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Inclusion levels of tree and herbaceous legumes on nutritive quality of grass silage: results from on-farm trials

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Abstract The escalating prices of protein concentrate supplements and increasing demand for dairy products in Ethiopia call for preservation of surplus forage during the growing season, to be fed to dairy cows at time of feed shortage. The objective of this on-farm study was to evaluate the nutritive quality of Napier and Desho grass silages with tree legume (sesbania) and herbaceous legumes (alfalfa, lablab, and faba bean) as legume components. Napier or Desho grasses were ensiled in bags with each legume at 0, 20, 30 and 40% inclusion levels (fresh weight). Replicates included nine and six farms in Mecha and Ad'aa districts, respectively. Silage bags were opened after 60 days, samples were taken for chemical analyses and evaluated for physical features. Data were analyzed using a completely randomized design in SPSS and differences among means separated using Duncan's. Silages were firm at Mecha and fairly firm at Ada'a. No mold appeared. pH values varied from 3.94 to 4.5 and increased ($p<0.001$) with increasing levels of legume inclusion. The crude protein values followed a similar pattern to that of the pH. The fiber contents, NDF ($p<0.001$) and ADF ($p<0.05$/$p<0.01$) declined while that of ADL increased ($p<0.001$) with increasing legume inclusion levels. The different legume-based silages were assessed as highly palatable and revealed that the use of tree and herbaceous legumes can improve the nutritive quality of grasses.

Keywords Ada'a · Mecha · Ethiopia · Pennisetum · Nutritive value · Seasonality

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Introduction

Two-thirds of the global rural population are engaged in mixed crop-livestock systems (MCLS), producing around 50, 60 and 75% of the world's cereals, meat, and milk (Paul et al. 2020). In Ethiopia, MCLS produce even 80 and 87% of the milk and meat, respectively. Seventy two percent of the national cattle population is in MCLS, with cattle being the dominant species for 70 to 90% of the livestock holding households (Shapiro et al. 2015). Crops and livestock play interdependent roles, with livestock providing draught power and manure for cropping, while crop residues provide feed for the livestock (Management Entity 2021).

In many developing countries, including Ethiopia, projections show that the demand for animal sourced foods (ASFs) will double by 2050 (Sattari et al. 2016), owing to human population growth, urbanization (Mekonnen et al. 2022), and rising incomes (van der Lee 2020). Thus, the increase in demand for roughages matching the increase in ASFs is equally inevitable. Nevertheless, scarcity of consistent availability of quantity and quality livestock feed is a major constraint of farmers in MCLS in Sub-Saharan Africa (SSA) including Ethiopia, especially during the dry season (Paul et al. 2020). Between 79 and 99% of the MCLS farmers experience feed shortages at some point of the year (Njarui et al. 2016). In Ethiopia, feed requirement is estimated at 126.35 million tons per year on dry matter basis, while the actual availability is 99.5 million tons per year (FAO 2018). Generally, feed costs cover 70% of the total cost of animal production (FAO 2019).

While there is a critical shortage of succulent green feed during the dry season and pasture production widely fluctuates, Ethiopian dairy farmers do not use improved feed conservation practices beyond hay making (Assefa et al. 2017). The increasing trend of dairy production in Ethiopia (Effa et al. 2017) and in other developing countries (Hawu et al. 2022) requires conservation of surplus forage during the growing season. Prices of commercial concentrate feed are also escalating over time. Animal feed inflation rate (45–55%) was higher than food inflation rate (24.1%) from July 2020 to July 2021 in Ethiopia (Negash 2022), justifying the need to look for alternatives. Most grasses used are low in protein values (Kebede et al. 2016) and diets based on grass only will not meet nutritional requirement of a lactating cow, thus requiring supplementation with sources of fermentable nitrogen and energy (Ofori and Nartey 2018). Tree and herbaceous legumes have multiple roles, including maintaining the natural resource base through soil stabilization, preventing soil erosion, contributing to soil fertility through microbial nitrogen fixation and organic matter, and climate change mitigation through carbon sequestration (Hanson and Ellis 2020).

