

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

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15
16 **Abstract**

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

31 **Key words:** Silage, mixture, yield, quality.

32 **1. Introduction**

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

37 Maize containing high dry matter with considerable energy [2], is the most popular
38 crop for silage making all over the world [3,4] In addition, maize silage meets almost all
39 the nutritional requirements of animals, and reduces the need for concentrate feeds by up
40 to 50% [5]. However, low protein content is the most important disadvantage in maize
41 silage. Previous researches show that crude protein of silage maize ranges from 7.0 to
42 8.0% [6-8]. The protein content of the maize silage can be increased by adding a protein-
43 rich legume such as soybean, cowpea. Titterton [9] reported that legume incorporation
44 increased the crude protein (CP) content from 7.7% to 15.3% in maize silage.

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

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

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

63 **2. Material and methods**

64 **2.1. Plant material**

65 Plant materials consisted of Arifiye variety of maize, Yeşilsoy variety of soybean and
66 Ülkem variety of cowpea was sown as binary mixtures with three seed rates (75 + 25%,
67 50 + 50%, and 25 + 75%). These plants were also planted separately as control.

68 **2.2. Experimental site and design**

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

78 Seed rate was calculated with regard to alone sowing rate of each plant; 12.000
79 plant/da for maize and 10 kg/da for soybean and cowpea. Row distance was arranged in
80 70 cm in sole cropping. Mixtures were sown in rows with 35 cm distances. The
81 experiment was set in three replicates in a randomized complete block design (RCBD).
82 After planting, 3 kg/da N and 8 kg/da P₂O₅ as fertilizer were applied. Then, 3 kg/da N
83 was applied as plants reach up to 40 - 50 cm. All the plots were irrigated five times during
84 the vegetation period. Sole maize and intercrops were harvested depending on maize at
85 the milk dough stage, the sole legumes when seed shape exactly formed in the bottom
86 pods.

87 **2.3. Silage yield, silage preparation, ensiling and silo opening**

88 The silage yield was calculated as kg/da from fresh weight and determined by harvesting
89 and weighing the plants that were in 2.8 m² area located center of the plots. The harvested
90 plants were chopped in 2 cm size, and they were filled into plastic jars according to the
91 mixture ratios. Silages were stored at 25 ± 2 °C and opened after 45 days of ensiling.

92 **2.4. Flieg score**

93 Flieg score was calculated by using pH and dry matter ratio as follows; Flieg Score = 220
94 + (2 x Dry Matter% - 15) - 40 x pH) [11]. The Flieg score ranged between 81 and 100

95 was considered to be very good, between 61 and 80 was considered to be good, between
96 41 and 60 was considered to be medium, between 21 and 40 was considered to be poor,
97 and between 0 and 20 was considered to be poorer silage quality and excluded from the
98 experiment.

99 **2.5. Organic acid analyses**

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

106 Malic, Citric, Succinic and Oxalic acids were as described considering Uden's [12]
107 method. Organic acids were determined by adding 100 or 200 ml water to each 100 g
108 sample and refreezing for 24 hours in plastic silage bags. A hydraulic press was then used
109 to extract the liquid after defrosting, followed by centrifugation of the extract at 2000 x g
110 for 5 min. Then, samples were analyzed in HPLC (Shimadzu; Kyoto, Japan) auto sampler
111 system model LC - 20AT equipped with four pumps and an SPDM20A diode array
112 detector (DAD).

113 **2.6. Mineral content analyses**

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

117 **2.7. Dry matter, crude protein, acid detergent fiber and neutral detergent fiber ratio** 118 **analyses**

119 The fresh weights of the samples taken from each jar were determined and they were
120 dried in a hot-air oven at 105 °C for 72 hours; consequently, dry matter ratio (DM) (%)
121 was calculated. Silage samples were dried at 65 °C until they reach up to constant weight.
122 Then, samples were grounded in a grain mill with 0.5 to 1 ml sieve. Nitrogen (N) contents
123 of samples were determined using the Kjeldahl apparatus (FOSS 984.13) and then, crude
124 protein content (CP) was calculated by multiplying the N concentration by a factor of
125 6.25. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) analyzes were
126 determined as specified by Van Soest [14] and Van Soest and Wine [15]. Relative feed

127 value (RFV) was estimated according to the following equations adapted from Rohweder
128 et al. [16].

