none protein nitrogen, acetic acid and propionic acid composition decreased in quadratic trends with increasing legume forage inclusion to minimum values of 5.8, 48.0 and 0.7g/kg at 14.7, 1.8 and 6.0% inclusion levels of lablab forage, respectively. On the other hand, following a quadratic trend, WSC composition decreased with increase in lablab forage, with a maximum of 28.9g/kg obtained at 7.6% inclusion level of lablab forage. Generally, CP, dry matter, INVOMD and metabolizable energy of the silage increased with increase in the inclusion levels of lablab silage. Using regression equations of the response curves, NDF and ADF decreased with increasing legume forage inclusion to minimum values of 349.3 and 172.1g/kg at inclusion levels of 16.1 and 17.1%, respectively. On the other hand in-vitro organic matter digestibility increased with the increase in the lablab forage inclusion to a maximum of 49.4%. However, mineral composition of the silage was not affected by lablab forage inclusion. Since the quality of silage for dairy cows depends on short chain volatile fatty acid, fibre and crude protein composition, inclusion of lablab forage to *Brachiaria hybrid cv mulato* II silage at a rate of 17.1% potentially yields the best results in lactating cows.

Keywords: *Brachiaria hybrid cv mulato*, silage, fermentation characteristics, lablab forage

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1.0 Introduction

Milk consumption has been identified as a key element in a healthy and balanced diet of such groups (Haug *et al.* 2007). Milk and dairy products can therefore play key roles in improving their nutrition nutrient deficient vulnerable groups. However, in Northern Uganda, the dairy subsector is dominated by smallholder farmers (FAO, 2004) characterized by primitive feed resource development technologies. Consequently, inadequate feeding has stood out to be the most limiting towards sustainable diary production (Nviiri *et al.,* 2014).

Due to its high biomass production and relatively higher nutritional attributes than other grass forages, *Brachiaria hybrid cv Mulato II* can form a basis for such smallholder zero-grazing systems (Kabirizi et al., 2013). However, this forage species is still limited by low CP which can be improved by supplementing with forage legumes (Tiemann *et al.,* 2008). In addition, the dry matter yield and nutritional value vary considerably with season and management with peak performance during the peaks of the growing seasons (Geofrey *et al.,* 2019). . This implies that, there is need for periodic harvesting and conservation during the growing seasons which renders ensiling the most appropriate conservation technology. Recognized for its high CP (Geofrey et al., 2019, *Lablab purpureus* has a great potential for bridging the existing protein gap (Aganga et al., 2003). In addition, such supplementation increases fibre degradation in the rumen by increasing nitrogen and short chain VFA profiles (Tiemann et al., 2008). However, feeds with such high CP content can only be effectively preserved using suitably customized methods given the offset in the buffer capacity and fermentative sugar composition (Ridwan *et al.,* 2015; Tittern & Bareeba, 2001).

Defined as a natural fermentation process in which lactic acid bacteria (LAB) convert water soluble carbohydrates (WSC) into volatile fatty acids (VFA) (Menardo *et al.,* 2015), ensiling can improve the feeding value of Brachiaria forages. This is attributed to the fact that, the resultant VFAs and lactic acid lower the forage pH while maintaining the moisture content resulting into maximum nutrient retention while inhibiting growth of less desirable organisms proliferation (Henderson 1993). Although fermentation characteristics of some silage types has been reported (Li & Nishino, 2013), the information on newly adopted Brachiaria hybrids is still limited. The objective of this study therefore was to assess lablab supplemented *Brachiaria hybrid cv Mulato II* silage basing on fermentation characteristics.

2.0 Materials and methods

2.1 Preparation of silage samples

The silage was made using wilted Brachiaria forage (*Brachiaria hybrid cv Mulato II)* and lab lab (*Lablab purpureus*) Legumes which were collected from the Ngetta ZARDI demonstration sites, in Lira district, Northern Uganda. Grasses and legume forages were chopped to lengths of approximately 5 cm prior to ensiling using a manually powered chopper. The forages were mixed into 4 treatments as follows; R0 (*Brachiaria hybrid cv mulato II* 100%), R1 (*Brachiaria hybrid cv mulato II* 90% + Lablab forage 10%), R2 (*Brachiaria hybrid cv mulato II* 80% +Lablab forage 20%), R3 *(Brachiaria hybrid cv mulato II* 70% + Lablab forage 30% by weight). All forage combinations were prepared in plastic jar silos to laboratory scale and then incubated at room temperature (±30°C) for 45 days. Adhesive tape was used to seal the container lids to ensure airtight and anaerobic conditions. A total of three replicates of one kilogram of each of the silage mixtures were prepared per treatment and the silage was evaluated in a completely randomized arrangement. The mini-silos were then stored at room temperature in the laboratory.

