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Effect of curd and cowpea (*Vigna unguilata*) inclusion on physical and proximate properties in maize (*Zea mays*) Silage

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Abstract

The current study had objective: To evaluate the nutritive value of maize silage prepared with the inclusion of bacterial inoculum (curd) and different levels of cowpea fodder. Major strains of LAB – forms to be *Lactobacillus plantarum*. The silage was made with maize (*Zea mays*) and cowpea (*Vigna unguilata*) fodder in varying proportions of 100:0, 90:10, 80:20 and 70:30 respectively, with or without the addition of Lactic Acid Bacteria (LAB-curd) in small containers of 10 kg and this was ensiled for 6 weeks. The *in-vitro* experiment consisted of 12 treatments. After ensiling, the various treatment groups were subjected to *in vitro* analysis such as dry matter, crude protein, ether extract and total ash. Majority of treatment groups showed yellowish green colour along with sweet odour and pH which varied from 3.92 to 4.11 with no fungal growth. The results indicated that silage reduced dry matter in range of 2.90 to 7.80% in comparison to fresh fodder DM and pH ($P<0.05$). The CP of silage treated with curd ranged from 7.38 to 13.24% depending on various inclusion levels. The inclusion of 30% cowpea with curd in maize silage resulted in a significant ($P<0.05$) increase in crude protein. From the results, it is concluded that, for appropriate levels of protein in fodder and silage, it is recommended to limit the non-legume and legume production to 70-90% and 30-10% respectively, along with the addition of LAB (curd). Inclusion of legumes in non-legume fodder has been found to be highly profitable and will be a solution to livestock nutritional imbalance in an environmentally sustainable way.

Keywords: Lactic acid bacteria (LAB), Cowpea, proximate principles, maize mixed silage.

Introduction

The health and production of cattle are strongly influenced by the amount of high-quality fodder that is fed to the animals. In previous decades, animals had ample access to an abundant supply of grass, crop leftovers and concentrate. However, the situation has now been altered. India's overall livestock population, as reported in the 20th Livestock Census of 2019, stands at 535.82 million, reflecting a 4.6% increase compared to the last Census conducted in 2012. The bovine population consists of cattle, buffalo, mithun and yak totaling 302.82 million individuals. It houses 57.3% of the global buffalo population and 14.7% of the global cow population. The rural areas of the country are home to around 74.26 million sheep and 148.8 million goats, as reported in the 20th Livestock Census of 2019. Addressing the difficulties of fodder and feed resource development is essential for the sustainability of animal husbandry in the country. The expected availability of fodder supplies, which was estimated to exceed 60% in the 1990s, has now decreased to approximately 50% of the overall requirement. Expanding the area of fodder crops is not feasible due to increasing competition from other land uses for cultivable land (Kumar and Agrawal *et al.*, 2008).

According to the IGFR Vision for 2050, the country is currently experiencing a shortage of 35.6% green fodder, 10.95% dry fodder and 44% concentrate feed ingredients.

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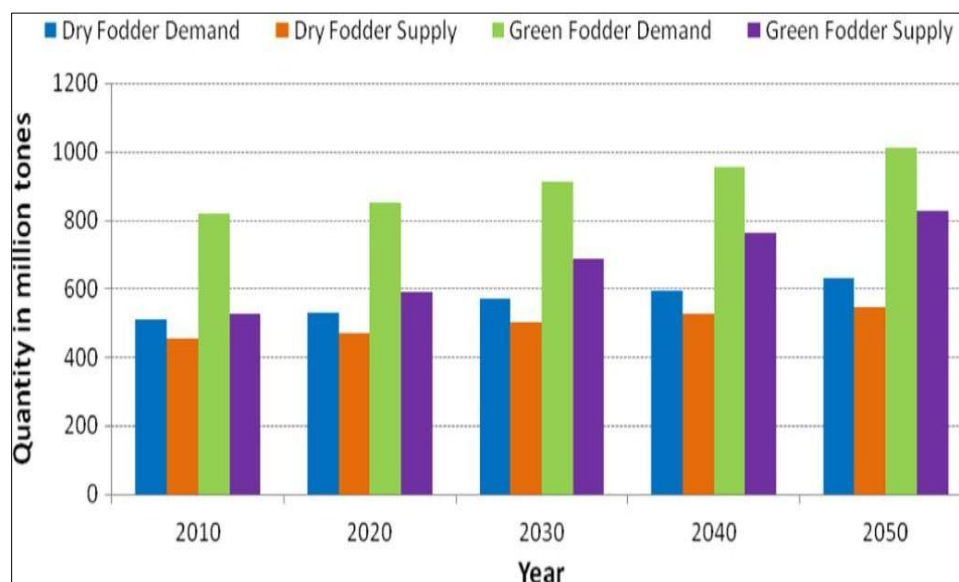


