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An assessment of ensiling potential in maize x legume (soybean and cowpea) binary mixtures for yield and feeding quality

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Abstract: In this study, maize and legume intercropping were evaluated in terms of silage yield and quality. Maize (*Zea mays* L. "M") was intercropped with soybean (*Glycine max* L. "S") and cowpea (*Vigna unguiculata* L. "C") as binary mixtues (maize + legume), and the seed rates were as follows: 100 + 0%, 75 + 25%, 50 + 50% and 25 + 75%. The harvested plants were chopped with the particle size of < 2, ensiled in 2 kg plastic jars and left fermentation at 25 ± 2 °C for 45 days. In this study, silage yield, dry matter ratio, pH value, crude protein ratio, acid detergent fiber, neutral detergent fiber, digestibility of dry matter, dry matter intake, total digestibil nutrient relative feed values, lactic acid, acetic acid, butyric acid, malic acid, citric acid, succinic acid, oxalic acid, potassium, phosphorus, calcium, and magnesium contents were determined. All the M + S mixtures showed high performance in terms of Flieg score and lactic acid content. The highest relative feed quality value was determined in the sole cowpea (156.4) and 25M + 75S% (148.5) mixture. As a result, intercropping maize with legumes resulted in superior silage quality without a reduction in yield.

Key words: Silage, mixture, yield, quality

1. Introduction

The availability of sufficient quality and quantity of roughages in animal production reduces the use of expensive concentrate feeds, which provides a great economic profit for farmers. Feeding costs constitute approximately 70% of the inputs in animal production, and 78% of this cost is roughages and 22% are concentrate feeds [1].

Maize containing high dry matter with considerable energy [2], is the most popular crop for silage making all over the world [3,4] In addition, maize silage meets almost all the nutritional requirements of animals, and reduces the need for concentrate feeds by up to 50% [5]. However, low protein content is the most important disadvantage in maize silage. Previous researches show that crude protein of silage maize ranges from 7.0 to 8.0% [6–8]. The protein content of the maize silage can be increased by adding a protein-rich legume such as soybean, cowpea. Titterton [9] reported that legume incorporation increased the crude protein (CP) content from 7.7% to 15.3% in maize silage.

To obtain high-quality silage, fermentation processes are extremely important and should be provided very well. The oxygen concentration in silo adversely affects fermentation and increases decomposition in silage by encouraging fungal activity. Organic acids formed by microorganisms such as beneficial bacteria (acetic acid, propionic acid, formic acid, benzoic acid, sorbic acid, citric acid, etc.) have the highest growth inhibition efficiency against fungi and yeasts in the silo. Besides, organic acid prevents the silage from spoiling.

Kowalczyk et al. [10] reported that the use of in-feed antibiotic growth promoters was banned in 2006, so organic acids can be used as an effective alternative to antibiotics. They also indicated that organic acids are the most reliable growth promoters among nonantibiotic growth promoters. For this reason, organic acids (fumarate, citric, succunic, and malate) are becoming increasingly popular as feed additives for animals. Plants can synthesize these organic acids by themselves; its amount can be low sometimes and should be added to the silo. However, the relatively high cost of organic acids limits their application opportunity in silage making and brings additional costs to the farmer's economy.

This study aims to improve the yield, nutritional value, and organic acid content in maize silage by intercropping maize with the soybean and cowpea at different seed rates.

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2. Material and methods

2.1. Plant material

Plant materials consisted of Arifiye variety of maize, Yeşilsoy variety of soybean, and Ülkem variety of cowpea were sown as binary mixtures with three seed rates (75 + 25%, 50 + 50%, and 25 + 75%). These plants were also planted separately as control.

2.2. Experimental site and design

This study was conducted during the summer season of 2018 (April 24th) on the Research Field of the Faculty of Agriculture and Natural Sciences, Bilecik Şeyh Edebali University in Bilecik, Turkey. The soil of the experimental area was analyzed by the Republic of Turkey Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute. It was clay-loam with low organic matter (1.32%) and high pH value (7.72). It also contained high phosphorus (24.94 kg/da) and potassium (161.7 kg/da). The average temperature was 20.68 °C, and the total precipitation was 170.9 mm in 2018 growing season. Long-term mean temperature and annual precipitation during the vegetation period (April–August) were 18.88 °C and 152.9 mm, respectively (Table 1).