129 Digestibility of dry matter% (DDM) = $88.9 - (0.779 \times \text{ADF})$

130 Dry matter intake% (DMI) = $120 / \text{NDF}$

131 Total digestibil nutrient% (TDN) = $(96.35 - (\text{ADF} \times 1.15))$

132 Relative feed value (RFV) = $(\text{DDM} \times \text{DMI}) / 1.29$

133 **2.8. Statistical analyses**

134 All data were statistically analyzed by repeated measure analysis in SPSS version 18.0
135 and means were separated by Duncan's Multiple Range Test [17].

136 **3. Results**

137 Dry matter ratio, pH, and Flieg score were significantly different ($P < 0.01$) between
138 treatments as seen in Table 2. The dry matter ranged between 24.89% (sole cowpea) and
139 34.45% (sole maize). The highest pH was determined as 5.32 (25M + 75C%), while the
140 lowest was determined as 4.35 (sole maize), 4.44 (sole cowpea) and 4.38 (75M + 25S%).
141 Flieg scores of silages ranged between 43.13 (25M + 75C%) and 99.88 (sole maize), and
142 all the silages studied varied between medium and very good quality class (Table 2).

143 Silage yield, crude protein (CP), acid detergent fiber (ADF) and neutral detergent
144 fiber (NDF) ratios were given in Table 3. Silage yield and CP were significantly different
145 ($P < 0.01$) between treatments, while ADF and NDF were not significant. Silage yield
146 was found to be high in 100M% (6246.1 kg/da), 75M + 25S% (6109.0 kg/da), 50M +
147 50S% (5164.5 kg/da), 25M + 75S% (4950.9 kg/da) and 75M + 25C% (5532.0 kg/da),
148 while the lowest silage yield was found in sole cowpea (3258.7 kg/da). The highest crude
149 protein ratio was determined as 18.85% in sole cowpea while the lowest was 11.42% in
150 sole maize. ADF and NDF ratios ranged between 27.39 (sole cowpea) - 33.18% (sole
151 maize) and 40.19 (sole cowpea) - 49.82% (sole maize), respectively.

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

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

161 Organic acids content in maize - legume mixtures were given in Table 5. There
162 were statistically significant differences ($P < 0.01$) between treatments regarding organic
163 acid content, except butyric and malic acids. The highest lactic acid content was
164 determined in the treatments of 25M + 75S% (56.24 g/kg), sole soybean (52.26 g/kg),
165 50M + 50S% (51.39 g/kg), sole maize (37.47 g/kg) and 75M + 25S% (32.90 g/kg). Acetic
166 acid ranged between 0.16 and 0.39 g/kg among treatments. The malic acid was listed from
167 high to low value according to the sole silages: maize > cowpea > soybean. Besides, the
168 malic acid of maize - soybean mixture silages was higher than maize - cowpea silages.
169 The highest citric acid was determined in 75M + 25S% (6.151 g/kg), 75M + 25C% (4.666
170 g/kg) and 50M + 50C% (5.215 g/kg), while the lowest was in sole maize (2.375 g/kg).
171 The content of succinic acid ranged between 0.274 (sole soybean) and 0.615 (75M +
172 25S%) g/kg. The highest oxalic acid was determined in sole maize with 0.170 g/kg, while
173 it was lowest as 0.034 (75M + 25C%) g/kg (Table 5).

174 Mineral contents of maize - legume mixture silages were given in Table 6. There
175 were statistically significant differences ($P < 0.01$) for all mineral elements among the
176 treatments. Potassium (K) content of silages ranged between 13.09 (sole maize) and 25.81
177 (75M + 25C%) g/kg. The highest content of phosphorus (P) was determined as 4.16 g/kg
178 in 25M + 75C% mixture. Phosphorus content was found to be low in sole maize, 75M +
179 25S%, and 50M + 50S% (2.49, 2.60 and 2.61 g/kg, respectively). The calcium and
180 magnesium content of silages were ranged from 2.82 to 13.02 g/kg and 1.71 to 4.83 g/kg,
181 respectively (Table 6).

182 **4. Discussion**

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

189 Acidity is an important parameter in the evaluation process of silage quality.
190 Acidity in silage is a feature that directly affects the fermentation process, and the increase

191 in acidity prevents the leaching of the nutrients such as protein. Researchers [19,20]
192 suggest that pH values of quality silage should be between 3.7 and 4.8. In the current
193 study, the pH in the mixture silages was similar to the suggested values except in maize
194 x cowpea mixtures. Besides, sole legume silages exhibited higher pH values than sole
195 maize. This may be due to higher buffering capacity, higher crude protein, and lower
196 carbohydrate contents of legumes. The Flieg score is calculated using dry matter content
197 (DM) and pH, and gives information on the quality of silage. Flieg scores of silage
198 determined in this study were found to be a medium, good, and very good quality class
199 of silage. In previous studies, Flieg score of legume + cereal mixture silages were ranged
200 from 61.80 to 95.06 [13, 17].

