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EFFECT OF ECOCOOL IN MIXED GRASS-LEGUME SILAGES ON MILK PRODUCTION OF FRIESIAN DAIRY COWS

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Abstract

Ecocool is comprised of Lactobacillus plantarum (NCIMB 40027). min 6 67x 10 cfu/g and Lactobacillus buchneri (NCIMB 30139). min 1 33x 10 cfu/g. A study to assess the potential contribution of Ecocool in inducing a faster and more efficient anaerobic start-up fermentation through L. plantarum and increasing aerobic stability at feed-out through L. buchneri in mixed grass-legume (Cenchrus purpureus var Kakamega I - Mucuna pruriens) silage at the ratio 3:1, respectively, was conducted at Mahwa National Zootechnical Station. Three forage pre-ensiling treatments were tested in this experiment namely, fresh non-ensiled herbage (control), ensiled without Ecocool (untreated) and ensiled with Ecocool (treated). Forage was ensiled for 1 day and fermentation was completed after 45 days, but the silo was opened after seven months. From 13th October to 24th November 2023, the three different forages were individually fed to nine lactating Friesian cows in a completely randomised design experiment (CRD). Three types of rations were assigned to each group of three dairy cows. To minimize animal selection bias, cows were balanced across treatments with respect to breed type, live weight, milk yield, lactation stage and parity. Daily milk yield was recorded and milk composition was analysed three times during the experiment. Silage analysis revealed that the untreated silage had the highest pH, indicating low quality silage while Ecocool treated silage had the lowest pH. Milk yield differed significantly among treatments (p <0.05). Cows allocated to silage treated with Ecocool had the highest average milk yield (15.75 l/day), compared to those on untreated silage (13.59 l/day), or control (12.35 l/day). Regarding milk quality assessment, there were no significant differences for almost all parameters analysed except proteins (P<0.05). The highest mean protein content was observed from cows fed Ecocool treated silage (3.58 ± 0.48 % DM) and the lowest was from cows assigned to the control (2.78 ± 0.15% DM).

Keywords: *Aerobic stability, Ecocool, Kakamega I, Milk yield, Mucuna, Silages*

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Introduction

In developing countries, including those in Africa, 60%-70% of the rural population depends on livestock (FAO, 2012), which contributes significantly to the livelihoods of many smallholder farmers (Zenda Za Begani et al., 2024). The contribution of the livestock sector to the agricultural gross domestic product of African countries is between 30 and 80 % (Erdaw, 2023) In dairy farming, feed alone constitutes more than 75% of investments because it ensures growth, production, reproduction and the acquisition of immunity (Maleko et al., 2019). However, in most African countries including Burundi, complex systemic challenges limit livestock productivity, of which the limited supply of quality feed is commonly represented as the greatest (Balehegn et al., 2021). The projected global increase in the demand for animal source foods, which will mostly be centred in low- and middle-income countries (LMIC), may allow smallholder livestock producers to increase their livelihoods, food security and nutrition (Baltenweck et al., 2020).

In Burundi, about 26% of households own at least one large livestock, and 60% own small livestock (Dolberg, 2008)(Security & Analysis, 2023). In the past decade, the Government of Burundi has been promoting dairy farming to improve income, nutritional security and livelihoods of smallholder farmers. Availability of high-quality feed is one of the main factors in the success of a production farm (Baris, 2023). The low availability of high-quality feed is a crucial constraint to improving milk yields and hence dairy income for smallholders, especially in the dry season (Duguma, 2022).

Currently in Burundi, climate-smart fodders crops such as Napier grass (*Cenchrus purpureus* var. Kakamega I) and *Mucuna pruriens* are gaining popularity due to their high forage productivity in tonnes DM per ha per year, respectively, of (30-48) (Ayele *et al.*, 2022); and (7-11) (Blomme *et al.*, 2022) that also is of high nutritive value. The high quantity of Napier and Mucuna herbage produced during the wet season can be conserved as silage, which will be fed to milking cows during the dry season to maintain high milk yields. Farmers will normally achieve high quality fermentation products and reduce ensiling losses. Additives are needed to improve silage fermentation and to avoid spoilage from undesirable bacteria, moulds and yeasts (Groseth *et al.*, 2024). Among the different additives, bacterial inoculants can help control and ensure good fermentation under the right ensiling conditions (Kumari et al., 2023). There are different inoculants which have been developed in order to improve the fermentation quality, aerobic stability and milk production for dairy cows (Ma et al., 2023).