Fig 1: Year wise demand and supply of green fodder and dry fodder

Nevertheless, the proportion of cultivated land dedicated to fodder is merely 4% of the total cultivated land, which amounts to 8.4 million hectares in the country. This allocation has remained constant over the past few decades (Dagar, 2017, Halli *et al.*, 2018 and Meena *et al.*, 2018). The cost of feed and fodder constitutes more than two-thirds of the overall expenses in animal production (Ginwal *et al.*, 2019). Therefore, any initiatives aimed at enhancing the availability of feed and fodder and reducing the cost of feed will lead to improved financial returns for livestock farmers.

As part of efforts to address this issue, several contemporary technologies have emerged, one of which is the preservation of forages during the wet season for use as feed during the dry season (Dube, 1995). Hay and silage are the typical preserved forms of fodder crops. Ensiling is a superior method of conserving feed compared to hay making. Silage is the product that results from the intentional fermentation of crops in the absence of oxygen, first described by Narayanan and Dabadghao in 1972. The process is referred to as ensilage, and the building used to contain and store the ensiled material is known as a silo. The fermentation process is initiated by microorganisms found in fresh herbage or by the use of additions and microbial inoculants. This process occurs in the absence of oxygen, known as anaerobic conditions. Various crops such as sorghum, maize, and super napier are commonly used for preparing silage. Utilizing substances can enhance the quality of silage. Silage additives refer to any substances that can potentially accelerate fermentation or enhance the nutritional content of silage. The inclusion of bacterial inoculants and exogenous additives. Small-scale silage preparation offers advantages, but it is often accompanied by quality challenges because to limited biomass availability and compaction constraints. The present study aimed to increase the level of protein and find out the level of better digestibility in the fodder by combining legume fodder such as cowpea (*Vigna unguiculata*) with commonly cultivated cereal fodder maize (*Zea mays*). The legume fodders were chosen to be mixed with the cereal fodders, in varying proportion to make silages.

Materials and Methods

Study was to assess and compare different inclusion levels of legume fodder with cereal fodders along with addition of curd, as silage inoculant (LAB) and the nutrient content of cereal and legume silages. This study was conducted at the Veterinary college in Department of Livestock Production Management, KVAFSU, Bengaluru.

- **Latitude:** 13° 01' 27.42" N
- **Longitude:** 77° 35' 3.59" E.

The laboratory silages will be prepared with and without the addition of curd as bacterial inoculum from combinations of non-legume maize (*Zea mays*) and a legume fodder variety cowpea (*Vigna unguiculata*). Curd at the level commensurate with the bacterial concentration will be added uniformly to the fodder before ensiling.

4% of Curd (*Lactobacillus plantarum*) will be sprinkled uniformly over chopped green fodder intended for making silage. The representative samples from fresh fodders and their respective silages will be collected randomly in a considerably larger quantity in triplicates for moisture estimation and laboratory analysis. The collected samples after moisture estimation will be ground in the laboratory by Wiley mill using a sieve of 2 mm diameter. The dried and ground samples will be stored in clean, labelled airtight containers for further analysis. The processed samples of fresh fodder as well as silages will be analyzed for physical characteristics- colour of the fermented fodder was documented according to Olorunnisomo and Adesina (2014), odour of the silage fodder was documented promptly upon opening the container lid. pH was determined using the Playne and McDonald (1966) method. Unsealed silage material was checked for any mould growth after opening the container lid Gouri (2012) and proximate composition as per the standard methods described by the Association of Official Analytical Chemists (AOAC, 1990).