The seed rate was calculated with regard to sowing rate of each plant alone: 12.000 plant/da for maize and 10 kg/da for soybean and cowpea. Row distance was arranged in 70 cm in sole cropping. Mixtures were sown in rows with a distance of 35 cm. The experiment was set in three replicates in a randomized complete block design (RCBD). After planting, 5 kg/da N and 8 kg/da P_2O_5 as fertilizer were applied. Then, 5 kg/da N was applied as plants reach up to 40–50 cm. All the plots were irrigated five times during the vegetation period. Sole maize and intercrops were harvested depending on maize at the milk dough stage, the sole legumes when seed shape exactly formed in the bottom pods.

2.3. Silage yield, silage preparation, ensiling, and silo opening

The Green forage yield was calculated as kg/da from fresh weight and determined by harvesting and weighing the plants that were in 2.8 m^2 area located center of the plots. The silage yield was calculated by reducing the silage losses by 25% over the green forage yields. The harvested plants were chopped in 2 cm size, and they were filled into plastic jars according to the mixture ratios. Silages were stored at 25 ± 2 °C and opened after 45 days of ensiling.

2.4. Flieg score

Flieg score was calculated by using pH value and dry matter ratio as fallows. Flieg Score = 220 + (2 × Dry Matter%–15) – 40 × pH) [11]. The Flieg score ranged between 81 and 100 was considered to be very good, between 61 and 80 was considered to be good, between 41 and 60 was considered to be medium, between 21 and 40 was considered to be poor, and between 0 and 20 was considered to be poorer silage quality and excluded from the experiment.

2.5. Organic acid analyses

The 20 g silage sample was taken from each jar and mixed with 100 mL of distilled water for 5 min by an electric blender and then filtered. The pH value of silage samples was determined by using a digital pH meter. Organic acid analysis (lactic acid, acetic acid, and butyric acid) of silages were performed on HPLC (Shimadzu, Kyoto, Japan) auto sampler system model LC - 20AT equipped with four pumps and an SPDM20A diode array detector (DAD).

Malic, citric, succinic and oxalic acids were as described considering Uden's [12] method. Organic acids were determined by adding 100 or 200 mL water to each 100 g sample and refreezing for 24 h in plastic silage bags. A hydraulic press was then used to extract the liquid after defrosting, followed by centrifugation of the extract at 2000 × g for 5 min. Then, samples were analyzed in HPLC

Table 1. Meteorological data of experiment area in the longterm and studied year.*

Months	Temperature (°C)		Precipitation (mm)		Moisture (%)	
	Long-term	2018	Long-term	2018	Long-term	2018
April	11.3	16.0	42.3	18.6	64.3	56.8
May	16.1	18.2	51.2	80.8	64.4	72.5
June	20.1	21.2	34.2	39.5	62.0	67.3
July	23.5	23.8	13.7	14.2	59.6	62.5
August	23.4	24.2	11.5	17.8	60.6	62.5
Average	18.88	20.68			62.18	64.32
Total			152.9	170.9		

* Tukish State Meterogical Service

(Shimadzu, Kyoto, Japan) auto sampler system model LC - 20AT equipped with four pumps and an SPDM20A diode array detector (DAD).

2.6. Mineral content analyses

The determination of potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg) in silages were performed by inductively coupled plasma mass spectrometry (ICP-MS) using a Thermo Scientific - iCAPQc (Bremen, Germany) [13].

2.7. Dry matter, crude protein, acid detergent fiber, and neutral detergent fiber ratio analyses

The fresh weights of the samples taken from each jar were determined, and they were dried in a hot-air oven at 105 °C for 48 h; consequently, dry matter ratio (DM) (%) was calculated. Silage samples were dried at 65 °C until they reach up to constant weight. Then, samples were grounded in a grain mill with 0.5 to 1 mL sieve. Nitrogen (N) contents of samples were determined using the Kjeldahl apparatus (FOSS 984.13) and then, crude protein content (CP) was calculated by multiplying the N concentration by a factor of 6.25. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) analyzes were determined as specified by Van Soest [14] and Van Soest and Wine [15]. Relative feed value (RFV) was estimated according to the following equations adapted from Rohweder et al. [16].