Among the popular grass species used by smallholder dairy farmers in developing countries for feeding their animals are Napier/elephant (*Pennisetum purpureum)* and Desho (*Pennisetum pedicellatum*) grasses. The nutritive quality of Napier or Desho grass can be improved through incorporation of legumes (Hawu et al. 2022). Nevertheless, there is no information on the nutritive quality of grass silage that incorporates protein rich tree and herbaceous legumes in Ethiopia. This study, therefore, examined the effect of inclusion of different levels of sesbania (*Sesbania sesban*), alfalfa (*Medicago sativa*), lablab (*Lablab purpureus*), and local faba bean (*Vicia faba*) in Napier or Desho grass silage on physical and nutritional quality parameters through on-farm research.

Materials and methods

The studies were undertaken in Ada'a (East Shoa zone, Oromia Region) and Mecha districts (West Gojam zone, Amhara Region), Ethiopia (Fig. 1). The study districts are among the 39 districts where the Building Rural Income through Inclusive Dairy Business Growth in Ethiopia project (BRIDGE) undertakes different interventions along the dairy value chain. Both districts are in the mid-altitude areas of Ethiopia and are characterized by MCLS. In Mecha, altitude ranges from 1800 to 2500 m above sea level (masl) and annual rainfall is between 820 and 1250 mm (Astewel and Yihenew 2018). Most soils are acidic Nitisols, with a pH of 4.7–5.4 (Mekonnen 2015). The mean annual temperature ranges from 17 to 30 °C. At Ada'a, the altitude ranges from 1540 to 3100 masl, soils are mainly pellic vertisols with pH 6.38 to 7.09, the mean annual rainfall is 867 mm; the minimum and maximum temperature ranges are 9–13 °C and 22–26 °C, respectively.

Selection of farms and forage materials

The studies used an action research approach, undertaking on-farm trials together with farmers. The farmers were selected through the BRIDGE project by staff of the district livestock officers, based on their willingness to participate in the study. Nine farmers in Mecha district and six in Ada'a district were identified and used as