2.1.2 Sample preparation for analysis

After 45 days of ensiling, the silos were opened and inspected visually and by smell. The moldy silage formed on the surface of the silos was removed. The silage from each mini-silo was then thoroughly mixed on a clean plastic surface

and a representative sample of 400g was then obtained from each mini-silo. 300g of the sample was then placed on metallic trays and dried in a forced air oven at 60^{o}C for 72 hours. The remaining 100g of the wet silage sample was blended at high speed for one minute with 500*ml* of distilled water using a kitchen blender (Phillips) and the mixture filtered through a Whatman No. 1 filter paper. A sample of 400*ml* of the filtrate was then placed in a well labeled 200*ml* plastic bottle and frozen at -20 ^{0}C for subsequent volatile fatty acids (VFAs) and lactic acid analyses.

2.3 Chemical analysis

After 6 weeks, dry matter (DM) of the silage was obtained by oven drying sample at 65^{0}C to a constant weight, after which dried samples were ground to pass through a 2 mm screen. Ash was determined in a muffle furnace at 600^{0} C for 6 hours. The DM was corrected for VFA loss (DMcorr) according to Weissbach and Kuhla (1995) as cited by Sonja et al. (2012): DMcor = 2.08 + 0.975DM (g/100g). Crude protein was analyzed using standard methods 920.39 and 984.13, respectively of AOAC (1990). The non-protein-nitrogen (NH3-N) in silage was determined by the procedure described by Filya (2003) using the 2200 Kjeltech auto distillation (Foss Tecator, Sweden), without the previous digestion step. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed using the procedure by Van Soest et al. (1991).

The pH was determined using a glass rod probe digital pH meter (Metler Teldo, Five®Go) dipped into 100*ml* of the filtrate obtained from the wet silage sample after allowing the filtrate to stand for five minutes. The WSC content of the silages was determined on dried samples using the anthrone-sulphuric acid colorimetric method as described by Van Soest and Robertson (1985). Approximately 0.2 g of the ground sample was transferred into a bottle of 200 *ml* of distilled water and the content shaken on a Dubnoff metabolic shaking incubator (Precision®) for one hour. The mixture was filtered through a 12.5 cm Whatmnan No.1 filter paper. 2*ml* of the filtrate was mixed with 10 ml of anthrone reagent using a vortex mixer, incubated into a boiling water bath for 20 minutes, cooled and absorbance of the mixture measured using a spectrophotometer at a wavelength of 625 nm. Then, the water soluble carbohydrate content was estimated using the standard curve of glucose.

After thawing of the frozen silage samples, 1 ml of 25% orthophosphoric acid was added to 5 ml of the silage sample and the mixture left to stand for at least 30 minutes and centrifuged at 10,000 rpm for 15 minutes. The samples in the centrifuge vials were frozen at -23 ^{o}C until analysis for VFAs using Gas chromatography (Perkin Elmer model 500). The GC (Perkin Elmer model 500) was equipped with a flame-ionization detector (FID). Hydrogen gas was used as a mobile phase at 12 kPa column head pressure. The injector temperature was set at 260^{o}C/min to 190^{o}C, then 20^{o}C and detector at 330^{o}C/min to 230^{o}C. The oven was programmed at 110^{o}C where it was kept isothermal for 1 minute before cooling for the proceeding run. A volume of 0.5 µl of each of the prepared silage samples were drawn using a 1 µl syringe and then injected onto the chromatograph. The components of interest were eluted after 7 minutes but a total of 10 minutes was required to remove other components from the column. The peaks of the individual VFAs in the silage were identified by comparing retention time with corresponding peaks of the volatile acid standard mix (SUPELCO, USA). Quantification of VFAs in silage was achieved by preparing standard solutions of acetic, propionic, and butyric and isobutyric acids in water at different concentrations of 200, 100, 50, 0; 0.085, 0.061, 0.297, 0; 30, 15, 7.5, 0; and 5, 2.5, 1.25, 0 M, respectively. The peak-area ratio to 2-Ethylbutyric acid was used for the standard acids above. The peak-area ratio was then plotted against the concentration of each of the standard acids. Silage VFA concentrations were determined by fitting the peak-area ratio as

previously described for the internal standard (2Ethylbutyric acid).