Digestibility of dry matter% (DDM) = 88.9 – (0.779 × ADF)

Dry matter intake% (DMI) = 120 / NDF

Total digestibil nutrient% (TDN) = (96.35 – (ADF*1.15))

Relative feed value (RFV) = (DDM * DMI) /1.29

2.8. Statistical analyses

All data were statistically analyzed according to the randomized plot design in SPSS version 18.0 (SPSSInc., Chicago, IL, USA) and means were separated by Duncan's multiple range test [17].

3. Results

Dry matter ratio, pH value, and Flieg score were significantly different ($p < 0.01$) between treatments as seen in Table 2. The dry matter ranged between 24.89% (sole cowpea) and 34.45% (sole maize). The highest pH value was determined as 5.32 (25M + 75C%), while the lowest was determined as 4.35 (sole maize), 4.44 (sole cowpea) and 4.38 (75M + 25S%). Flieg scores of silages ranged between 43.13 (25M + 75C%) and 99.88 (sole maize), and all the silages studied varied between medium and very good quality class (Table 2).

Silage yield, crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) raitos were given in Table 3. Silage yield and CP were significantly different ($p < 0.01$) between treatments, while ADF and NDF were not significant. Silage yield was found to be high in 100M% (6246.1 kg/da), 75M + 25S% (6109.9 kg/da), 50M + 50S% (5164.5 kg/da), 25M + 75S% (4950.9 kg/da) and 75M + 25C% (5532.0 kg/da), while the lowest silage yield was found in sole cowpea (3258.7 kg/da). The highest crude protein ratio was determined as 18.85% in sole cowpea while the lowest was 11.42% in sole maize. ADF and NDF ratios ranged between 27.39 (sole cowpea) – 33.18% (sole maize) and 40.19 (sole cowpea) – 49.82% (sole maize), respectively.

Digestibility of dry matter (DDM), dry matter intake (DMI), total digestibil nutrient (TDN), and relative feed values (RFV) were given in Table 4. Dry matter intake ($p < 0.01$) and RFV ($p < 0.05$) values were significantly different between treatments, DDM and TDN values were not significant. DDM values of silages ranged from 63.05% (sole maize) to 67.56% (sole cowpea). The highest DMI was determined as 2.99% in sole cowpea, while the lowest was as 2.41% in sole maize. Total digestibil nutrient values ranged between 58.2 and 64.9. The highest RFV

Table 2. Dry matter ratio, pH value, Flieg score and quality classs of maize - legume silages.

Treatments	Dry matter ratio (%)**	pH value**	Flieg score**	Quality class
100M%	34.45 a	4.35 d	99.88 a	Very good
100S%	33.55 a	4.59 c	88.38 b	Very good
100C%	24.89 c	4.44 d	77.31 d	Good
75M + 25S%	33.55 a	4.38 d	97.05 a	Very good
50M + 50S%	33.33 a	4.60 c	87.53 bc	Very good
25M + 75S%	32.89 a	4.68 c	87.71 bcd	Very good
75M + 25C%	34.44 a	4.84 b	80.15 cd	Good
50M + 50C%	28.45 b	4.85 b	67.75 d	Good
25M + 75C%	25.55 c	5.32 a	43.13 e	Medium

**: $p < 0.01$; M: Maize; S: Soybean; C: Cowpea.

Table 3. Silage yield, crude protein, acid detergent fiber and neutral detergent fiber ratio of maize - legume silages.

Treatments	Silage yield (kg/da)**	Crude protein ratio (%)**	Acid detergent fiber (%)	Neutral detergent fiber (%)
100M%	6246.1 a	11.42 f	33.18	49.82
100S%	3873.3 cd	16.76 b	30.02	45.67
100C%	3258.7 d	18.85 a	27.39	40.19
75M + 25S%	6109.9 a	11.91 ef	30.41	45.68
50M + 50S%	5164.5 abc	12.49 ef	30.02	44.46
25M + 75S%	4950.9 abc	14.85 c	28.31	41.88
75M + 25C%	5532.0 ab	13.04 de	31.63	47.69
50M + 50C%	4249.2 bcd	14.04 cd	29.97	44.41
25M + 75C%	3667.7 cd	15.54 bc	29.05	44.02

**: $p < 0.01$; M: Maize, S: Soybean; C: Cowpea; CP: Crude protein ratio.

Table 4. Digestibility of dry matter, dry matter intake, total digestibil nutrient and relative feed value values of maize - legume silages.