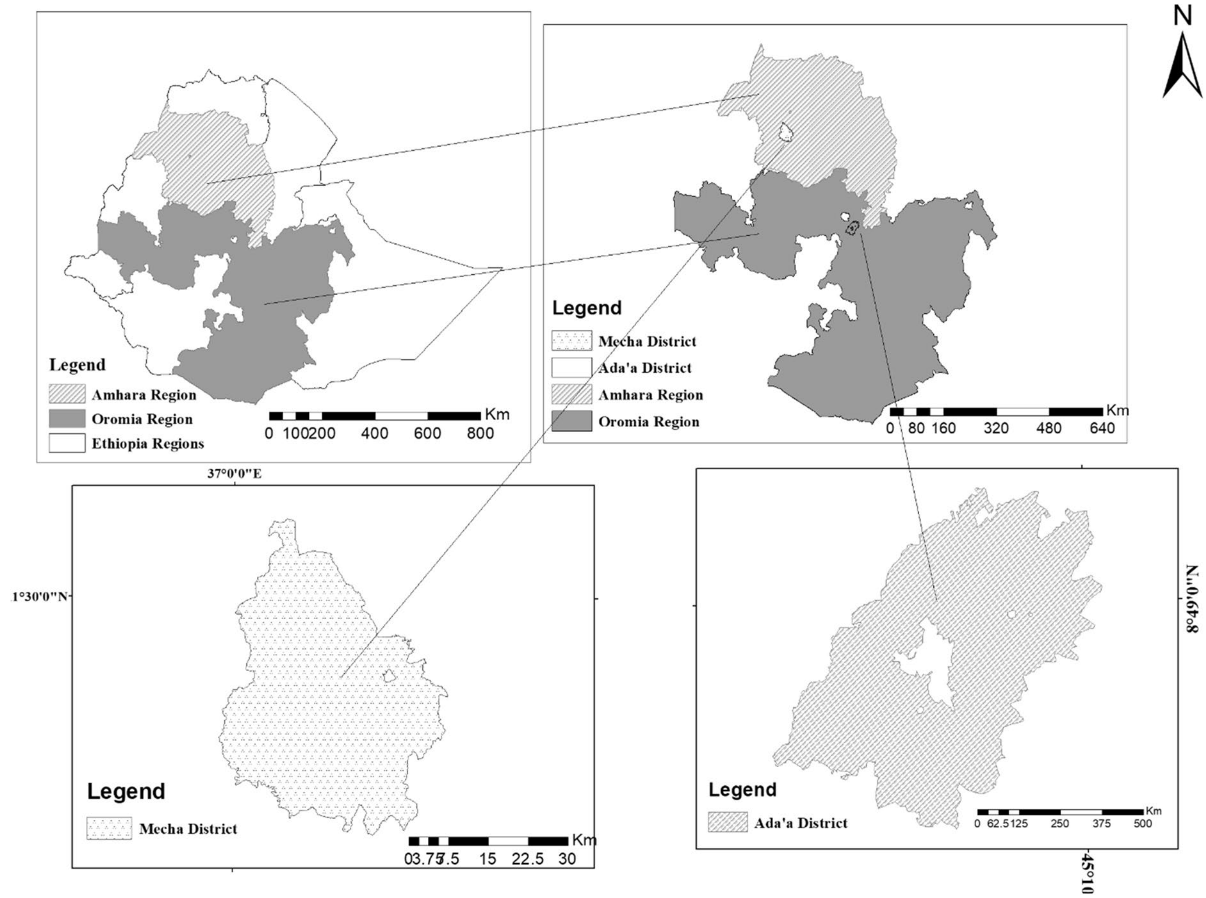

Fig. 1 Location of the study areas

replications. The farmers in each district were living near to each other. Thus, in each district, the farm-sites selected for planting the forage materials were similar in terms of agroecological conditions with respect to soil type, altitude, rainfall, temperatures, and slope. Napier and Desho grasses and alfalfa and lablab were planted in the plots of farmers in July 2021 during the main growing season. The splits of Desho and Napier grasses were planted at a rate of 20,000 and 10,000 plants ha^{-1}, respectively. The seeding rates for alfalfa and lablab were 10 and 20 kg ha^{-1}, respectively (Zewdu 2007). The choice of the legumes is based on their popularity in each district.

Experimental design and silage making procedures

The studies were intended to investigate the potential effect of each legume on its ensiling effect on the specific grass. Thus, the experiment was planned in a completely randomized design (CRD). In Ada'a district, the study examined silages from Desho grass (Areka variety) alone or with the inclusions of three levels of alfalfa or lablab (20, 30 and 40% by fresh weight). Similarly, in Mecha district, the study examined silages of Napier grass (ILRI accession 16,791) alone or with inclusion levels of alfalfa, sesbania and faba bean legumes (20, 30 and 40% by fresh weight) (Table 1). The faba bean herbage was purchased from a farmer who planted a local faba bean variety during the main growing season of 2021, as farmers commonly feed animals the herbage when they think the planting does not have good potential for seed production. Sesbania herbage was harvested from already existing sesbania trees planted about 2 and half years ago by farmers in the surrounding areas.

Table 1 Types of silages and levels of inclusions of the different forage legumes

Levels of inclusion of the legumes	Ada'a district silage mixtures	Mecha district silage mixtures
0	Desho grass	Napier grass
20	Desho-alfalfa	Napier-alfalfa
30	Desho-alfalfa	Napier-alfalfa
40	Desho-alfalfa	Napier-alfalfa
20	Desho-lablab	Napier-Sesbania
30	Desho-lablab	Napier-Sesbania
40	Desho-lablab	Napier-Sesbania
20		Napier-Faba bean
30		Napier-Faba bean
40		Napier-Faba bean

All herbage materials used in silage making (except sesbania) were harvested 5 to 7 cm above the ground using hand sickles. The Napier grass was harvested when the plant was at 1 to 1.2 m height, and the Desho grass at 0.8 to 1.0 m. The legumes were harvested when they were 15 to 25% flowering. At Mecha, the harvested herbages were wilted for about 6 to 24 h, depending on the moisture content, and were chopped to a length of about 3 to 5 cm, using a chopping machine (Brathma chopper made in Brazil by Trapp company, model TRP 80 G). At Ada'a, herbage was hand chopped to a similar length. The wilted and chopped grasses and legumes were weighed and mixed at different proportions, as indicated in Table 1. Molasses was added at 3% by weight of the herbage and was diluted after weighing with 1 to 2 L of water, as the molasses used was too thick for mixing. It was mixed with the herbage mixtures prior to ensiling. The silage was prepared using plastic bags, while compacting properly to exclude air using feet, hands and local compacting materials. For each treatment, two plastic bags were prepared per farmer, each weighing 100 to 120 kg. The plastic bags containing the ensiled feed were kept in a safe place under a shed.