Lactic content of silages was quantified using the colorimetric method as developed by Barker and Summerson (1941) and modified by Kimberly and Taylor (1996). In order to prevent interference by proteins during lactic acid determination, deproteinisation of silage samples was done using the procedure as described by Madrid et al. (1999). To a portion of 5 ml of the silage filtrate, 2.5 ml of 0.15M Ba $(OH)_2$ and 2.5 ml of 0.2M $ZnSO_4$ were added. The solution was mixed and centrifuged at 3000 rpm for 20 minutes. Following deproteinisation, the standard lactic acid curve was developed by adding zero to thirty micrograms of lactic acid into different test tubes. The serial dilutions were made in differences of 5 micro grams increments per 1000 ml. To make the lactic acid solution, 1.065 g of dry Lithium lactate were dissolved in water to make 1000 *ml*. To 1 ml of the resultant lactic acid solution, 3 ml of concentrated (82%) sulphuric acid were added and the mixture was vortexed. The mixture was then incubated in a steam bath at 95-100 ^oC for 10 minutes before cooling the mixture to room temperature. Then, 50 µl of copper sulphate solution (4% $CuSO_4$) were added followed by 100 µl of p-phenylphenol (1.5% p-phenylphenol in 95% alcohol) followed by vortexing for 1 min at room temperature. The mixture was left to stand for 30 minutes at room temperature before reading the absorbance at 570 nm using a spectrophotometer (JENWAY 6405 UV/Vis; UK).

2.4 Evaluation of silage quality

Silage samples were analyzed using the proximate standard procedures of AOAC (1997), and the fiber fraction content protocol by Van Soest et al. (1991). The CP and ether extract content (EE) were analyzed using FOSS equipment (Kjeltec 8400 analyzer unit and Soxtec 2050, Hoganas, Sweden). Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), hemicellulose, and cellulose were analyzed using Fibertec apparatus (FOSS Fibertec 2010, Hoganas, Sweden). Other parameters tested, include temperature using what?, pH using Cyberscan pH310 Eutech, concentration of lactic acid by the titration method, NH_3-N by the Conway method, WSC by a colorimetric method (Dubois et al., 1956), VFA using GC-FID (Bruker Scion 436, Fremont, CA, USA) and TP and TT by the Folin-Ciocalteu method (Makkar, 2003).

2.5 Data analysis

Data on chemical composition were analyzed using the PROC GLM procedure of SAS (2003). Where significant differences were detected, the LSMEANS statement was used to separate the LS means using the PDIFF option (SAS, 2003). Regressions were used to test for linear and quadratic effects of inclusion of graded levels of legume forages.

The following model was used;

$Yij = \mu + Lj + eij$

Where yij is the overall observation level of inclusion of *lablab purpureus* forage L, µ is the overall mean, Lj is effect of jth inclusion of graded levels of lablab forage (j= 0%, 10%, 20% and 30% on DM basis) and eij is the random error.

3.0 Results

The effect of lablab legume inclusion on short chain volatile fatty acids (VFAs), lactic acid composition, residual carbohydrates and non-protein nitrogen is presented in Table 3.1 and figures; *1a* and *1b*. The pH of the silage increased with the increase in lablab forage inclusion. However, according to the regression curve (*fig. 1a*), the maximum possible pH increase could not be obtained within the treatment limits. The inclusion of lablab forage influenced the composition of residual water soluble carbohydrate, lactic acid, acetic acid and propionic acid as well as silage pH. Whereas the composition of water soluble carbohydrate (WSC) decreased with lablab forage inclusion levels, that of non-protein nitrogen and lactic acid and propionic acid increased.