Treatments	Digestibility of dry matter (%)	Dry matter intake (%)**	Total digestibil nutrient (%)	Relative feed value*
100M%	63.05	2.41 f	58.2	117.7 c
100S%	65.51	2.63 d	61.8	133.4 b
100C%	67.56	2.99 a	64.9	156.4 a
75M + 25S%	65.21	2.63 d	61.4	132.8 b
50M + 50S%	65.51	2.70 cd	61.8	137.1 b
25M + 75S%	66.85	2.87 b	63.8	148.5 a
75M + 25C%	64.26	2.52 e	60.0	125.3 c
50M + 50C%	65.55	2.70 cd	61.9	137.3 b
25M + 75C%	66.27	2.73 c	62.9	140.0 b

*: $p < 0.05$; **: $p < 0.01$; M: Maize; S: Soybean; C: Cowpea.

value was determined in sole cowpea with 156.4, and 25M + 75S% (148.5) mixture. Besides, the lowest RFV value was determined as 117.7 in sole maize and 125.3 in 25M + 75C%

Organic acids content in maize - legume mixtures were given in Table 5. There were statistically significant differences ($p < 0.01$) between treatments regarding organic acid content, except butyric, and malic acids. The highest lactic acid content was determined in the treatments of 25M + 75S% (56.24 g/kg), sole soybean (52.26 g/kg), 50M + 50S% (51.39 g/kg), sole maize (37.47 g/kg) and 75M + 25S% (32.90 g/kg). Acetic acid ranged between 0.16 and 0.39 g/kg among treatments. The malic acid was listed from high to low value according to the sole silages: maize > cowpea > soybean. Besides, the malic acid of maize - soybean mixture silages was higher than maize - cowpea silages. The highest citric acid was determined in 75M + 25S% (6.151 g/kg), 75M + 25C% (4.666 g/kg) and 50M + 50C% (5.215 g/kg), while the lowest was in sole maize (2.375 g/kg). The content of succinic acid ranged between 0.274 (sole soybean) and 0.615 (75M + 25S%) g/kg. The highest oxalic acid was determined in sole maize with 0.170 g/kg, while it was lowest as 0.034 (75M + 25C%) g/kg (Table 5).

Mineral contents of maize - legume mixture silages were given in Table 6. There were statistically significant differences ($p < 0.01$) for all mineral elements among the treatments. Potassium (K) content of silages ranged between 13.09 (sole maize) and 25.81 (75M + 25C%) g/kg. The highest content of phosphorus (P) was determined as 4.16 g/kg in 25M + 75C% mixture. Phosphorus content was found to be low in sole maize, 75M + 25S%, and 50M +

Table 5. Organic acids of maize - legume silages (g/kg).

Treatments	LA**	AA**	BA	MA	CA**	SA**	OA**
100M%	37.47 ab	0.25 bcd	0.42	0.479	2.375 e	0.346 cd	0.170 a
100S%	52.26 a	0.19 cd	0.58	0.251	2.742 de	0.274 d	0.126 b
100C%	24.43 bc	0.16 d	0.49	0.314	4.047 bcd	0.405 bcd	0.096 bc
75M + 25S%	32.90 abc	0.27 bc	0.08	0.684	6.151 a	0.615 a	0.080 cde
50M + 50S%	51.39 a	0.19 cd	0.13	0.276	3.983 b-e	0.398 bcd	0. 041 ef
25M + 75S%	56.24 a	0.21 cd	0.18	0.279	3.639 cde	0.364 bcd	0. 084 cd
75M + 25C%	22.86 bc	0.39 a	0.12	0.169	4.666 abc	0.467 abc	0. 034 f
50M + 50C%	14.90 bc	0.35 ab	0.22	0.238	5.215 abc	0.522 abc	0. 049 def
25M + 75C%	11.44 c	0.18 cd	0.06	0.212	3.622 cde	0.476 abc	0.050 def

**: $p < 0.01$; M: Maize; S: Soybean; C: Cowpea; LA: Lactic acid; AA: Acetic acid; BA: Butyric acid; MA: Malic acid; CA: Citric acid; SA: Succinic acid; OA: Oxalic acid.