Silage sampling and assessment

After 55 to 60 days of ensiling, the plastic bags were opened one by one for the evaluation of the physical features of the silages (color, aroma, mold appearance, and texture) and fermentation characteristics (DM, pH and temperature). The physical features were inspected and subjectively judged under field conditions by a team who were trained on how to judge silage physical features. The color (1 = dark or deep brown; 2 = light brown; 3 = pale yellow; 4 = yellowish green) and aroma (1 = putrid or rancid; 2 = pleasant; 3 = sweet; 4 = very sweet) were rated according to the method used by Muhammad et al. (2009), while the texture was assessed using a scale of 0 to 4, where 0 = slimy; 1 = very soft; 2 = soft; 3 = fairly firm; 4 = firm (Ososanya and Olorunnisomo 2015). The pH of the samples was determined using a digital pH meter (pH meter Hanna with electrode, range 0–14 pH ± 1 pH cat.no ELC1691) in the field and in a laboratory. The temperature of the silage at each site was measured using a thermometer with an accuracy of 0.1 °C.

The palatability of the different silages was evaluated through the observation of the feeding behaviour of the cows in the field and interviews with the farmers, and evaluating the consumption of silage after each feed delivery.

Laboratory analysis

Immediately upon opening, silage samples were taken from each of the treatments per farmer (two samples), put in plastic bags tied properly, and kept in a refrigerator. The samples, oven dried at a temperature of 65 °C for 72 h, were used for laboratory analysis to determine chemical composition after grinding them to pass a 1-mm sieve. The samples were analyzed for DM, ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). Total ash content was determined by oven drying the samples overnight at 105 °C and by combusting the samples in a muffle furnace at 550 °C for 6 h (AOAC 1990). Nitrogen (N) content was determined following the micro-Kjeldahl digestion, distillation and titration procedures the CP content was estimated by multiplying the nitrogen content by 6.25. The structural plant constituents (NDF, ADF and ADL) were determined according to the procedure of Van Soest and Robertson (1985).

Statistical analysis

Qualitative information regarding the study obtained from farmers was summarized and interpretated qualitatively. This included the scoring results of the physical characteristics of the silage, such as color, aroma, mold appearance and texture. Fermentation characteristics and chemical composition data of the silages were analyzed using the SPSS general linear model (GLM) procedures for analysis of variance. The data were subjected to one-way ANOVA test and the difference among the treatment means separated using Duncan's multiple range test. The statistical model was: $Y_{ij} = \mu + T_i + E_{ij}$, where Y_{ij} = the observation ij, μ = the overall mean, T_i = the effect due to treatment i, and E_{ij} = the experimental error. Probability values less than 0.05 ($p < 0.05$) were considered significant.

Results

Physical features and fermentation qualities of silages

The results of the physical features and fermentation qualities of the silages at both locations are shown in Tables 2 and 3 and Figs. 2 and 3. Accordingly, the color of Desho and Napier grass silages were pale yellow while that made from the grass-legume inclusions were yellowish green. The other physical characteristics of the different silages were similar, except for texture, which is 'fairly firm' at Ada'a and 'firm' at Mecha.. At Ada'a, the mean DM of the silages ranged from 30.27 to 31.94% while at Mecha it varied between 31.88 to 32.56%. The overall analysis showed that there was no significant difference in DM content ($p > 0.05$) among the treatments in each district (Figs. 2 and 3). The pH of the silages varied from 3.98 to 4.44 at Ada'a and from 3.93 to 4.47 at Mecha. The pH of the silages prepared with the inclusion of the legume was higher ($p < 0.001$) than the pH of the silage prepared from the grass alone and increasing the level of each legume resulted in increased pH ($p < 0.001$). The temperatures of the silages, which fell with the temperature range of the study areas, was not affected ($p > 0.05$) by the inclusion of each legume and the levels of inclusion of the legumes. It ranged from 20 to 20.33 °C at Ada'a and 19.89 to 20.11 °C at Mecha.

Chemical composition of the different silages

The chemical composition of the silages is presented in Tables 3 and 4. The CP contents of the silages significantly increased ($p < 0.001$) with increased legume inclusion levels (Desho grass CP = 8.68%; CP of average legume inclusion levels = 13.34%; Napier grass CP = 7.2%; CP of average legume inclusion levels = 13.94%) and the ash content followed a similar pattern except at the 40% inclusion level, where the values declined. The fiber contents of the silages, NDF ($p < 0.001$) and ADF ($p < 0.05$/$p < 0.01$) declined in response to legume inclusion at different levels, while ADL increased with increasing forage legume inclusion levels (Tables 4 and 5).