201 Despite the importance of quality, yield still maintains its importance in silage crops
202 due to the high level of roughage requirement. In this sense, efforts to increase the yield
203 in silage plants continue intensively all over the world. However, the relationship between
204 yield and quality in silage plants should never be ignored. Because, animal productivity
205 and health are the result of the combination and interaction of both parameters. Our result
206 showed that intercropping produced more desired results for almost all the investigated
207 quality parameters. Maize x legume intercropping was more yielding than sole legumes.
208 In addition, it can be said that the maize is a determinant factor in yield for the mixtures.
209 The performances of the legumes in the mixtures were also different, silage yield and
210 quality in maize x soybean intercropping was generally higher than maize x cowpea
211 intercropping, with the significant effect of seed rates. Silage yield was significantly ($P <$
212 0.01) different amongst the treatments and it varied from 3258.7 (sole C) to 6246.1 (sole
213 M) kg/da. For maize x legume intercropping, similar differences in yield were previously
214 reported by Alaca and Parlak [21] that ranging between 462 and 9700 kg/da.

215 Researchers reported that the use of legumes in silage increases the quality of the
216 ensiled mass and the protein content [22,23]. Therefore, in the current study, protein
217 content was higher in sole legumes and mixtures than sole maize. Başaran et al. [13]
218 reported that the protein content of grasspea + cereal mixture silages was ranged from
219 12.18% to 22.68%.

220 Acid detergent fiber (ADF) and neutral detergent fiber (NDF) are important for
221 rumen degradation and influence animal performance. Higher ADF in forage is related
222 with the low the energy value, while the more NDF is with the low animal intake [24,

223 25]. Kaplan et al. [26] indicated that low ADF and NDF contents of forage crops are
224 usually desired since these materials complicate digestion and consequently decrease the
225 quality. Therefore, in the quality forages of ADF should be ranges from 20 to 30%, while
226 the NDF ranges from 30 to 40%¹. In the present study, it is determined that ADF and
227 NDF ratios of silages between desired limits except for NDF of sole maize. Sole maize
228 silages ADF and NDF content had higher than sole soybean and cowpea. This may be
229 due to the low fiber content in the legumes. Besides, the increasing rate of legumes in the
230 mixtures caused lower ADF and NDF content in silage.

231 Sole soybean and cowpea silages exhibited higher DDM, DMI, TDN, and RFV
232 values than sole maize (Table 4). This may be due to the higher ADF and NDF contents
233 of maize. Besides, DDM, DMI, TDN, and RFV values were decreased with increasing
234 ratio of maize in the mixtures. The Relative Feed Value (RFV) is the widely used index
235 of feed quality worldwide and is based on estimates of feed intake from NDF
236 content and digestibility from ADF content. Accordingly, the RFV value for beginning
237 quality standard was > 151, for first quality standard was 151 – 125, for the second quality
238 standard was 124 – 103, third quality standard was 102 – 87, fourth quality standard was
239 86 – 75 and fifth quality standard was < 75 represented the forage quality [16]. The RFV
240 values determined in the study showed that examined silages between the second and
241 beginning quality classes. Can et al. [17] reported that RFV values of *Bituminaria*
242 *bituminosa* + oat mixture silages was ranged between 86.60 and 159.89.

243 The formation of quality silage depends on lactic acid content, and it should be
244 more than 20.0 g/kg [27]. Accordingly, the lactic acid contents in the silage samples of
245 the present study were relatively high with reference to the critical value, except for
246 50M+50C% and 25M + 75C%. In addition, it was seen that addition of soybean to corn
247 silage increases lactic acid content compared to cowpea. König et al. [28] reported that
248 lactic acid of red clover - grass silage ranged between 23 - 133 g/kg.

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

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

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

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

268 Kung et al. [38] reported that the citric acid contain active ingredients with
269 antimycotic activity for livestock. Citric acid has a function in stimulating rumen
270 fermentation and improving animal performance [39]. Uden [12] indicated that citric acid
271 is used to keep the pH between 4 - 6 during fermentation of silage, while Ke et al. [40]
272 reported that application of citric acid in silage decreased the pH value, limited proteolysis
273 and improved fermentation quality. Playne and McDonald [41] found that citric acid of
274 Italian ryegrass silage between 1 - 25 g/100 g DM. In the current study, the citric acid of
275 silages ranged between 2.375 and 6.151 g/kg. Besides, this study showed that the amount
276 of citric acid increased adding legumes to maize silage.