Sampling design: In the current experiment, laboratory silages of combinations of one non-legume (maize- 100, 90 ,80 and 70%) and one legume (Cowpea – 0, 10, 20 and 30 %) with and without the addition of curd will be prepared in triplicates. The laboratory silages will be prepared in 10 litres plastic/polyethylene containers as per the standard protocols for laboratory silage preparation (Gouri, 2012) and sealed for a period of six weeks. After six weeks, laboratory analysis will be conducted with samples collected from the treatments each with three replicates.

Table 1: Treatment combinations of maize–cowpea system with inoculum and curd application

Inclusion of Cowpea with maize Inoculum	M+C (100+0)	M+C (90+10)	M+C (80+20)	M+C (70+30)
No additive	T1(Control)	T2	T3	T4
With curd	T5	T6	T7	T8

Laboratory Analysis

The fresh fodder and silage samples will be analysed for physical properties viz., colour, odour, pH etc. and for proximate principles (AOAC, 1990) viz., dry matter, crude protein and ether extract.

Statistical analysis

The data related to different factors were statistically analyzed with one-way ANOVA using Graph pad Prism (8.4.3) and two-way ANOVA with the assistance of R statistical software. Tukey's test was used to compare the means, with a significance level of $P < 0.05$.

Results and Discussion

The silages made from maize (*Zea mays*) combined with cowpea (*Vigna unguiculata*) legume in varying ratios, exhibited a yellowish green coloration, these results corroborate the conclusions of

Trisnadewi and Cakra, (2020). All the silages, regardless of the treatments, exhibited a sweetish odour due to the combination of maize (*Zea mays*) and cowpea (*Vigna unguiculata*) legume at different levels. No fungal growth was seen in any of the silage varieties or their various inclusion levels. Comparable pH values to those documented by Htet *et al.* (2016) were observed in the silage treatments, which varied from 3.92 to 4.11. The amount of fodder biomass that may have been stored in the container ranged from 5.34 to 5.56 kg for silages made from maize mixed with cowpea legume in different inclusion levels along with addition of curd. The amount of silage biomass obtained from maize and cowpea silage ranged from 5.27 to 5.48 kg. Legumes generally exhibit resistance to pH lowering due to the presence of buffering substances including calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^{+}), which counteract the organic acids generated during fermentation and thereby prevent pH reduction (Playne and McDonald, 1966). Furthermore, legumes have a low DM and soluble carbohydrate content (Hawu *et al.*, 2022). The pH values of legume silages reported by Kung (2018) ranged from 4.2 to 4.7. In the present study, the addition of cowpea (*Vigna unguiculata*) resulted in a significant ($P < 0.05$) decrease in the dry matter content of the maize silages. The possible cause could be the lower concentration of dietary fiber in the legume utilized. There was a statistically significant decrease ($P < 0.05$) in the dry matter (DM) content of the fresh fodders used for silage and six weeks of silage fodder.

Dry matter (DM):

In No additive group, DM per cent was significantly ($P < 0.05$) higher in M+C (70+30) group in comparison to other treatment in both fresh fodder and silage. Lower value is observed in M+C (100+0) group in both fresh fodder and silage. The Curd group, consisting of M+C (90+10) had the highest percentage of DM in both fresh fodder and silage and M+C (100+0) had the lowest percentage of DM in both fresh fodder and silage.