Table 2 Physical features and pH of the silages prepared from Desho grass alone or with the inclusion of different levels of forage legumes in Ada'a district, Ethiopia (n = 6 for each treatment)

Type of silage	Legume inclusion level	Physical features				
		Color	Aroma	Texture	Mold appearance	pH
Desho grass	0	Pale yellow	Sweet	Fairly firm	No mold	3.98[a]
Desho-alfalfa	20	Yellowish green	Sweet	Fairly firm	No mold	4.12[b]
Desho-alfalfa	30	Yellowish green	Sweet	Fairly firm	No mold	4.26[c]
Desho-alfalfa	40	Yellowish green	Sweet	Fairly firm	No mold	4.44[d]
SEM						0.04
p value						$p < 0.001$
Desho grass	0	Pale yellow	Sweet	Fairly firm	No mold	3.98a
Desho-lablab	20	Yellowish green	Sweet	Fairly firm	No mold	4.09b
Desho-lablab	30	Yellowish green	Sweet	Fairly firm	No mold	4.22c
Desho-lablab	40	Yellowish	Sweet	Fairly firm	No mold	4.38d
SEM						0.04
p value						$p < 0.001$

Means with different letters in each legume along a column are significantly different ($p < 00.0.05$); SEM = standard error of the mean

Table 3 Physical features and pH of the silages prepared from Napier grass alone or with the inclusion of different levels of forage legumes in Mecha district, Ethiopia (n = 9. for each treatment)

	Legume inclusion level	Physical features				
		Color	Aroma	Texture	Mold appearance	pH
Napier	0	Pale yellow	Pleasant	Firm	No mold	3.93a
Napier-alfalfa	20	Yellowish green	Sweet	Firm	No mold	4.10b
Napier-alfalfa	30	Yellowish green	Sweet	Firm	No mold	4.20c
Napier-alfalfa	40	Yellowish green	Sweet	Firm	No mold	4.38d
SEM						0.03
p value						$p<0.001$
Napier	0	Pale yellow	Pleasant	Firm	No mold	3.93a
Napier-sesbania	20	Yellowish green	Sweet	Firm	No mold	4.13b
Napier-sesbania	30	Yellowish green	Sweet	Firm	No mold	4.30c
Napier-sesbania	40	Yellowish green	Sweet	Firm	No mold	4.47d
SEM						0.04
p value						$p<0.001$
Napier	0	Pale yellow	Pleasant	Firm	No mold	3.93a
Napier-Faba bean	20	Yellowish green	Sweet	Firm	No mold	4.06b
Napier-Faba bean	30	Yellowish green	Sweet	Firm	No mold	4.18c
Napier-faba bean	40	Yellowish green	Sweet	Firm	No mold	4.31d
SEM						0.03
p value						$p<0.001$

Means with different letters in each legume along a column are significantly different ($p<0.0.05$)

Discussion

Physical features and fermentation quality

The difference in color between the grass-alone silages and the grass-legume mixture silages observed in our study could be attributed to the inclusion of legumes in the latter. The difference in texture of the silages at Mecha and Ada'a is attributed to the difference in chopping technique, i.e., hand chopping in Ada'a vs. machine chopping in Mecha, where the machine chopped the herbage to finer texture, which helps proper packing and compacting. The physical characteristics, temperatures and dry matter values of the silages reported in the current studies are within the acceptable range for a good quality silage (Khota et al. 2018; Dongxia et al. 2019). pH is a major indicator of good silage, when assessing silage quality; generally, the lower the pH, which is the case in our studies (grass silages = 3.93 to 3.98; grass-legume silages = 4.09 to 4.47), the greater the preservation and the more stable the silage (Jusoh et al. 2016). Matlabe et al. (2022) argued that pH values lower than 4.8 could be considered appropriate for good quality silage production. Different research findings ascribed increments of pH values in response to legumes inclusion. Their inclusion at different levels is related to the high buffering capacity of the legumes, primarily due to cation existence (K^+, Ca^{2+} and Mg^{2+}) (Wang et al. 2017) though this was not investigated in our studies. The cations encounter organic acids, formed by the fermentation, neutralizing them and preventing the occurrence of pH drop (da Silva Brito et al. 2020).