277 McDonald et al. [42] indicated that succinic acid is well-known agent for silage
278 fermentation and it is produced by several bacterial species. Succinic acid is effective for
279 the various diseases of the livestock and it contributes to the development of body growth
280 of livestock. Zeikus et al [43] reported that succinic acid increased the concentration of
281 propionate in the rumen and acted as an energy source for animals. Uden [12] found that
282 succinic acid of silage maize and legumes ranged between 0.1 and 0.9 g/kg. Succinic acid
283 values obtained from the present study (0.274 - 0.615 g/kg DM) are consistent with Uden
284 [12].

285 Nakata [44] indicated that oxalic acid accumulates in many plants as calcium
286 oxalates, and it plays a role in calcium regulation with detoxification. Oxalic acid is an
287 anti-nutrient, and its overconsumption can cause, depression, weakness, difficulty in
288 breathing and death in animals [45]. However, oxalic acid can be metabolized in the
289 rumen, and 40 g/day can be tolerated by the sheep [46]. On the other hand, Rolinec et al
290 [47] indicated that oxalic acid content greater than 100 g/kg DM could be considered
291 potentially dangerous, while Panda and Sahu [48] observed that the total oxalic acid
292 intake at the level of 5.8 g/kg DM intake was harmless to bulls, but an increase to 11.9
293 g/kg created a negative balance of calcium. In this study, oxalic acid was ranged between
294 0.034 - 0.170 g/kg DM and was low levels in the mixtures compared to the sole silages.
295 Hejduk and Dolezal [49] reported that oxalic acid content of pure rumex silages was 41.1
296 g/kg, while 50% rumex + 50% grassland silage was 18.1 g/kg.

297 Suttle [50] reported that forage crops are an important part of livestock production,
298 as they represent the basic source of essential minerals in cattle nutrition. Potassium has
299 functions at the cellular level as the principle intracellular cation, and plays an important
300 role in osmotic pressure regulation and water balance in the animal's body, while,
301 phosphorus is involved in every metabolic reaction and energy transfer with in the animal
302 body. [51,52]. Calcium is the most pervasive mineral in an organism, and it is the main
303 component of bones and teeth. Besides, calcium is one of the most important nutrients
304 influencing productions, reproduction in the cattlee. [53]. Arnoud [54] indicated that
305 magnesium is used in the dairy cow's diet to maintain a correct blood Magnesium level
306 and to ensure an optimal ruminal pH (between 6.2 and 6.5) and allowing the correct
307 functioning of the ruminal digestion mechanisms. Accordingly, Kidambi et al. [55] and
308 Tekeli and Ates [56] reported that roughage require at least 8.0 g/kg of K, 2.1 g/kg of P,
309 3 g/kg of Ca and 1.0 g/kg of Mg. Within this respect, in this study, the nutrients of all
310 silages were at the desired level except for Ca of 100M%. Mut et al. [57] reported that
311 silages of alfalfa and companion crops mixtures K, P, Ca, and Mg content ranged between
312 15.03-30.47, 2.67-7.97, 8.16-12.07, and 2.27-4.48 g/kg, respectively. Besides, legumes
313 have a richer nutrient content than cereals [58]. Therefore, in the current study, mineral
314 nutrients of the sole soybean, cowpea, and mixture silages were higher than sole maize.
315 Önal Aşçı and Acar [59] indicated that K content of forage crops have higher than other
316 macro mineral nutrients.

317 **5. Conclusion**

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

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

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

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Tables507 **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		

508 * Turkish State Meteorological Service

509 **Table 2.** Dry matter ratio, pH, Flieg score and quality class of maize - legume silages

Treatments	Dry matter ratio (%)**	pH**	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

510 **: P < 0.01; M: Maize; S: Soybean; C: Cowpea.

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520 **Table 3.** Silage yield, crude protein, acid detergent fiber and neutral detergent fiber ratio
521 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

522 **: P < 0.01; M: Maize, S: Soybean; C: Cowpea; CP: Crude protein ratio;

523 **Table 4.** Digestibility of dry matter, dry matter intake, total digestibil nutrient and relative
524 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

525 *: P < 0.05; **: P < 0.01; M: Maize; S: Soybean; C: Cowpea.

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528 **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

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

531 **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

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

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