Table 6. Mineral contents of maize - legume silages (g/kg).

Treatments	Potassium**	Phosphorus**	Calcium**	Magnesium**
100M%	13.09 e	2.49 c	2.82 e	1.71 d
100S%	22.81 ab	3.03 b	11.87 ab	4.83 a
100C%	21.63 bc	3.25 b	13.02 a	4.39 a
75M + 25S%	17.71 d	2.60 c	7.19 d	3.05 c
50M + 50S%	18.37 cd	2.61 c	7.48 d	3.22 bc
25M + 75S%	21.44 bc	3.04 b	10.72 bc	4.35 a
75M + 25C%	25.81 a	3.08 b	7.27 d	3.09 c
50M + 50C%	23.28 ab	3.38 b	9.63 c	3.75 b
25M + 75C%	19.22 cd	4.16 a	12.31 a	4.31 a

**: $p < 0.01$; M: Maize; S: Soybean; C: Cowpea.

50S% (2.49, 2.60, and 2.61 g/kg, respectively). The calcium and magnesium content of silages were ranged from 2.82 to 13.02 g/kg and 1.71 to 4.83 g/kg, respectively (Table 6).

4. Discussion

The silage quality is highly complex and can be affected by many parameters. Panyasak and Tumwasorn [18] indicated that good quality silage should contain 25% – 40% dry matter. If the silage contains more than 40% dry matter, palatability decreases with the high cellulose and hemicellulose content. In addition, if the silage contains low dry matter content (< 25%), most of the carbohydrate may be leached. Dry matter content of silages ranged between 24.89% (sole cowpea)–34.45% (sole maize).

Acidity is an important parameter in the evaluation process of silage quality. Acidity in silage is a feature that directly affects the fermentation process, and the increase in acidity prevents the leaching of the nutriens such as protein. Researchers [19,20] suggest that pH values of quality silage should be between 3.7 and 4.8. In the current study, the pH value of the mixture silages was similar to the suggested values except in maize x cowpea mixtures. Besides, sole legume silages exhibited higher pH values than sole maize. This may be due to higher buffering capacity, higher crude protein, and lower carbohydrate contents of legumes. The Flieg score is calculated using dry matter content (DM) and pH value, and gives information on the quality of silage. Flieg scores of silage determined in this study were found to be a medium, good, and very good quality class of silage. In previous studies, Flieg score of legume + cereal mixture silages ranged from 61.80 to 95.06 [13,17].

Despite the importance of quality, yield still maintains its importance in silage crops due to the high level of roughage requirement. In this sense, efforts to increase the yield in silage plants continue intensively all over the world. However, the relationship between yield and quality in silage plants should never be ignored. Because, animal productivity and health are the result of the combination and interaction of both parameters. Our result showed that intercropping produced more desired results for almost all the investigated quality parameters. Maize x legume intercropping was more yielding than sole legumes. In addition, it can be said that the maize is a determinant factor in yield for the mixtures. The performances of the legumes in the mixtures were also different, silage yield and qualiy in maize x soybean intercropping was generally higher than maize x cowpea intercropping, with the significant effect of seed rates. Silage yield was significantly ($p < 0.01$) different amongst the treatments and it varied from 3258.7 (sole C) to 6246.1 (sole M) kg/da. For maize x legume intercropping, similar differences in yield were previously reported by Alaca and Özaslan Parlak [21], which ranges between 462 and 9700 kg/da.

Researchers reported that the use of legumes in silage increases the quality of the ensiled mass and the protein content [22,23]. Therefore, in the current study, protein content was higher in sole legumes and mixtures than sole maize. Başaran et al. [13] reported that the protein content of grasspea + cereal mixture silages ranged between 12.18% and 22.68%.