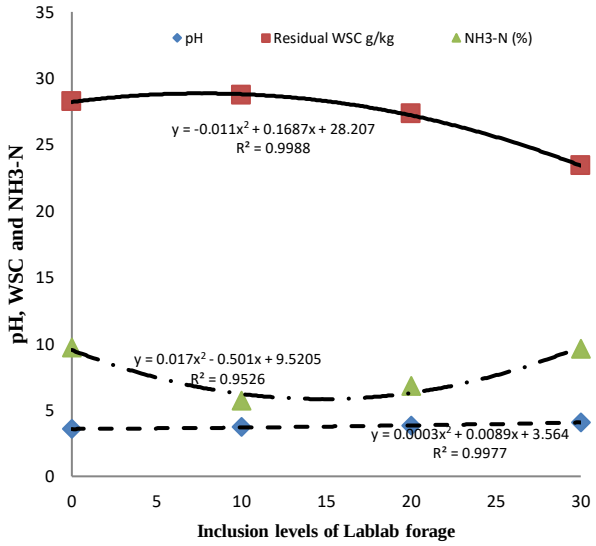
Using the regression equations of the response curves (Fig 1a and 1b), non-protein nitrogen, acetic acid and propionic acid composition decreased with increasing legume forage inclusion to minimum values of 5.8g/kg, 48.0g/kg and 0.7g/kg at 14.7, 1.8 and 6.0% inclusion levels of lablab forage, respectively, following quadratic trends. On the other hand, following a quadratic trend, WSC composition decreased with increase in lablab forage, with a maximum of 28.9g/kg obtained at 7.6% inclusion level of lablab forage.

Table1: Effects of lablab legume inclusion levels on the fermentation characteristics of silage

	Inclusion levels of lablab legume forage					
	0	10	20	30	SEM	p-value
pH	3.560^{b}	3.69^{a}	3.83^{ab}	4.06^{a}	0.12	***
Residual WSC (g/kg)	28.24^{a}	28.7^{a}	27.30^{a}	23.38^{b}	0.84	*
NH3-N (%)	9.690^{d}	5.70^{c}	6.8^{b}	9.60^{a}	0.29	**
Lactic acid (g/kg)	11.80^{d}	29.4^{c}	33.6^{b}	49.3^{a}	0.49	***
Acetic acid (g/kg)	47.20^{c}	53.4^{b}	55.7^{b}	74.85^{a}	1.487	***
Propionic acid (g/kg)	0.599^{c}	0.90^{b}	0.90^{b}	1.44^{a}	0.043	**
Lactic acid: acetic acid	0.259^{d}	0.50^{c}	0.56^{b}	0.606^{a}	0.0086	**

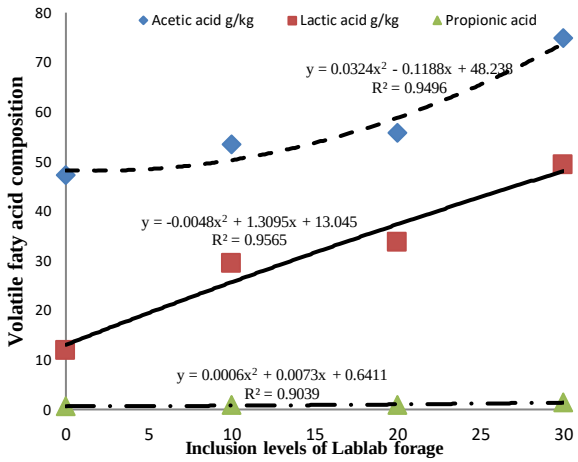
P values were significant at *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant at P > 0.05; SEM, standard error of mean.

Fig 1.A



Relationship between lablab forage inclusion and pH, WSC and NH_3-N

Fig1.B



Relationship between lablab forage inclusion and volatile acid composition

The effect of lablab legume inclusion on the nutrient composition of silages is presented in Table 3.2. Generally, CP, dry matter, *INVOMD* and metabolizable energy of the silage increased with increase in the inclusion levels of lablab silage. Specifically, using regression equations of the response curves (Fig 2), NDF and ADF decreased with increasing legume forage inclusion to minimum values of 349.3g/kg and 172.1g/kg at inclusion levels of 16.1% and