(Okoye et al., 2023) confirmed that different types of inoculants can effectively improve the quality of silage during and after fermentation by promoting the growth of beneficial microbes and preventing the growth of pathogenic microorganisms. Inoculants which contain *L. buchneri* improve the aerobic stability of silage, consequently milk yield of cows feeding on the same silage. (Kok et al., 2024) found that silage inoculated with a combination of *Lactococcus lactis* and *Lentilactobacillus buchneri* improved milk production efficiency and nutrient digestibility in Friesians dairy cows. Inoculants like lactic acid bacteria (LAB) decrease the pH by stimulating a rapid (but controlled) fermentation of water-soluble carbohydrates (WSC) into lactic acid (Fan et al., 2021). According to (Monteiro et al., 2021), the cows receiving the silage treated with inoculant had greater milk production and greater lactose concentration compared to the control. The aim of preservation is to retain quality of silage and avoiding ensiling losses. If the fermentation doesn't occur efficiently, bad bacteria can persist and be responsible to convert lactic acid to butyric acid. This can produce unpalatable silage and up to a 17% loss of the original sugar content in the silage (Ávila & Carvalho, 2020).

Muck et al., (2018) found that cows eating inoculated silage had higher milk yields and lactose concentration compared to no inoculated silages. If fermentation is poor, undesirable bacteria such as Clostridium will persist and they will convert lactic acid to butyric acid (Liao et al., 2022). Presence of butyric acid makes the silage unpalatable. Usually, such silages will have lost 17% of the original sugar content (Ávila & Carvalho, 2020). Silage inoculant help to reduce dry matter losses, improve feed value, optimize feed intake and lower potential risk for animals (Gallo et al., 2021). Hence, the objective of our study was to evaluate the effect of the Ecocool in mixed grass-legume silages on milk production of Friesians dairy cow

Materials and Methods

Description of Study area

The experiment was conducted at the Mahwa National Research Station located in Gitega province, Ryansoro commune (about 100 km from Bujumbura) at an altitude of 1850m. Average annual temperature is 25º C. In Mahwa, during the entire year, the rain falls for 271.1 days and collects up to 1766mm of precipitation. The geographic coordinates are longitude of 29 º 46′ 73″ and latitude of 3º 48′ 11″ as shown in Figure 1 below.

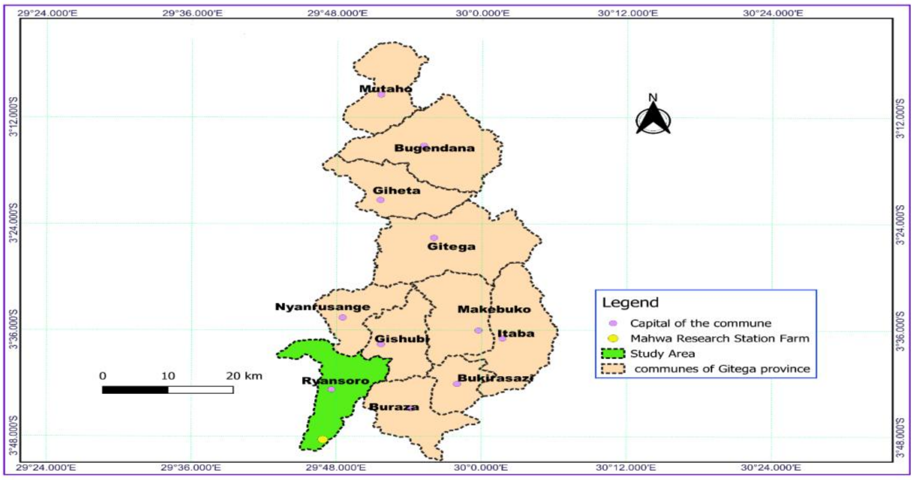

Fig.1 Location of Mahwa National Zootechnical Station (own map)

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Selection of animals
Nine Friesian dairy cows were selected and divided into three groups; each group was composed of three cows. Selection was based on age, average daily milk yield, stage of lactation and animal live weight. The three treatment diets were randomly allotted to the groups. The experiment had a Completely Randomised Design (CRD).

Before the start of the experiment, each animal was treated agaisnt external and internal parasites. The animals were fed two times per day, at 8:00 a.m. and 2:30 p.m. Only cows that were between 10 and 40 days in lactation, averaging 540 kg (±20 kg) of live weight and 4 years of age were considered. Age and weight were also taken into consideration to homogenise the target sample. Constitution and identification of homogenous groups of these cows was made using stratified sampling method according to the parameters cited above and then randomly selected.

Silage making
Two types of silages, each with a capacity of approximately 3 m^3 were prepared. One consisted of Kakamega I - *Mucuna pruriens* mixture (3:1 ratio). Another consisted of Kakamega I - *Mucuna pruriens (3:1)* inoculated with Ecocool that comprises *L. plantarum* (NCIMB 40027) min 6 67x 10 cfu/g and *L. bucneri* NCIMB 30139) min 1 33x 10 cfu/g.