Acid detergent fiber (ADF) and neutral detergent fiber (NDF) are important for rumen degradation and influence animal performance. Higher ADF in forage is related with the low the energy value, while the more NDF is with the low animal intake [24,25]. Kaplan et al. [26] indicated that low ADF and NDF contents of forage crops are usually desired since these materials complicate digestion and consequently decrease the quality. Therefore, in the quality forages, ADF should range from 20 to 30%, while the NDF ranges from 30 to 40%.[1] In the present study, it is determined that ADF and NDF ratios of silages between desired limits except for NDF of sole maize. Sole maize silages ADF and NDF content had higher than sole soybean and cowpea. This may be due to the low fiber content in the legumes. Besides, the increasing rate of legumes in the mixtures caused lower ADF and NDF content in silage.

Sole soybean and cowpea silages exhibited higher DDM, DMI, TDN, and RFV vaules than sole maize (Table 4). This may be due to the higher ADF and NDF contents of maize. Besides, DDM, DMI, TDN, and RFV values were decreased with increasing ratio of maize in the mixtures. The relative feed value (RFV) is the widely used index of feed quality worldwide and based on estimates of feed intake from NDF content and digestibility from ADF content. Acordingly, the RFV value for beginning quality standard was > 151, for the first quality standard was 151–125, for the second quality standard was 124–103, for the third quality standard was 102–87, for the fourth quality standard was 86–75 and for the fifth quality standard was < 75 represented the forage quality [16]. The RFV values determined in the study showed that examined silages between the second and beginning quality classes. Can et al. [17] reported that RFV values of *Bituminaria bituminosa* + oat mixture silages ranged between 86.60 and 159.89.

The formation of quality silage depends on lactic acid content, and it should be more than 20.0 g/kg [27]. Accordingly, the lactic acid contents in the silage samples of the present study were relatively high with reference to the critical value, except for 50M+50C% and 25M + 75C%. In addition, it was seen that addition of soybean to corn silage increases lactic acid content compared to cowpea. König et al. [28] reported that lactic acid of red clover - grass silage ranged between 23–133 g/kg.

Acetic acid indicates the spoiling in silage; therefore, the amount of acetic acid in the silage should not exceed 8 g/kg [29]. In the present study, the acetic acid content in studied samples was lower than the critical value (8 g/kg). Başaran et al. [13] found that acetic acid content ranged between 0.001%–0.187% in grass pea - cereal silages.

Butyric acid is the substance with the greatest inhibitory effect on lactic acid bacteria and yeast growth. Thus, it is undesirable in the silage [30,31]. However, its amount between 1.0 and 6 g/kg would not affect the silage quality. In the current study, the butyric acid content of all silages was lower than this critical value. Seppälä et al. [32] found that the butyric acid of faba bean and field pea silages ranged from 0.53 to 0.60 g/kg.

Malic acid can improve the ruminal environment and increase propionate production. Some researchers have indicated that malic acid could increase rumen's pH value, improve microbial N, and increase feed digestibility [33,34]. Besides, malic acid improved the milk yield of cows [35]. Stallcup [36] reported that cows given 70 g/day of malic acid had higher milk yield. Sniffen et al. [37] evaluated the effect of malic acid supplementation on lactation performance of mid-lactation dairy cows and determined higher milk yield in cows given supplemental malic acid. Uden [12] reported malic acid content ranged between 0.4 and 0.6 g/kg in maize. In the present study, malic acid ranged between 0.169–0.684 g/kg.

Kung et al. [38] reported that the citric acid contains active ingredients with antimycotic activity for livestocks. Citric acid has a function in stimulating rumen fermentation and improving animal performance [39]. Uden [12] indicated that citric acid is used to keep the pH value between 4–6 during fermentation of silage, while Ke et al. [40] reported that application of citric acid in silage decreased the pH value, limited proteolysis and improved fermentation quality. Playne and Mcdonald [41] found

[1] Understanding Your Forage Test (2020). Elden Cole [online]. Website http://extension.missouri.edu/webster/documents/resources/agriculture/UnderstandingYourForageTest.pdf/. [accessed 20 May 2020].

that citric acid of Italian ryegrass silage between 1–25 g/100 g DM. In the current study, the citric acid of silages ranged between 2.375 and 6.151 g/kg. Besides, this study showed that the amount of citric acid increased adding legumes to maize silage.