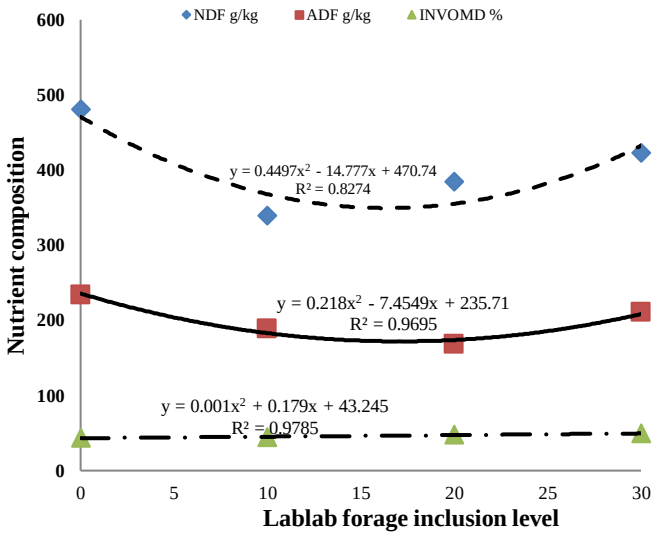
17.1%, respectively, following a quadratic trend. *In-vitro* organic matter digestibility on the other hand increased with the increase in the lablab forage inclusion to a maximum of 49.4%. However, mineral composition of the silage was not affected by lablab forage inclusion.

Table 2: Effects of legume inclusion levels on the nutrient characteristics of silage

	Inclusion levels of legumes					
	0	10	20	30	SEM	p-value
DM (g/kg)	163.0^{c}	175.3^{bc}	190.5^{b}	207.13^{a}	6.34	***
CP (g/kg)	77.74^{c}	99.8^{b}	135.9^{a}	138.8^{a}	6.37	***
NDF (g/kg)	480.38^{a}	339.0^{d}	384.0^{c}	422.5^{b}	11.94	***
ADF (g/kg)	233.8^{a}	188.7^{b}	168.1^{c}	210.2^{b}	12.93	***
Ash (g/kg)	66.35^{a}	67.30^{a}	69.70^{a}	70.33^{a}	2.18	ns
ME (MJ/kg DM)	5.3^{b}	5.69^{b}	6.8^{a}	7.011^{a}	0.212	*
INVOMD (%)	43.4^{c}	44.67^{a}	47.7^{ab}	49.38^{a}	1.51	**

DM, Dry matter; CP, Crude protein; NDF, Neutral detergent fiber; ADF, Acid detergent fiber; ME, Metabolizable energy; INVOMD, In-vitro Organic matter digestibility; P values were significant at *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant at P>0.05; SEM, Standard error of mean.

Fig 2.



Relationship between lablab forage inclusion and nutrient characteristics of silage

4.0 Discussions

The study explored the effect of lablab forage inclusion on the fermentation characteristics of Brachiaria silage after 45 days of ensiling. This is the first study of this kind and the findings will provide a protocol for ensiling Brachiaria forages protein boosted by lablab forages to be utilized in livestock nutrition to form a platform for sustainable dairy production. Specifically, emphasis was placed on the four key nutrients which affect the digestibility and utilization of fiber the key determinants in dairy production.

The study revealed high increase in pH as result of lablab forage inclusion. Whereas legumes like lablab are recognized for their high protein content (Murphy et al., 1999) they are not natural ensilage materials (Titterton and Bareeba, 2000). This is attributed to the fact that, legumes

have low water soluble carbohydrates and high buffering capacity. Consequently, the buffer effect largely contributed to by the proteins renders ensiling difficult since pH reduction is a prerequisite for proper silage formation (Manyawu et al., 2003; Tyroyá and Výborná, 2011). This implies that, the addition of legume forages prior to ensiling has to be accompanied by the addition of high sugar feed ingredients like molasses to lower the buffered capacity and proteolysis (Titterton and Bareeba 2000). Similarly, Kato et al. (2004) reported an increase in crude protein composition of silages when graded levels of legume forages were added to maize fodder.

There was a reduction in NDF and ADF and this can be attributed to the fact that, lablab foliage contains lower fibre fractions than Brachiaria forage. This reduction is in agreement with that reported by Sibanda et al. (1997), who reported lower NDF content with increased addition of *Desmodium uncinatum* to maize silage. NDF enhances dry matter intake by dairy cattle. This implies that, addition of lablab forage at a rate of 16.1% results into maximum silage dry matter intake by dairy cattle. However, the NDF of 349.3g/kg is still higher than the recommended intake for dairy cattle of 250-330g/kg DM (NRC, 2001).