Forage (Kakamega I) was harvested at 6-8 weeks of regrowth at 15 cm above the ground and *Mucuna pruriens* was harvested at the beginning of flowering. After harvesting, both were wilted to increase DM content and chopped immediately to avoid losses of water-soluble sugars. Chopping was done using a mechanised forage chopper fitted with a 3 mm screen. During ensiling, overlapping plastic sheeting was placed on the floor and sides of the silo to make it airtight, once filled. Fodder was spread layer after layer in a silo of approximately 3m^3 each and compacted by trampling with human feet. For the silo with Ecocool inoculant, the bottle was shaken to obtain a homogeneous mixture. Using the product instructions (150 g of Ecocool mixed with 200 liters of water to treat 100 tons), solution was prepared. A batch of 9 g of Ecocool was mixed with 12 liters of water to treat 3 tons of herbage. While ensiling, the solution was applied by successively spraying it over each ±15cm layer of chopped mixed-forage during ensiling. Thus, the two silos were filled with the chopped fodder to 50cm above ground level and then covered with a polyethylene film. Thereafter, polythene film was covered with loose soil to ensure complete from air and water entry. The two silos were incubated for 45 days before opening. The prepared treatment silages are shown in Table 1 below.

Experimental design
The experiment tested feeding quality of three silage-based diets, namely: T1 control or negative control, T2 or positive control for silage without Ecocool and T3 Silage with Ecocool.

Table 1: Basal and supplement herbage and proportion of mixture of concentrate given to experimental cows

Feed /Treatment	**T1 (Control)**	**T2**	**T3**
Basal forage	Chopped Kakamega I (20 kg)	Chopped Kakamega I (20 kg)	Chopped Kakamega I (20 kg)
Supplementary	mixed Kakamega I and Mucuna (3:1) chopped (20 kg)	mixed Kakamega I and Mucuna (3:1) silage (20 kg)	mixed Kakamega I and Mucuna (3:1) silage treated with Ecocool (20kg)
Concentrate	6 (kg per cow per day)	6 (kg per cow per day)	6 (kg per cow per day)
Maize flour	18	18	18
Rice bran	17	17	17
Cotton seed cake	23	23	23
Bone meal	1.5	1.5	1.5
Premix	0.5	0.5	0.5
Palm kernel cake	19	19	19
Limestone	2	2	2
Salt	1	1	1
Wheat Bran	18	18	18
Total	**100 kg**	**100 kg**	**100 kg**

The cow in all treatment groups received 20kg of Kakamega I fresh forage as basal feed. Mixture silage of Kakamega I and Mucuna respectively at a ratio of 3:1 and concentrate were then provided as supplementary feed. The experiment had a completely randomised design. Cattle had free access *(ad libitum*) to clean water throughout the feeding trial. The mineral licking blocks were available at will and permanently hung in the barn. Silages and concentrate were fed separately twice daily and silages were offered after concentrate feeding. Each dairy cow received a total of six kg of concentrate mixture per day (three kilogrammes in the morning and in the evening, respectively) to meet nutrient requirements. Milk production from the nine lactating cows were daily recorded (at 8:00 a.m. and at 4:00 p.m.).

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Data collection

Fresh herbage material was sampled at day 1, to determine chemical composition at inception of fermentation. Thereafter, silage samples were collected after 8 weeks of fermentation. Twenty-six (26) samples of silage and fresh samples (two for fresh material sample, twelve for untreated silage and twelve for treated silage) which total to 26 kg were placed in plastic containers and immediately stored in a freezer at -20 ºC at the National Veterinary Laboratory, and later moved to a cold room (2 -8 ºC), for transfer to the ILRI Mazingira laboratory in Kenya. Samples were analysed for volatile fatty acids, DM, crude protein, moisture, ammonium, nitrate and phosphorous contents. The silos were then opened for animal feeding. Sampling for aerobic stability was done on day 1, 6, 11, 16, 21 and 26 during feeding out.

Milk yield and feed intake were recorded at 8.00 a.m. and 4.00 p.m. and 8.00 a.m. and 2.00 p.m., respectively. Milk samples were tested for fat, salt, protein, Solid-not fat (SNF) and lactose contents and also conductivity, using a lactoscan Combo at National Veterinary Laboratory, in Bujumbura. Silage samples were analysed through gas liquid chromatography for volatile fatty acids (acetic, propionic, butyric, Iso- valeric and valeric acid) and proximate analysis for DM (oven drying at 105º C for 24 hours), crude protein (Kjeldahl method), water content, ammonium, nitrate and phosphorous.

Data analysis

Data from experiment (milk production and weight gain) were analysed using R Software as shown in the following model:

$$Y_{ik}=\mu+\alpha_i+\varepsilon_{ik}$$

where:

Yik: Milk yield

µ: Overall mean

αi: Effect of Ecocool inoculation

εij: Random error component

Results and Discussion

In this study, the dairy cows fed mixed grass-legume silage treated with Ecocool had increased milk production than cows fed untreated mixed grass-legume silage. The figure 2 shows that at onset of the experiment all cows had similar milk yields (P>0.05).