Chemical composition of the silages

The increase in ash content in response to legume inclusion could be an indication of high mineral concentration (Kebede et al. 2016). The ash content of the grass silages (Desho = 11.63%; Napier = 12.67%) is comparable with the findings of Zewdu (2005), Nurhayu et al. (2021) and Tesfaye et al. (2019). Concentration of ash in forage varies due to factors such

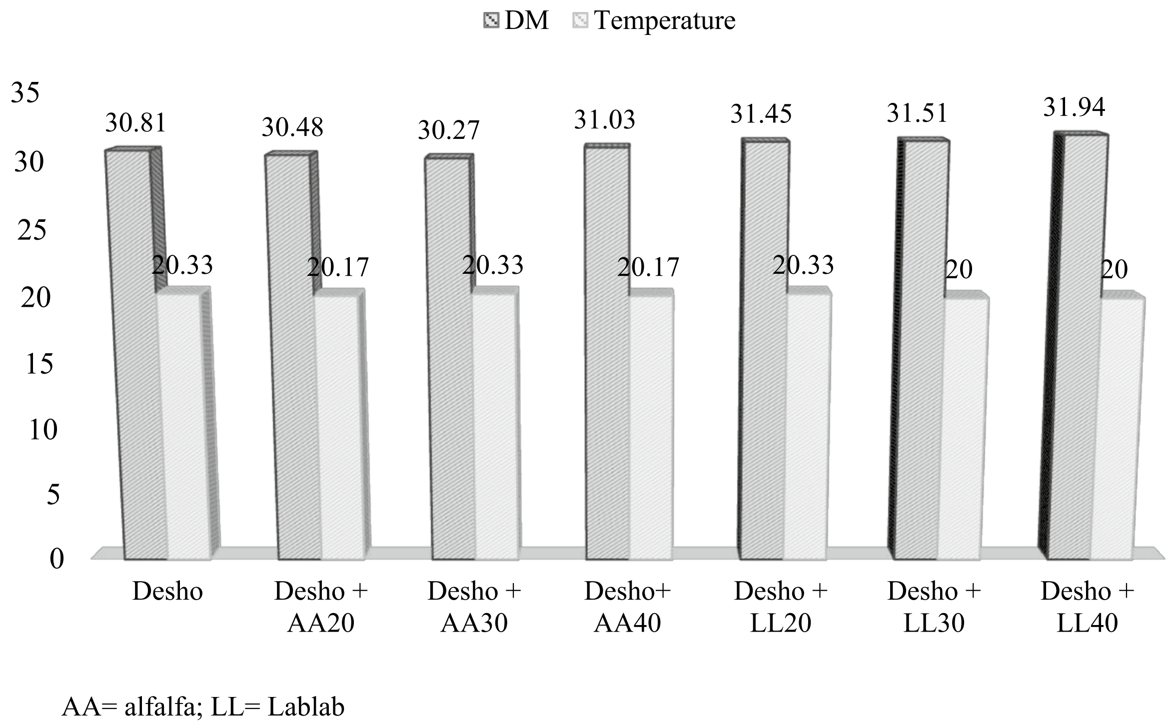

Fig. 2 Dry matter (%) and temperatures (°C) of silages at Ada'a; n = 6 for each treatment