There was a general decrease in the water soluble carbohydrate of the silage with increase in lablab forage inclusion level. This probably implies that protein nitrogen rather than soluble carbohydrate limited the ensiling quality of Brachiaria forage. Consequently, the WSC present in the silage was readily available to the lactic acid bacteria to facilitate the formation of stable silage. Therefore by putting more sugars like molasses followed by additional legume forages improves the silage nutritive value. This is attributed to the fact that the proteins would buffer the excessive volatile acid production producing more acetate and propionate. Moreover, acetate and propionate are precursors of milk butter fat and lactose respectively hence improved milk yield.

There was decrease in NH_3-N content of the silage to a minimum of 14.7% inclusion level of lablab forage. This may imply that at this optimum inclusion level, there is minimum hydrolysis of proteins to non-protein nitrogen. Therefore adding lablab forage beyond this optimum would result into increased buffer capacity and less stable silage. Therefore the crude protein of this silage would be improved by adding more soluble carbohydrates together with lablab forages. This would inactivate plant proteolysis and suppress protein hydrolysis (Kang, 2010) a consequence of rapid pH reduction as the lactic acid bacteria anaerobically hydrolyze the soluble carbohydrates to lactic acid. Nevertheless, the silage produced at the optimum inclusion level of the lablab forage was within the acceptable range (>10% NH_3-N) (Kung and Shaver, 2001)

The observed lactic acid concentration in both silages was higher than 10 g/kg DM and is considered to provide sufficient acidity to prevent proliferation of the undesirable Clostridia and entero-bacteria microorganisms (Kung, 2010). The low lactic acid to acetic acid ratio also indicates that fermentation was dominated by hetero-fermentative lactic acid bacteria which are less efficient in producing lactic acid (Segler, 2003b). This type of fermentation is interpreted to mean that there was an increase in acetic acid production at the expense of lactic acid. However, in addition to lactic acid concentration resulting from the fermentation of WSC, initial acidity of the pineapple by-products could be attributed to presence of organic acids mainly citric, ascorbic and tartaric possibly prevented undesirable fermentation process (Falade et al ., 2003). Since (Wright et al. 2000), reported a negative relationship between lactic acid content and DM intake of silages the lower lactic acid content obtained in these Brachiaria silages study would possibly encourage higher DM intake by dairy cattle.

5. Conclusion

Since the quality of silage for dairy cows depends on volatile fatty acid profiles, fibre and crude

protein composition, inclusion of lablab forage to *Brachiaria hybrid cv mulato II* silage at a rate of 17.1% yields optimum results in lactating cows. Furthermore, the improvement of silage protein beyond 17.1% inclusion without offsetting the buffer effect can only be done with improvement on WSC of the silage.