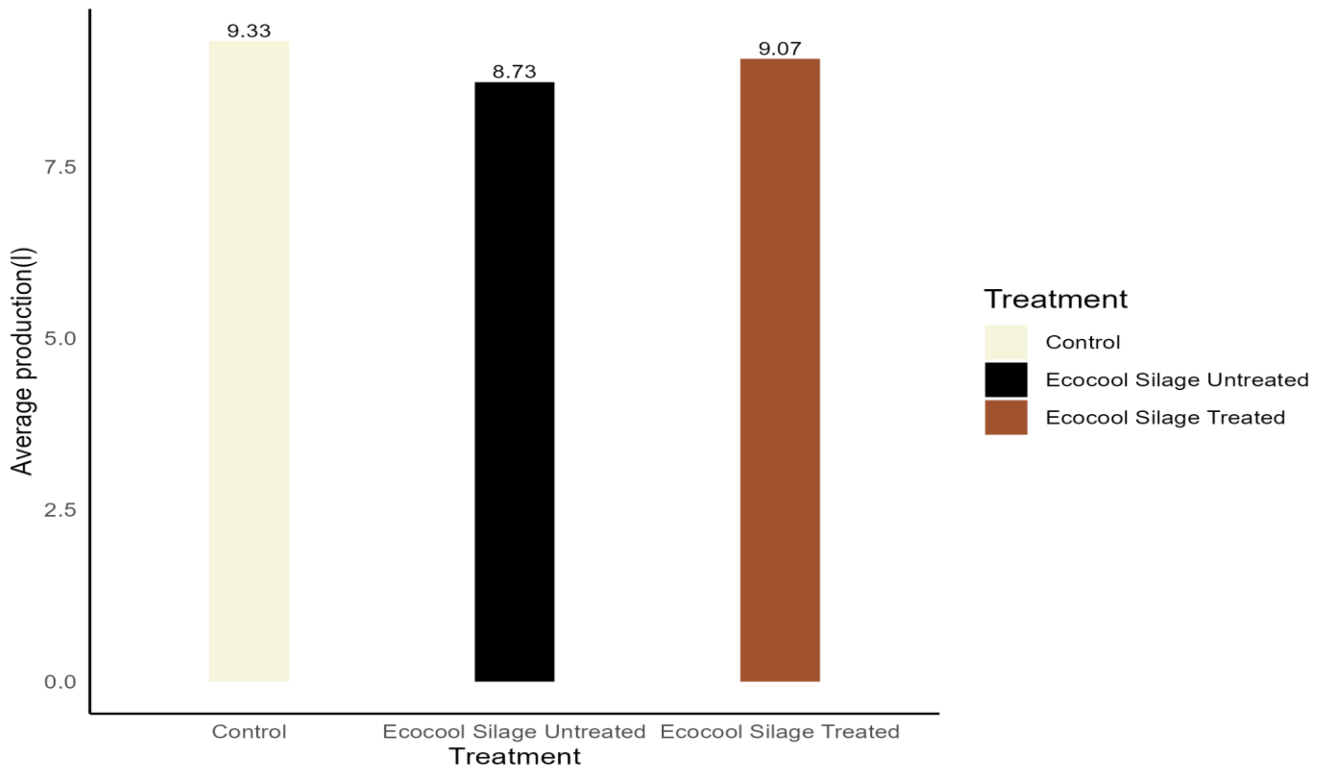

Fig.2 Comparison of pre-trial daily milk yield of Friesian cows selected for the experiment that compared benefits to inoculating Napier grass - *M. pruriens* mixtures with Ecocool (*L. plantarum* and *L. buchneri*) at ensiling on milk production.

The figure 3 shows that cows fed with inoculated (Ecocool) silage had significantly (P>0.01) higher milk yields than these fed fresh forage and silage without Ecocool. Cows fed by Ecocool silage treated had significantly (P<0.05) higher milks than cows fed by silage without Ecocool. Cows fed by silage without Ecocool tended to produce more milk than cows fed fresh forage. Grøseth et al., (2024) find that the use of silages treated with different additives had no effect in milk yield.