The dry matter (DM) proportion in the silage fodders was substantially lower ($P < 0.05$) than that of the fresh fodders that were utilized in silage making. The dry matter (DM) contents in all silages consistently reduced during the ensiling process when compared to the chemical composition of the materials before ensiling. This could be attributed to the fact that the readily decomposable components (such as water-soluble carbohydrates) of silage were enzymatically converted by microorganisms into silage acids, carbon dioxide and ethanol during the process of silage fermentation (Chen *et al.*, 2019). A comparable finding was made in a study conducted by Chen *et al.* (2019), where they substituted Sorghum with alfalfa legume fodder in varying quantities. Use of LAB (curd) in present study in silage is in align with the results from Tabacco *et al.* (2011) study where it was quoted that the dry matter (DM) losses significantly increased during aerobic deterioration in the control and *Lactobacillus plantarum* inoculum (LP) silages, surpassing the initial stored DM values of after days of ensiling for maize and sorghum, respectively. Similar results were reported by Gouri *et al.* (2023), where DM per cent significantly decreased in maize stover silage with *Lactobacillus* culture addition. This is in contrary to results by Huyen *et al.* (2020) where it was stated that the silage fermentation quality of both rain-treated and fresh ryegrass was enhanced in the present study through the reduction of dry matter losses induced by the LAB (curd) inoculant treatments.

Crude protein (CP)

In No additive group, higher value is observed in M+C (70+30) in both fresh fodder and silage. Lower value is observed in M+C (100+0) in both fresh fodder and silage. In silage - in Curd, M+C (70+30) had the highest CP while M+C (100+0) has lowest CP in study.

The findings in the present study were consistent with results obtained by Phiri *et al.* (2007) and Chen *et al.* (2019) where it was stated that decrease in crude protein (CP) content in the silage compared to fresh fodders can be attributed to the breakdown of protein during the ensiling process. This breakdown leads to increased levels of non-protein nitrogen in the silage compared to the herbage before ensiling. Similar results were seen in Jolapure (2022). But present findings are contrary to findings of Htet *et al.* (2021), who observed a rise in the CP content after the fodders were ensiled. The present results were concurrent with the study conducted by Gordon in 1989, it was found that diets made with inoculated (curd) silage had a higher crude protein (CP) level. Similar was reported in Gang *et al.* (2020) study, where significant increase in CP with LAB (curd) inoculums. While study by Trabi *et al.* (2017) were in contrary which stated that

there were no significant differences ($P < 0.05$) observed in the levels of crude protein (CP) between the treatments following ensiling.

Ether extract (EE)

In No additive group, higher value is observed in M + C (100+0) in fresh fodder. While in silage, the EE per cent was significantly ($P < 0.05$) higher in M+C (80+20) group. The EE per cent was significantly ($P < 0.05$) lower in M+C (90+10) group in both fresh fodder and silage. In curd group, M+C (100+0) has the highest in both fresh fodder and silage, while lowest is seen in M+C (70+30). it is interpreted that current study found statistically significant ($P < 0.05$) difference among M+C (100+0), M+C (90+10) and M+C (70+30) groups on comparison between fresh fodder and their respective silage.

The present findings of silage are in agreement with the findings of Amodu *et al.* (2008), who observed no influence on EE concentration when legume was included. But this result contradicts the EE content in groups with a higher proportion of legume showed a consistent linear increase, which aligns with the findings reported by Chen *et al.* (2019). The outcome was comparable to the finding reported by Htet *et al.* (2021). Similar results were observed in Gouri *et al.* (2023). No significant change in EE content with *Lactobacillus* addition. But contrary results were seen in Lei *et al.* (2017) study, here there was variation in EE.

Total ash (TA)

In No additive group, the percentage of TA did differ significantly ($P < 0.05$) higher in M+C (90+10) group in fresh fodder and in silage, higher is seen in M+C (90+10). Significantly ($P < 0.05$) lower difference is observed in M+C (70+30) in fresh fodder and in silage, lower value is seen in M+C (70+30). In curd treated group, the percentage of TA differ non-significantly ($P > 0.05$) higher in M+C (90+10) group in fresh fodder and in silage, M+C (90+10) was significantly ($P < 0.05$) higher. While significantly ($P < 0.05$) lower value is seen in M+C (80+20) in fresh fodder and in silage it is in M+C (100+0).