References

1. Aganga, A. A., and S. O. Tshwenyane. "Lucerne, Lablab and Leucaena leucocephala forages: production and utilization for livestock production." *Pakistan Journal of Nutrition* 2, no. 2 (2003): 46-53.
2. Falade, Olumuyiwa S., Olusoga R. Sowunmi, Adewale Oladipo, Ayo Tubosun, and Steve RA Adewusi. "The level of organic acids in some Nigerian fruits and their effect on mineral availability in composite diets." *Pak. J. Nutr* 2, no. 2 (2003): 82-83.
3. FAO, FAOSTAT. "FAO Statistical Data." (2004).
4. Henderson, Nancy. "Silage additives." *Animal Feed Science and Technology* 45, no. 1 (1993): 35-56.
5. Geofrey, Nviiri, Okello Horace, Turyagyenda Laban, Kigozi Abasi, and Mugerwa Swidiq. "Periodic Variations of Lablab purpureus Productivity Harvested at Different Growth Stages: A Case Study in Mid Northern Uganda. *International Journal of Science and Research* Volume 8 Issue 6, (2019).
6. Kabirizi, Jolly, Emma Ziiwa, Swidiq Mugerwa, Jean Ndikumana, and William Nanyennya. "Dry season forages for improving dairy production in smallholder systems in Uganda." *Tropical Grasslands-Forrajes Tropicales* 1, no. 2 (2013): 212-214.
7. Kammes, K. L., G. B. H. Heemink, K. A. Albrecht, and D. K. Combs. "Utilization of kura clover-reed canarygrass silage versus alfalfa silage by lactating dairy cows." *Journal of dairy science* 91, no. 8 (2008): 3138-3144.
8. Kato, H., F. B. Bareeba, C. Ebong, and E. Sabiiti. "Fermentation characteristics and nutrient composition of browses ensiled with maize fodder." *African Crop Science Journal* 12, no. 4 (2004): 393-400.
9. Kung Jr, Limin. "Understanding the biology of silage preservation to maximize quality and protect the environment." In *Proceedings, 2010 California Alfalfa & Forage Symposium and Corn/Cereal Silage Conference*, pp. 1-2. 2010.
10. Li, Yanbing, and Naoki Nishino. "Effects of ensiling fermentation and aerobic deterioration on the bacterial community in italian ryegrass, guinea grass, and whole-crop maize silages stored at high moisture content." *Asian-Australasian journal of animal sciences* 26, no. 9 (2013): 1304.
11. Manyawu, G. J., S. Sibanda, C. Mutisi, I. C. Chakoma, and P. N. B. Ndiweni. "The effect of pre-wilting and incorporation of maize meal on the fermentation of Bana grass silage." *Asian-australasian journal of animal sciences* 16, no. 6 (2003): 843-851.
12. Menardo, Simona, Paolo Balsari, Ernesto Tabacco, and Giorgio Borreani. "Effect of conservation time and the addition of lactic acid bacteria on the biogas and methane production of corn stalk silage." *BioEnergy Research* 8, no. 4 (2015): 1810-1823.
13. Murphy, Andrea M., and Pablo E. Colucci. "A tropical forage solution to poor quality ruminant diets: A review of Lablab purpureus." *Livestock Research for Rural Development* 11, no. 2 (1999): 1999.
14. Nviiri, Geofrey, H. Okello, P. Nakyewa, and G. A. Maiteki. "Extent of availability of major nutrients from selected Cereal Crop Residues to dairy ruminants as an alternative Dry Season Forage in Northern Uganda." *Journal of Advances in Agriculture, 4* 1 (2014).
15. Ridwan, Roni, Iman Rusmana, Yantyati Widyastuti, Komang G. Wiryawan, Bambang Prasetya, Mitsuo Sakamoto, and Moriya Ohkuma. "Fermentation characteristics and microbial diversity of tropical grass-legumes silages." *Asian-Australasian journal of animal sciences* 28, no. 4 (2015): 511.
16. Sas, S. A. S., and STAT User'S. Guide. "Version 9.1." *SAS Institute Inc., Cary, NC* (2003).
17. Seglar, Bill. "Fermentation analysis and silage quality testing." (2003).
18. Sibanda, S., R. M. Jingura, and J. H. Topps. "The effect of level of inclusion of the legume Desmodium uncinatum and the use of molasses or ground maize as additives on the chemical composition of grass-and maize-legume silages." *Animal Feed Science and Technology* 68, no. 3-4 (1997): 295-305.
19. Tiemann, Tassilo T., Carlos E. Lascano, H-R. Wettstein, Andrea Corina Mayer, Michael Kreuzer, and Hans Dieter Hess. "Effect of the tropical tannin-rich shrub legumes Calliandra calothyrsus and Flemingia macrophylla on methane emission and nitrogen and energy

balance in growing lambs." *Animal* 2, no. 5 (2008): 790-799.

20. Titterton, M., and F. B. Bareeba. "Grass and legume silages in the tropics." *FAO Plant Production and Protection Papers* (2000): 43-50.
21. Tyrolová, Y., and A. Výborná. "The effects of wilting and biological and chemical additives on the fermentation process in field pea silage." *Czech Journal of Animal Science* 56, no. 10 (2011): 427-432.
22. Van Soest, PJ van, J. B. Robertson, and B. A. Lewis. "Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition." *Journal of dairy science* 74, no. 10 (1991): 3583-3597.
23. Wright, D. A., F. J. Gordon, R. W. J. Steen, and D. C. Patterson. "Factors influencing the response in intake of silage and animal performance after wilting of grass before ensiling: a review." *Grass and Forage Science* 55, no. 1 (2000): 1-13.