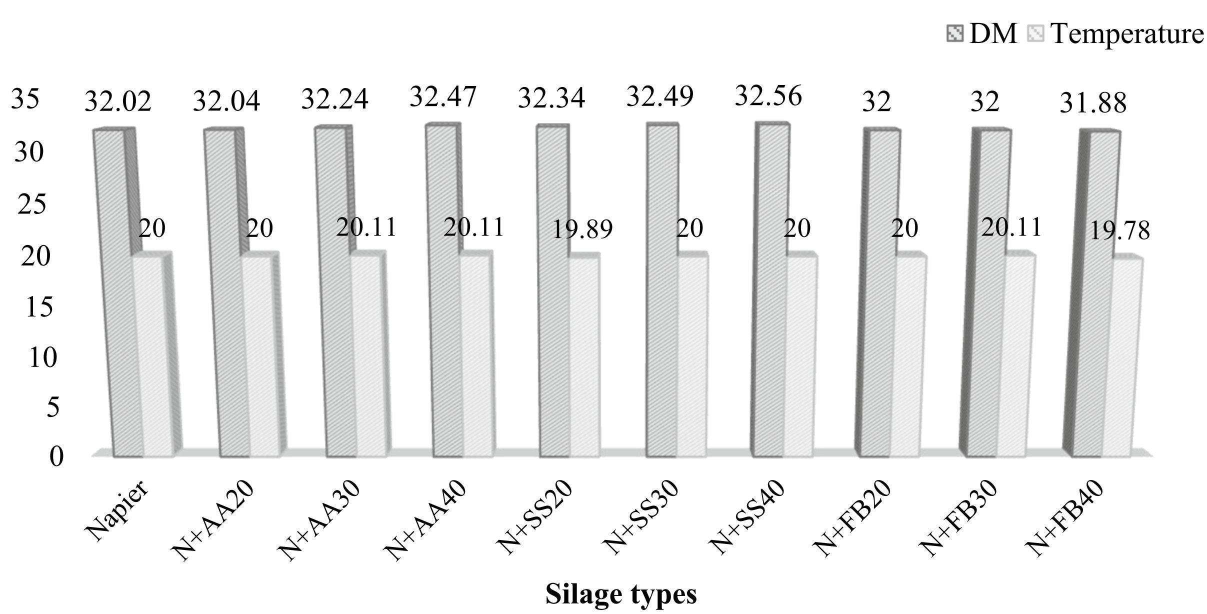

Fig. 3 Dry matter (%) and temperatures (°C) of the silages at Mecha district; n = 9 for each treatment; N = Napier; SS = *Sesbania sesban*; FB = Faba bean; AA = Alfalfa)

Table 4 Chemical composition (% DM) of Desho grass with the inclusion of different levels of forage legumes in Ada'a district of east Shoa zone, Ethiopia (n = 6 for each treatment)

Type of silages	Legume inclusion levels (%)	Chemical composition				
		Ash	Crude protein	Neutral detergent fiber	Acid detergent fiber	Acid detergent lignin
Desho grass	0	11.63a	8.68a	61.17a	35.5a	7.10a
Desho-alfalfa	20	13.67b	12.50 b	57.50b	34.8ab	9.80b
Desho-alfalfa	30	15.33c	13.60 c	54.50c	34.01b	10.90c
Desho-alfalfa	40	12.18a	14.90 d	50.01d	33.80b	11.30d
p value		< 0.001	< 0.001	< 0.001	< 0.01	< 0.001
SEM		0.33	0.49	0.74	0.22	0.35
Desho grass	0	11.63a	8.86a	61.17a	35.5a	7.10a
Desho-lablab	20	13.87b	11.41b	58.50b	34.90ab	9.95b
Desho-lablab	30	15.67c	13.43c	55.0c	34.32ab	11.30c
Desho-lablab	40	12.37a	14.20d	52.76d	33.78b	11.60c
p values		< 0.001	< 0.001	< 0.001	< 0.05	< 0.001
SEM		0.34	0.47	0.81	0.23	0.38

Means with different letters in each legume along a column are significantly different ($p < 0.0.05$)

Table 5 Chemical composition (% DM) of Napier grass silages with or without the inclusion of different levels of forage legumes in Mecha district of West Gojam Zone, Ethiopia (n = 9 for each treatment)

Type of silages	Legume inclusion levels (%)	Chemical composition				
		Ash	Crude protein	Neutral detergent fiber	Acid detergent fiber	Acid detergent lignin
Napier grass	0	12.67a	7.2a	62.07a	30.6a	7.01a
Napier-alfalfa	20	14.78b	12.40b	58.06b	30.27a	10.01b
Napier-alfalfa	30	15.33c	14.24c	55.06c	29.90ab	11.16c
Napier-alfalfa	40	12.82a	14.72d	50.06d	29.40b	11.50c
SEM		0.20	0.51	0.80	0.13	0.31
p values		< 0.001	< 0.001	< 0.001	< 0.01	< 0.001
Napier grass	0	12.67a	7.20a	62.07a	30.6a	7.01a
Napier-sesbania	20	14.53b	13.82b	58.80b	30.10ab	10.40b
Napier-sesbania	30	16.43c	14.90c	54.72c	29.7b	11.40c
Napier-sesbania	40	12.97a	15.84d	48.43d	29.40b	11.66c
SEM		0.26	0.58	0.87	0.13	0.32
p values		< 0.001	< 0.001	< 0.001	< 0.01	< 0.001
Napier grass	0	12.67a	7.20a	62.07a	30.6a	7.01a
Napier-faba bean	20	13.80b	12.10b	59.30b	30.29a	9.80b
Napier-Faba bean	30	15.30c	13.40c	56.20c	29.90ab	11.00c
Napier-faba bean	40	13.01a	14.01d	52.50d	29.50b	11.30c
SEM		0.18	0.46	0.64	0.14	0.38
p values		< 0.001	< 0.001	< 0.001	< 0.05	< 0.001