In Curd-LAB treated silage groups, the present findings were in agreement to Lei *et al.* (2017), where it was quoted that the marginally increased levels of ash during the experiment can be due to the elimination of dry matter (DM) due to the release of CO_2 and VFAs. This loss is explained as a percentage of the DM. But present results were contrary with results from Khan *et al.* (2023) study the lowest ash concentrations in the ensiled CS with inoculant when compared to other conditions ($P < 0.05$).

Table 2: Physical properties of maize silage prepared by the addition of different levels of cowpea fodder

Parameter	MC	M+C (90+10)	M+C (80+20)	M+C (70+30)
Colour	Yellowish green	Yellowish green	Yellowish green	Yellowish green
Smell	Sweet aroma	Sweet aroma	Sweet aroma	Sweet aroma
Mould growth visibility	Nil	Nil	Nil	Nil
Weight (kg) of fresh fodder – Fodder	5.43 ± 0.006	5.38 ± 0.024	5.39 ± 0.043	5.44 ± 0.023
Weight (kg) of fresh fodder – Silage	5.34 ± 0.024	5.32 ± 0.011	5.32 ± 0.040	5.31 ± 0.024
Weight (kg) of dry matter – Fodder	1.27 ± 0.003	1.27 ± 0.005	1.30 ± 0.017	1.37 ± 0.005
Weight (kg) of dry matter – Silage	1.15 ± 0.003	1.21 ± 0.003	1.25 ± 0.011	1.29 ± 0.005
Dry matter % – Fodder	23.45 ± 0.63a	23.56 ± 0.63a	24.16 ± 0.63a	25.15 ± 0.63a
Dry matter % – Silage	21.62 ± 0.29a	22.77 ± 0.72a	23.44 ± 0.54a	24.27 ± 0.52b
pH	4.13 ± 0.63a	4.11 ± 0.63a	4.11 ± 0.63a	4.11 ± 0.63a

Note: Means in the same row with no common superscripts differ significantly ($P < 0.05$).

Table 3: Physical properties of maize silages prepared by the addition of different levels of cowpea fodder and curd

Parameter	MC	M+C (90+10)	M+C (80+20)	M+C (70+30)
Colour	Yellowish green	Yellowish green	Yellowish green	Brownish green
Smell	Sweet aroma	Sweet aroma	Sweet aroma	Sweet aroma
Mould growth visibility	Nil	Nil	Nil	Nil
Weight (kg) of fresh fodder – Fodder	5.43 ± 0.006	5.45 ± 0.129	5.50 ± 0.052	5.55 ± 0.078
Weight (kg) of fresh fodder – Silage	5.34 ± 0.024	5.38 ± 0.133	5.31 ± 0.059	5.48 ± 0.087
Weight (kg) of dry matter – Fodder	1.187 ± 0.003	1.34 ± 0.030	1.33 ± 0.014	1.35 ± 0.020
Weight (kg) of dry matter – Silage	1.18 ± 0.003	1.34 ± 0.030	1.33 ± 0.014	1.35 ± 0.020
Dry matter % – Fodder	21.84 ± 0.63a	24.57 ± 0.63a	24.20 ± 0.63a	24.30 ± 0.63a
Dry matter % – Silage	20.63 ± 0.63a	23.45 ± 0.27bc	23.15 ± 0.70ac	23.08 ± 0.72ac
pH	3.92 ± 0.58a	3.96 ± 0.58a	3.94 ± 0.60a	3.94 ± 0.60a

Note: Means in the same row with no common superscripts differ significantly ($P < 0.05$).

Table 4: Dry matter (%) content of varying proportion of maize and cowpea fresh fodder and laboratory silages prepared with and without addition of curd

Treatment	M+C (100+0)	M+C (90+10)	M+C (80+20)	M+C (70+30)
Fresh fodder – No additive DM (%)	23.45 ± 0.63a	23.56 ± 0.61a	24.16 ± 0.62a	25.15 ± 0.63a
Fresh fodder – With curd DM (%)	21.84 ± 0.62a	24.57 ± 0.60a	24.20 ± 0.61a	24.30 ± 0.62a
Silage – No additive DM (%)	21.62 ± 0.28a	22.77 ± 0.72a	23.44 ± 0.54a	24.27 ± 0.52ab
Silage – With curd DM (%)	20.63 ± 0.62ab	23.45 ± 0.27ab	23.15 ± 0.70a	23.08 ± 0.72abc

Note: Means in the same row with no common superscripts differ significantly ($P < 0.05$).

