# Effects of Different Synthetic and Organic Fertilizer Applications on the Micromorphological Characteristics of Maize (*Zea mays* L.) Leaves and Some Silage Quality Traits

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#### ABSTRACT

**Background:** Fertilization is an important cultural practice used to support plant growth and development. Fertilizers provide plants with the nutrients they need, which help them to photosynthesize, grow and develop. Fertilizers are divided into two groups: organic and synthetic. Organic fertilizers are derived from organic materials such as manure, compost and bat guano. Synthetic fertilizers are produced using chemical methods.

**Methods:** This study was conducted to determine the effects of different synthetic and organic fertilizer applications on silage quality and some micromorphological characteristics in maize plants. The study was conducted using a randomized complete block design with three replications. The RX-9292 silage maize variety was used as the plant material. Six different organic and synthetic fertilizer types with different contents (Control, Synthetic Fertilizer-1 (20 kg N, 8 kg P and 8 kg K per decare), Synthetic Fertilizer-2 (20 kg N, 10 kg P and 10 kg K per decare), Poultry Manure, Cattle Manure and Vermicompost). were used in the study. The ADF (%) (Acid Detergent Insoluble Fiber), NDF (%) (Neutral Detergent Insoluble Fiber), ADL (%) (Acid Detergent Insoluble Lignin), SDMR (%); (Silage Dry Matter Content) properties of the silage obtained were then examined. Additionally, the thickness of the cuticle, number and size of stoma-epidermis cells and stoma index o0f the leaves were measured and observed using Scanning Electron Microscopy. **Result:** As a result of the measurements and observations, it was found that both synthetic and organic fertilizer applications increased the yield and quality values in silage maize, but synthetic fertilizers and the importance of organic fertilizer applications for animal health, organic fertilization is thought to be more appropriate.

Key words: Cell size, Chemical fertilizer, Epidermal thickness, Organic fertilizer, Silage maize, Stomata.

## INTRODUCTION

Maize is the most cultivated plant in the world in terms of production area after wheat and rice among the family of cereals (FAO, 2023). Maize has more industrial uses than any other plant amongst the industrial crops. It is a source of raw material for many products such as human nutrition, animal nutrition (as green-dry grass or after silage production), starch or starch-based products and oil production (Yıldırım and Ay, 2023). In Turkey, in addition to the use of highly productive cultivated breeds of animals, quality roughage is also of great importance for the development of animal husbandry (Gökkuş and Oral, 2022). The quality of meat and milk obtained from animals is related to the quality of their food as well as their feeding program. The taste, aroma, nutritional content and even the toxicity of an animal food is dependent on the food consumed by the animal. Therefore, plants used in animal nutrition need to be grown more safely. Structural carbohydrates in roughages are divided into two groups as NDF (Neutral Detergent Insoluble Fiber) and ADF (Acid Detergent Insoluble Fiber). While monogastric animals cannot digest these structural carbohydrates in roughages, ruminants can digest these structural carbohydrates thanks to cellulolytic microorganisms (Tekçe and Gül, 2014).

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Therefore, in a quality roughage, it is desired that ADF ratio to be around 30% and NDF to be 40% and below. Fertilization is the most important step to obtain high quality

products in silage corn cultivation. Fertilizer both supports the development of plants and supports the plant against many stress factors (Kördikanlıoğlu and Gülümser, 2021). One of the factors affecting plant growth is photosynthesis. Leaf characteristics are the main factors affecting photosynthesis rate (Kyzy and Yıldırım, 2023). Especially the number, size and structure of stomata, as well as epidermis cell size and cuticle thickness are effective on photosynthesis rate (Kadıoğlu and Turgut, 1999; Kyzy et al., 2023). The main objective of this study is to determine the effect of organic fertilizers and synthetic fertilizers on silage quality and yield characteristics of the plant and thus to determine the advantage of organic fertilizers compared to synthetics (chemical fertilizers). Thus, people can turn to cleaner, reliable and sustainable resources for nature and living things. It is also an important study in terms of helping the development of organic or sustainable agriculture systems in animal enterprises.

#### MATERIALS AND METHODS

# Setting up the experiment, silage making and silage analysis

This study was conducted in Antalya, Elmalı in 2022 under the conditions of average precipitation of 20.42 mm and temperature of 19.05°C during the maize growth period. The research was carried out at the Molecular Biology and Genetics Laboratory of the Faculty of Arts and Sciences at Ordu University and the Laboratories of the Faculty of Veterinary Medicine at Ondokuz Mayıs University. The experiment was established in a randomized block design with three replications using RX 9292 silage corn variety, which has high cob yield and fast drying feature. Each plot was 6.3 m<sup>2</sup>, with 70 cm between rows and 18 cm within rows. Hand planting was done on April 21<sup>st</sup>.