Means with different letters in each legume along a column are significantly different ($p < 0.0.05$)

as plant developmental stage, morphological fractions, climatic conditions, soil characteristics and fertilization regime (Zewdu 2007). The decrease in the ash content with 40% inclusion of the legumes might be related to the fermentation process which will require further investigation. However, pattern similar to our findings is reported by Xue et al. (2020) and Nurhayu et al. (2021).

Crude protein content of about 60 to 70 g/kg DM (Smith 1993) is required for maintenance of ruminants and low-quality forages are defined as those with less than 80 g/kg DM CP (Leng 1990). Low quality forages adversely affect rumen microbial activity (Van Soest 1982). Accordingly, the silage prepared from these grasses can serve to cover maintenance requirements of ruminants and will require addition of legumes in the silage for increased milk production. According to EADD (2013), a ration comprising 13 and 15% CP is required for 10 and 15 L of milk yield per day, respectively. Our results revealed that most silages will meet the CP requirement for 10 L of milk while alfalfa and sesbania silages will meet the CP requirement for 15 L of milk production per day, reflecting higher CP in these forage legumes.

Albayrak and Turk (2013) explained that forage legumes are known to have lower fiber content than grasses, while grasses also generally contain more NDF and ADF. As can be expected naturally from grass-legume mixtures, our studies indeed indicated that the increasing forage legumes levels resulted in decreasing NDF and ADF contents. The reduction of the fiber contents may be explained by the hydrolysis of NDF-bound N (Jaakkola et al. 2006) during fermentation. Lower NDF and ADF values imply good-quality silage (Senjaya et al. 2010). The results of these studies complement the works of Xue et al. (2020) and Nurhayu et al. (2021). Generally, grasses tend to increase the fiber fractions, indicated by NDF and ADF, of mixtures owing to the abundant cell wall materials (Buxton and Redfearn 1997). Xue et al. (2020) showed that increasing alfalfa proportion in grass-legume mixture silages decreased NDF, ADF, hemicellulose, and cellulose concentrations, suggesting that forage mixtures improve nutritive values and reduce the need for purchased protein supplements in ruminant rations (Adesogan et al. 2004; Xue et al. 2020). The increase in lignin content of legumes inclusion at different levels is also reported by Xue et al. (2020) and Nurhayu et al. (2021). Lignin is considered as an anti-nutritional component in forage, as it has a negative impact on the nutrient availability of plant fiber (Moore and Jung 2001).

Conclusions

As it was hypothesized, the inclusion of different forage legumes at different levels in a grass-based silage is a good option for making better quality silage, as can be seen from the physical features, and fermentation qualities such as pH, and chemical composition of the experimental silages (increased CP and decreased fiber fractions). Thus, these results are helpful in indicating how better-quality silages can be prepared, which will be more accessible to farmers than the commercial protein supplements that are highly priced and less accessible for farmers in rural areas of developing countries and others.

These results show that the preparation of silages from grass-legume mixtures has a dual advantage: it improves the deficiency of protein in grass silage and it improves the low level of carbohydrates in legumes. Thus, the studies revealed that the use of tree and herbaceous legumes can improve the nutritive quality of grass silages. Depending on the type of livestock to be considered, 20 to 40% levels of inclusion of legumes in grass silages can be considered though the 30 to 40% forage legume inclusion levels appear to offer good feeding for milk production from crossbred cows. It is recommended that feeding experiments with milking cows be undertaken using the suggested levels of inclusion to document evidence in milk response.

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Author contributions AE, AAA, JVdL designing experimental plan; AE data analysis and draft writeup; AAA, JVdL manipulating and reading the manuscript; AE, FN, HA and HD implementation of the experiment and data collection; LB reading the manuscript.

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Data availability All data generated or analyzed during the studies are included in this draft paper; supplementary information can be accessed on a reasonable request from the corresponding author.

Declarations

Conflict of interest The authors declare no competing interests of any kind.

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