McDonald et al. [42] indicated that succinic acid is a well-known agent for silage fermentation, and it is produced by several bacterial species. Succinic acid is effective for the various diseases of the livestock and it contributes to the development of body growth of livestock. Zeikus et al. [43] reported that succinic acid increased the concentration of propionate in the rumen and acted as an energy source for animals. Uden [12] found that succinic acid of silage maize and legumes ranged between 0.1 and 0.9 g/kg. Succinic acid values obtained from the present study (0.274–0.615 g/kg DM) are consistent with Uden [12].

Nakata [44] indicated that oxalic acid accumulates in many plants as calcium oxalates, and it plays a role in calcium regulation with detoxification. Oxcalic acid is an antinutrient, and its overconsumption can cause depression, weakness, difficulty in breathing, and death in animals [45]. However, oxalic acid can be metabolized in the rumen, and 40 g/day can be tolerated by the sheep [46]. On the other hand, Rolinec et al. [47] indicated that oxalic acid content greater than 100 g/kg DM could be considered potentially dangerous, while Panda and Sahu [48] observed that the total oxalic acid intake at the level of 5.8 g/kg DM intake was harmless to bulls, but an increase of the total oxalic acid to 11.9 g/kg created a negative balance of calcium. In this study, the amount of oxalic acid ranged between 0.034–0.170 g/kg DM and was at low levels in the mixtures compared to the sole silages. Hejduk and Dolezal [49] reported that oxalic acid content of pure rumex silages was 41.1 g/kg, while 50% rumex + 50% grassland silage was 18.1 g/kg.

Suttle [50] reported that forage crops are an important part of livestock production as they represent the basic source of essential minerals in cattle nutrition. Potassium has functions at the cellular level as the principle intracellular cation and plays an important role in osmotic pressure regulation and water balance in the animal's body, while phosphorus is involved in every metabolic reaction and energy transfer with in the animal body [51,52]. Calcium is the most pervasive mineral in an organism, and it's the main component of bones and teeth. Besides, calcium is one of the most important nutrients influencing productions, reproduction in the cattlee [53]. Arnoud [54] indicated that magnesium is used in the dairy cow's diet to maintain a correct blood magnesium level and to ensure an optimal ruminal pH value (between 6.2 and 6.5) and allowing the correct functioning of the ruminal digestion mechanisms. Accordingly, Kidambi et al. [55] and Tekeli and Ates [56] reported that roughage require at least 8.0 g/kg of K, 2.1 g/kg of P, 3 g/kg of Ca, and 1.0 g/kg of Mg. Within this respect, in this study, the nutrients of all silages were at the desired level except for Ca of 100M%. Mut et al. [57] reported that silages of alfalfa and companion crops mixtures K, P, Ca, and Mg content ranged between 15.03–30.47, 2.67–7.97, 8.16–12.07, and 2.27–4.48 g/kg, respectively. Besides, legumes have a richer nutrient content than cereals [58]. Therefore, in the current study, mineral nutrients of the sole soybean, cowpea, and mixture silages were higher than the sole maize. Önal Aşçı and Acar [59] indicated that K content of forage crops have higher than in other macro mineral nutrients.

5. Conclusion

In recent years, developments and awareness in animal husbandry have increased the interest in more efficient and quality silage production. Today silage quality is evaluated depending on many parameters such as dry matter digestibility, protein, organic acid content, etc. Maize is the most important silage crop in the world but has some negative aspects such as low protein content. In this sense, many efforts have been performed to improve the quality in maize silage. One of the most used methods to increase the protein content is intercropping maize with legumes.

Our results showed that maize x legume intercropping caused significant improvement in the protein content, mineral content, DMI, and RFV values of silage. Moreover, it did not cause a decrease in yield. However, this effect was closely depended to legumes and seed rates. Accordingly, when yield and quality parameters were evaluated together, it was concluded that soybean would be more suitable for intercropping with maize at the seed rate of 75 + 25%.

In addition, this study showed that maize x legume intercropping caused a significant ($p < 0.01$) variation in the organic acid content of silage, especially in the LA, AA, CA SA, OA contents. The effects of organic acids on the quality, storage of silage, and animal health have been discussed in numerous studies. However, organic acids may result in changing effects in the rumen depending on amount, proportions of organic acids and chemical content of silage, which directly determined by plants. For this reason, there is a need for in-vivo studies to determine real effects of these organic acids on animal productivity and health. These studies are exremly importrant to support our findings and spread of maize x legume intercropping for silage making.

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