Six different fertilizer applications were used: control, synthetic fertilizer-1 (20 kg N, 8 kg P and 8 kg K per decare), synthetic fertilizer-2 (20 kg N, 10 kg P and 10 kg K per decare), poultry manure (250 kg/da), cattle manure (300 kg/da) and vermicompost (300 kg/da) (Yıldırım and Yılmaz 2023; Yıldırım *et al.*, 2023).

Harvesting was carried out at the milk maturity stage (Fig 1). Leaf samples were taken from 10 plants. Dry matter, NDF (Neutral Detergent Insoluble Fiber), ADF (Acid Detergent Insoluble Fiber), ADL (Acid Detergent Insoluble Lignin), stoma width, length and number, epidermis cell number, stomatal index and cuticle thickness were analyzed. For silage, corn was chopped into pieces, compressed into jars and kept in the dark for 60 days. Silage analyzes were performed on samples taken from the middle parts of the jars. ADF, NDF and ADL analyzes were performed according to the method of Van Soest *et al.* (1991) using an Ankom device. Digestible dry matter ratio was calculated using the equation of Horrocks and Valentine (1999).

**Plant dry matter ratio (%):** 500 g samples were dried in a drying oven at 70°C for 48 hours.

#### Anatomical and micromorphological analysis

To investigate the effects of six different fertilization methods on maize leaves, leaf sections were taken and made into permanent preparations. Photographs were taken from the preparations and cell dimensions were measured. Stoma and epidermal cells were counted and the stomatal index was calculated. The upper and lower surfaces of the leaves were examined with SEM (Scanning Electron Microscope). For imaging, the samples were coated with gold and visualized using a Hitachi SU 1510 scanning electron microscope (Meidner and Mansfield, 1968).

## Statistical analysis

Data analysis was performed using the JMP (John's Macintosh Project) software package. A one-way analysis of variance (ANOVA) was performed for pairwise comparisons. In the ANOVA, significant differences were compared and lettered using the Tukey test, taking into account the homogeneity of variances.

## **RESULTS AND DISCUSSION**

The study investigated the effects of synthetic and organic fertilizer applications on silage quality and micromorphological characteristics of silage maize in Antalya ecological conditions in 2022. According to Table 1, 2 and 3, fertilizer types significantly influenced Acid Detergent Insoluble Fiber (ADF), Neutral Detergent Insoluble Fiber (NDF), Acid Detergent Insoluble Lignin (ADL), Silage Dry Matter Content (SDMR), stomatal length, stomata number, upper leaf surface epidermis, lower leaf surface epidermis and stomatal length (p<0.01). Cuticle thickness, stomata width and lower leaf epidermis length were also significant (p<0.05). Other micromorphological features measured were not statistically significant.

Fertilizer effects on NDF (%) varied: vermicompost had the highest at  $57.31\pm0.02\%$ , while synthetic fertilizer-2 had the lowest at  $38.90\pm0.10\%$ . All fertilizer types formed distinct groups (Table 1). ADF values ranged from  $30.55\pm0.01\%$ for vermicompost to  $20.11\pm0.06\%$  for synthetic fertilizer-2. ADL mean values ranged from  $3.44\pm0.04\%$  for cattle manure to  $2.06\pm0.01\%$  for synthetic fertilizer-2. Silage dry matter varied from  $27.69\pm0.02\%$  for poultry fertilizer to  $22.19\pm0.02\%$  for the control group (Table 1).

Fertilizer treatments showed no significant difference in cuticle thickness ( $\mu$ m): Control group, 4.32±0.41  $\mu$ m; synthetic fertilizer-1, 4.17±0.67  $\mu$ m; synthetic fertilizer-2, 4.20±0.70  $\mu$ m; poultry manure, 5.61±0.80  $\mu$ m; cattle manure, 5.74±0.77  $\mu$ m; vermicompost, 5.19±0.48  $\mu$ m (Table 2). Stomatal cell width ( $\mu$ m) on the upper surface remained consistent across treatments: Control, 8.83±0.77  $\mu$ m; synthetic manure-1, 8.95±0.72  $\mu$ m; synthetic manure-2, 8.69±0.35  $\mu$ m; poultry manure, 10.12±0.87  $\mu$ m; cattle

manure, 10.06±0.29 µm; vermicompost, 8.81±0.93 µm (Table 2). Stomatal length (µm) on the upper surface varied from 46.76±1.57 to 37.83±2.44 µm (Table 2), with synthetic fertilizer-2 having the highest and vermicompost the lowest values. Synthetic fertilizer-2, poultry manure and synthetic fertilizer-1 did not differ significantly, while vermicompost was grouped with control and cattle manure.

When examining the effects of treatments on the number of stomata on the upper surface, mean values ranged from  $98.33\pm10.41$  to  $58.33\pm2.89$  µm, with the highest value obtained from cattle manure. Fertilizer types grouped similarly were poultry manure, vermicompost, synthetic fertilizer-1 and control, respectively (Table 2). Mean values of the number of epidermis on the upper surface varied from  $351.67\pm11.55$  to  $265.00\pm26.46$  µm, with cattle Manure having the highest and synthetic fertilizer-2 the lowest values. Vermicompost and synthetic fertilizer-1 did not differ significantly from cattle manure (Table 2).