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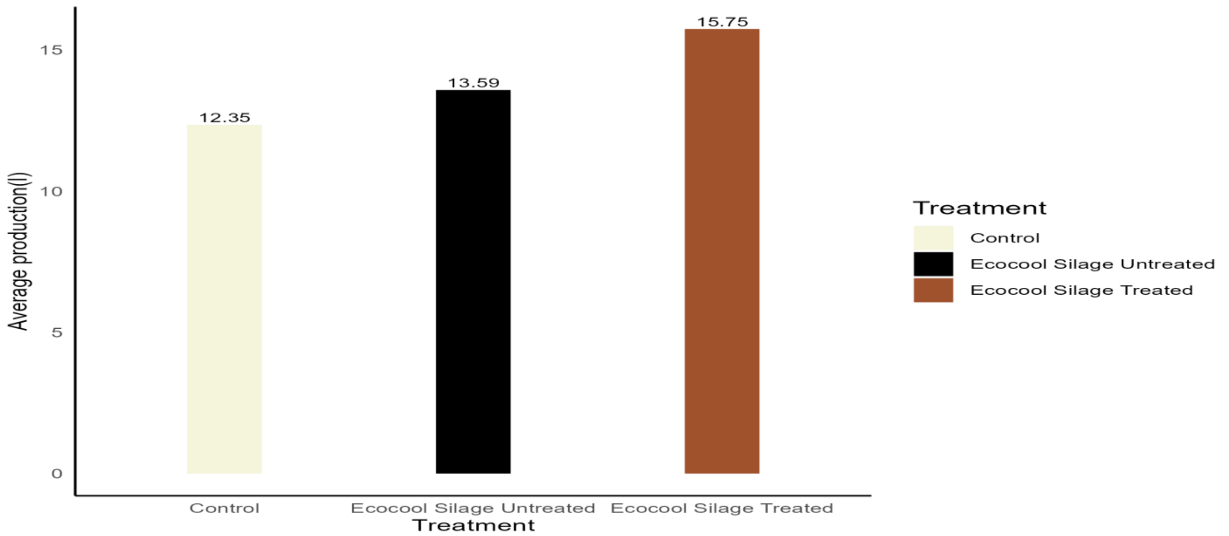

Figure 3: Benefits of inoculating and ensiling Napier grass -*M. pruriens* mixtures with Ecocool (*L. plantarum* and *L. buchneri*) on milk yield of Friesian cows.

The difference between cows consuming fresh material and those consuming untreated silage would have been due to the fermentation during ensiling which causes the increase in the dry matter of silage. For the case of silage treated with Ecocool, there is an added value which can be attributed to the Ecocool effect by reducing the loss of proteins during ensiling and spoilage of the silage. There have been a range of responses to feeding inoculated silages, including none (Jiang & Adesogan, 2017) trend trend towards increased milk yield (Bayatkouhsar et al., 2011; Ellis et al., 2016) and noticeably higher milk yield from two similar studies which have conducted in USA with an average milk increase of 2.47 litters per day per cow for the first and 3.10 litters per day per cow for the second (Wallberg, 2011). The milk yield performance is shown in Table 2 and Figure 4 below.

Table 2: Milk production of cows fed silages with or without ECOCOOL treatment

Items	T1 Control	T2 Untreated silage	T3 Treated silage	P-value	CV(%)
Initial	9.33[a]	8.73[a]	9.07[a]	0.3419	11.38
Milk production average	12.35[b]	13.59[b]	15.75[a]	0.0028	5
% of changes	35.29[b]	55.77[ab]	73.60[a]	0.01	13.40