Table 5: Crude protein (%) content of varying proportion of maize and cowpea fresh fodder and laboratory silages prepared with and without addition of curd

Treatment	M+C (100+0)	M+C (90+10)	M+C (80+20)	M+C (70+30)
Fresh fodder – No additive CP (%)	7.29 ± 0.64a	7.92 ± 0.59a	10.33 ± 0.64ab	14.08 ± 0.59b
Fresh fodder – With curd CP (%)	7.48 ± 0.59a	8.31 ± 0.64ab	12.06 ± 0.59ab	13.68 ± 0.64a
Silage – No additive CP (%)	7.10 ± 0.64a	7.80 ± 0.64a	10.20 ± 0.64ab	13.10 ± 0.58b
Silage – With curd CP (%)	7.38 ± 0.64a	8.29 ± 0.64ab	11.80 ± 0.64ab	13.24 ± 0.64a

Table 6: Ether extract (%) content of varying proportion of maize and cowpea fresh fodder and laboratory silages prepared with and without addition of curd.

Fodder Type	Treatment	M+C (100+0)	M+C (90+10)	M+C (80+20)	M+C (70+30)
Fresh fodder – EE (%)	No additive	2.43 ± 0.15 ^{abc}	1.14 ± 0.03 ^a	1.60 ± 0.05 ^b	1.84 ± 0.05 ^c
Fresh fodder – EE (%)	Curd	1.83 ± 0.09 ^a	1.41 ± 0.06 ^a	1.57 ± 0.07 ^a	1.32 ± 0.05 ^a
Silage – EE (%)	No additive	1.47 ± 0.04 ^{abc}	1.44 ± 0.03 ^a	1.94 ± 0.05 ^b	1.61 ± 0.04 ^c
Silage – EE (%)	Curd	3.16 ± 0.09 ^a	2.27 ± 0.06 ^a	2.06 ± 0.06 ^a	1.96 ± 0.07 ^a

Note: M+C refers to Maize + Cowpea proportions. Superscripts represent statistical significance markers as shown in the original image.

Table 7: Total ash (%) content of varying proportion of maize and cowpea fresh fodder and laboratory silages prepared with and without addition of curd.

Fodder Type	Treatment	M+C (100+0)	M+C (90+10)	M+C (80+20)	M+C (70+30)
Fresh fodder – TA (%)	No additive	6.64 ± 0.03 ^{ab}	6.88 ± 0.01 ^a	6.46 ± 0.01 ^{ab}	6.25 ± 0.01 ^c
Fresh fodder – TA (%)	Curd	6.47 ± 0.01 ^{cab}	6.58 ± 0.01 ^{ca}	6.33 ± 0.01 ^{bc}	6.46 ± 0.01 ^b
Silage – TA (%)	No additive	6.88 ± 0.01 ^b	6.97 ± 0.01 ^c	6.79 ± 0.01 ^{cab}	6.69 ± 0.01 ^{cb}
Silage – TA (%)	Curd	6.04 ± 0.03 ^c	7.19 ± 0.01 ^b	—	—

Conclusion

In conclusion, laboratory silages mixed with legume exhibited a higher nutritive value compared to laboratory silage consisting solely of cereal fodder. In order to get the highest protein content, it is advisable to include cowpea legume at a rate of 30% in the formulation. The legume mixed with maize along with curd had the best protein value when 30% of cowpea (*Vigna unguiculata*) was incorporated. The laboratory silages containing cowpea legume mixed with maize were determined to have higher nutritional content. In order to achieve an ideal balance protein and ether extract in the diet, the proportion of non-legumes and legumes might be limited at 90-70% and 10-30%. Including legumes along with non-legume fodders is highly suggested to dairy farmers as an effective solution to combat nutritional deficiencies.

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