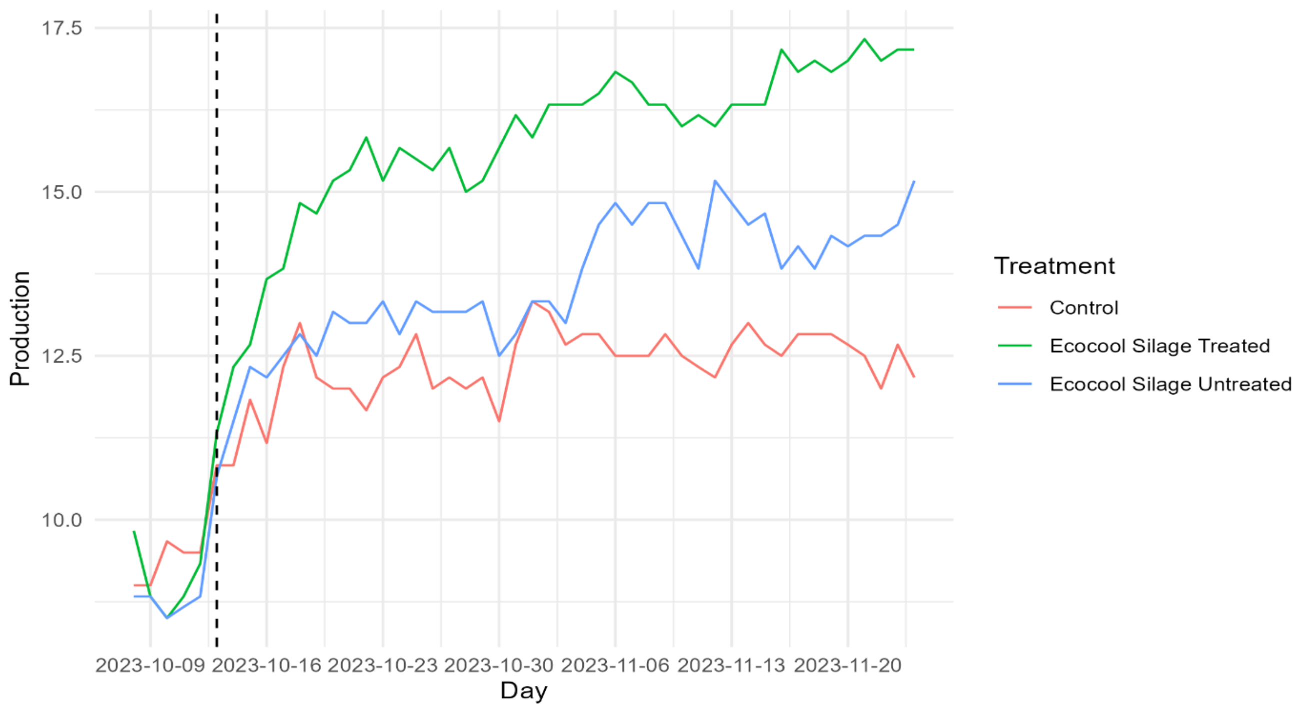

Fig.4 Comparison of milk evolution between treatments

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The figure 4 shows that there is a difference between the milk production curve from untreated silage and that of Ecocool treated silage.

The milk was assessed for fat, salt, protein, sugars and solids-not fat

Table 3: Milk quality assessment before and during experimentation

Treatment	Fat (%)	Salt (%)	Proteins (%)	Lactose (%)	Solid-Not- Fat (%)
Initial					
T1	3.76[a]±0.66	0.62[a]±0.02	2.87[a]±0.14	3.46[b]±0.34	7.64[a]±0.33
T2	3.49[a]±0.37	0.64[a]±0.01	2.87[a]±0.04	4.30[a]±0.06	7.85[a]±0.11
T3	3.69[a]±0.19	0.65[a]±0.03	2.88[a]±0.12	4.31[a]±0.18	7.85[a]±0.33
P- value	**0.84**	**0.47**	**0.56**	**0.01**	**0.44**
Intermediate					
T1(Control)	3.48[a]±0.10	0.62[a]±0.03	2.78[b]±0.15	3.84[a]±0.54	7.62[a]±0.40
T2	3.71[a]±0.62	0.66[a]±0.20	2.97[b]±0.10	4.46[a]±0.16	8.15[a]±0.30
T3	4.17[a]±0.20	0.65[a]±0.46	3.58[a]±0.48	4.35[a]±0.28	7.94[a]±0.50
P- value	**0.16**	**0.34**	**0.04**	**0.16**	**0.34**
Final					
T1(Control)	4.22[a]±0.55	0.60[a]±0.04	2.70[a]±0.15	4.05[b]±0.23	7.42[a]±0.39
T2	3.87[a]±0.33	0.67[a]±0.03	3.00[a]±0.13	4.34[ab]±0.05	8.22[a]±0.38
T3	4.35[a]±0.17	0.64[a]±0.03	3.20[a]±0.37	4.44[a]±0.09	7.87[a]±0.32
P- value	**0.47**	**0.13**	**0.19**	**0.08**	**0.17**

Mean and standard deviation

T1: Group of cows that was fed a base diet of green fodder (control); **T2:** Group of cows that was fed with silage without Ecocool; **T3:** Group of cows that was fed with silage with Ecocool

The study showed that there is no significant difference in fat, salt, proteins, lactose, conductivity and no solid fat between the milk of cows that were fed on treated silage and that which is not treated. This is in line with what said (Rossow et al., 2020) that no significant differences were observed in milk fat, protein or lactose concentrations, but a trend towards higher production was observed with Ecosyl treatment. (Huhtanen et al., 2015) find that the relationship between milk yield and milk composition are different from those genotype and phenotype. Milk yield is controlled by dietary management. In the contrary according to Craig et al., (2022), milk fat and protein concentration dropped with milk yield increase. The higher milk yield resulted in significantly higher production of all three components. The milk composition (butter fat, salt, protein, lactose, conductivity and solids-not-fat) from lactating cows fed on treated silage did not show any significant differences compared to cows consuming untreated silage or fresh forage. Parmar et al., (2020) confirmed that together with other elements including composition, feed treatment, seasonality, processing environment, the animal's quality and genetics characteristics have a major influence of the milk yield and quality. The results reported by Guliński & Kłopotowska, (2019) confirm the inverse relationship between Friesian milk yield and milk basic component content. From general knowledge, there are no studies that have been done on silage made from a mixture of grasses and legumes (*C. purpureus* and *M. pruriens*) preserved with Ecocool. But there are some references to studies done with Lucerne and those with corn.

The results of the analysis of the three silage samples, chopped fresh forage, untreated and Ecocool treated silage, are presented in the following Table 4.

Table 4: Volatile fatty acid and chemical composition of dietary feed used in the experiment

Characteristic	T1(Control)	**T2**, N = 6[1]	**T3**, N = 6[1]	p-value[2]
Water Content	89.1	83.42 ±1.00	83.64 ±0.27	0.6
Dry matter	10.9	16.36 ±0.27	16.58 ±1.00	0.6
Phosphorous	85.0	732 ±205	742 ±266	>0.9
pH	7.8	6.30 ±1.02	5.13 ±0.52	0.039
Crude protein	8.7	9.81 ±0.63	10.07 ±0.57	0.5
Nitrate	6.4	8.1 ±3.6	11.3 ±5.7	0.3
Acetic acid	0.01	0.12 ±0.13	0.16 ±0.14	0.6
propionic	0.01	0.027 ±0.027	0.043 ±0.031	0.3
Butyric acid	0.01	0.085 ±0.037	0.082 ±0.038	0.9

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Characteristic	T1(Control)	**T2**, N = 6[1]	**T3**, N = 6[1]	**p-value**[2]
Iso-valeric acid	0.002	0.003 ±0.005	0.007 ±0.005	0.3
Valeric acid	0.002	0.003 ±0.005	0.012 ±0.010	0.11
[1]Mean (SD)				

[2]One-way analysis of means.

Considering the parameters analyzed, the silage preserved with Ecocool did not show a significant difference except for the pH (P<0.05). This is in line with what is reported by Jatkauskas & Vrotniakiene, (2012), a study which concluded that due to a stronger lactic acid brought on by the homofermentative lactic acid bacteria, the inoculated silage significantly impacted the grass-legume silage quality attributes, including a low pH. The presence of a high concentration of butyric acid on samples of silage treated and untreated with Ecocool could be due to overstaying and may be exposure of the samples during transport and storage. This is demonstrated by the experiment which gave contrary results to the results of the laboratory. It is known that among the effects of Ecocool, there is the decrease in the amount of butyric acid and the increase in acetic acid. For these results, we observed an opposite case and this can be justified by the fact that the silage samples are kept for a long time at the National veterinary laboratory. The difference in concentration of acetic acid was not significant between silage treated with Ecocool and that without Ecocool. An accelerated decrease in pH during the ensilage process has a strong influence on reducing the growth of harmful bacteria (butyric, enterobacteriaceae). As a result, there is a decrease in organic matter loss and a decrease in protein degradation (proteolysis) during storage. The incorporation of homofermentative lactic acid bacteria into silage is one solution to achieve this.

According to the principle of neutrality (acid-base), the addition of homofermentative lactic acid bacteria lowers the pH as soon as they are applied and also makes it possible to direct favorable (lactic) fermentations that take place at low pH (< 5). Heterofermentative lactic acid bacteria work by transforming soluble sugars into lactic acid, acetic acid, alcohol, CO_2 and 1,2-propanediol. These bacteria are less effective than homofermentative bacteria in reducing pH. They are used for forages that are sufficiently rich in sugar. Among the heterofermentative bacteria, the most widely used strains are *L. buchneri*, *L. brevis* and *Propionibacterium acidipropionici*. According to (Boizumault, 2015), the use of bacterial inoculant in most cases leads to improvement in animal performance and the use of heterofermentative bacteria appears to be less directly related to animal performance. From these results, we observed that all parameters are not significant; water content (p-value = 0.6 ≥ 0.05), DM (p-value =0.3 ≥0.05), DW (p-value =9 ≥0.05), crude protein (p-value=0.5 ≥ 0.05), ammonium (p-value =0.2 ≥ 0.05) and nitrate (p-value = 0.3 ≥ 0.05) except the pH with a p-value of 0.031≤ 0.05.

To evaluate the effect of bacterial inoculants on fermentation and aerobic stability of silages, most experiments are carried out under controlled conditions at a constant temperature, usually between 20°C and 25°C. Results obtained under controlled conditions could lead to different results than when bacterial inoculants are tested under experimental conditions on the farm, where silages are kept with lower/higher or more variable temperatures. However, the small amounts of plant material typically used in laboratories make it difficult to accurately mimic the temperature fluctuations that occur in the peripheral layers of silos due to higher temperature fluctuations and faster daily temperature variations. The different species of microorganisms naturally present in silages or used as inoculants have different tolerances at low and high temperatures (Bernardes et al., 2018) and their activity could be limited or compromised under external conditions. Duponte et al., (2016) reported that the quality of the silage depends closely on the degree of acidity obtained during fermentation. According to Cai, (2020), low pH values were associated with stable and good quality silages.

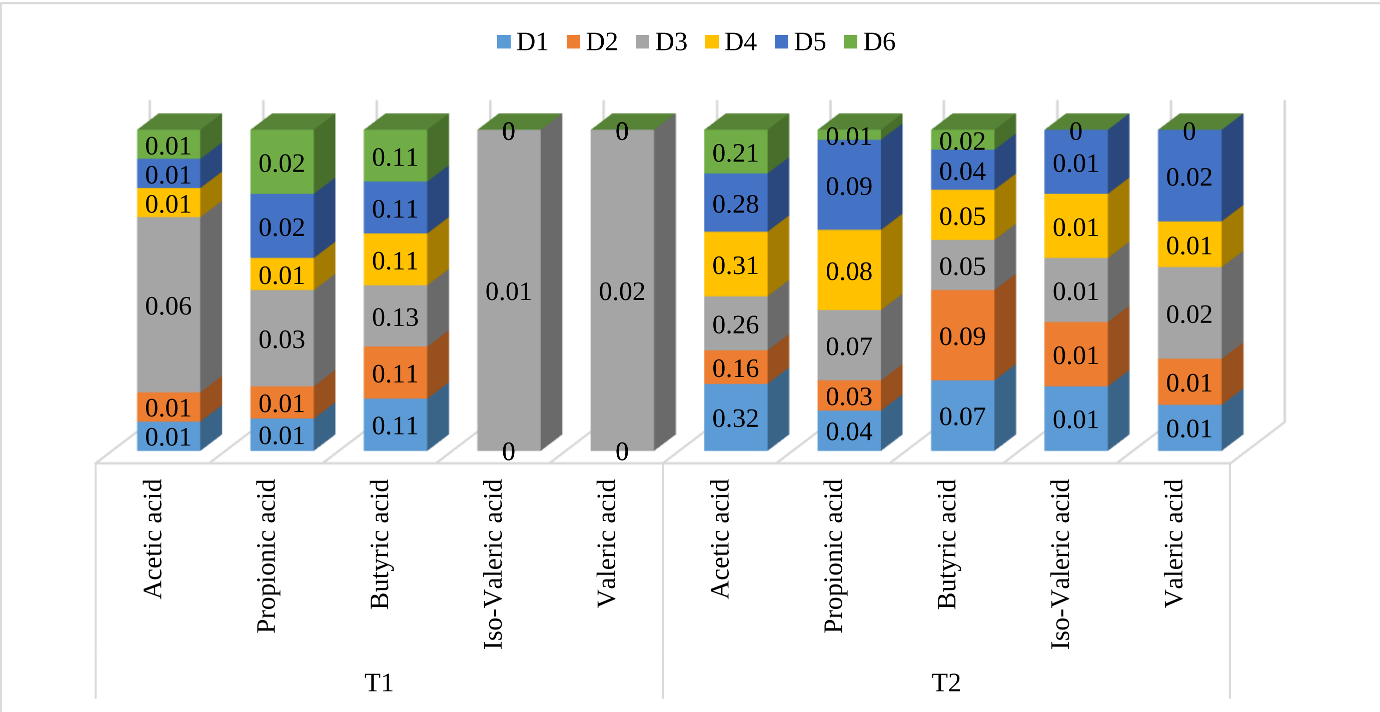

Fig.5 Aerobic stability assessment between T1 and T2 respectively silage untreated and silage treated

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D1= Day 1; **D2**=Day 6; **D3=** Day 11; **D4=** Day 16; **D5=** Day 21 and **D6=** Day 26. Between taking one sample and the next, there was an interval of 5 days.

T1: Treatment one (Silage untreated); **T2:** Treatment two (Silage treated with Ecocool)

From this figure, an increase in acetic acid production was observed in silage treated with Ecocool compared to silage without Ecocool. In contrast, this increase is not significant if we refer to the ANOVA test. Propionic acid was also slightly elevated in silage treated with Ecocool compared to untreated silage. This is similar to the results found by Kleinschmit & Kung (2006) which shows that silages treated with *L. buchneri* increases their acetic acid levels, which consequently reduces the ratio of lactic acid/acetic acid compared to untreated silages. This is due to the fact that some of the lactic acid is metabolized into acetic acid. Agarussi et al., (2022) reported that a slight increase in acetic acid production due to the use of *L. buchneri* is considered normal and has no negative effect on dietary intake. There is a concordance between the results of our experiment and the observations made by Auerbach et al., (2020) showing that silage can quickly deteriorate after exposure to air. According to Irawan et al., (2021) confirmation of the positive effects of heterofermentative bacteria alone or applied in combination with homofermentative bacteria on the aerobic stability of different types of silage by acting on yeast inhibition is observed. According to Zhao et al., (2018), both aerobic and the quality of silage fermentation have been effectively enhanced by additives. It is known that the presence of yeasts and molds develop the aerobic deterioration of the silage. Şehirli & Saydam, (2016) reported that acetic and propionic acids act in the inhibition of this deterioration.

Conclusion

Silage is an answer to feeding problems, and supplies feed especially during the dry season and prevent the loss of nutrients from the forage. To achieve production of good quality silage, several parameters must be taken into consideration, including the type of forage (containing the high nutritional value), the cutting period (maximum biomass and not lignified), wilting and the entire silage process from chopping, compaction, covering to the silage harvesting. The Ecocool inoculant has a significant effect in the increase of milk production.

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Conflict
The authors have no conflict of interest and accept the submission and publication of this manuscript.

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