Epidermis width on the upper surface was not statistically significant across treatments. Mean values were as follows: Control,  $34.82\pm1.63 \mu m$ ; synthetic fertilizer-1,  $30.73\pm1.24 \mu m$ ; synthetic fertilizer-2,  $32.49\pm1.42 \mu m$ ; poultry manure,  $28.82\pm1.95 \mu m$ ; cattle manure,  $31.85\pm3.27 \mu m$ ; vermicompost,  $33.60\pm4.93 \mu m$  (Table 2). Similarly, epidermis length on the upper surface was not statistically

significant across treatments. Mean values were: Control, 106.36±20.47 µm; synthetic fertilizer-1, 110.84±10.11 µm; synthetic fertilizer-2, 116.52±6.52 µm; poultry manure, 111.05±11.10 µm; Cattle Manure, 91.60±14.12 µm; vermicompost, 97.29±4.89 µm (Table 2). Stomatal index on the upper surface was also found to be statistically insignificant across treatments, with mean values ranging from 23.95±3.63 to 18.24±1.07 SI (Table 2).

Glandular hairs were observed on the lower leaf surface with deeper periclinal and anticlinal walls. Comparing lower leaf epidermal cell widths, the smallest was in synthetic fertilizer-1 and the largest in synthetic Fertilizer-2. The longest epidermal cells were in synthetic Fertilizer-2 and the shortest in poultry manure treatments. Stomatal width decreased in poultry manure and vermicompost treatments compared to the control, with the lowest stomatal length in the vermicompost treatment. Stomatal density was highest in cattle manure and lowest in synthetic manure-1 and -2 treatments. Stomatal index was higher in poultry manure treatment than others (Table 3, Fig 2 and 3).

This study investigated the effects of different organic and synthetic fertilizers on silage quality and leaf micromorphological characteristics of silage maize. NDF

Table 1: Means and significance levels of ADF (%), N	NDF (%), ADL (%), SDMR (%), of silage maize.
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			Mean ± SD	F	р
NDF (%)	Control	b	56.38±0.08	4.119	0.001*
	Synthetic fertilizer/1	d	41.77±0.07		
	Synthetic fertilizer/2	f	38.90±0.10		
	Poultry manure	е	41.45±0.05		
	Cattle manure	С	49.37±0.02		
	Vermicompost	а	57.31±0.02		
ADF (%)	Control	b	29.20±0.20	7.034	0.001*
	Synthetic fertilizer/1	е	20.96±0.03		
	Synthetic fertilizer/2	f	20.11±0.06		
	Poultry manure	d	21.95±0.02		
	Cattle manure	С	28.10±0.10		
	Vermicompost	а	30.55±0.01		
ADL (%)	Control	С	3.27±0.02	1.765	0.001*
	Synthetic fertilizer/1	е	2.18±0.02		
	Synthetic fertilizer/2	f	2.06±0.01		
	Poultry manure	d	2.72±0.02		
	Cattle manure	а	3.44±0.04		
	Vermicompost	b	3.35±0.03		
SDMR (%)	Control	f	22.19±0.02	13.6	0.001*
	Synthetic fertilizer/1	d	24.11±0.04		
	Synthetic fertilizer/2	С	25.17±0.03		
	Poultry manure	а	27.69±0.02		
	Cattle manure	е	22.36±0.03		
	Vermicompost	b	25.61±0.04		

\*p<0.05, \*\*Tukey Test. a-f: No difference between groups with the same letter. ADF (%): Acid detergent insoluble fiber, NDF (%): Neutral detergent insoluble fiber, ADL (%): Acid detergent insoluble lignin, SDMR (%): Silage dry matter content.

		Mean±SD	F	Р
Cuticle thickness (µm)	Control	c 4.32±0.41	3.737	0.028'
	Synthetic fertilizer/1	c 4.17±0.67		
	Synthetic fertilizer/2	c 4.20±0.70		
	Poultry manure	a 5.61±0.80		
	Cattle manure	a 5.74±0.77		
	Vermicompost	b 5.19±0.48		
Epidermis width (µm)	Control	34.82±1.63	1.803	0.187
	Synthetic fertilizer/1	30.73±1.24		
	Synthetic fertilizer/2	32.49±1.42		
	Poultry manure	28.82±1.95		
	Cattle manure	31.85±3.27		
	Vermicompost	33.60±4.93		
Epidermis length (μm)	Control	106.36±20.47	1.75	0.198
	Synthetic fertilizer/1	110.84±10.11		
	Synthetic fertilizer/2	116.52±6.52		
	Poultry manure	111.05±11.10		
	Cattle manure	91.60±14.12		
	Vermicompost	97.29±4.89		
Stoma width (µm)	Control	8.83±0.77	2.658	0.077
	Synthetic fertilizer/1	8.95±0.72		
	Synthetic fertilizer/2	8.69±0.35		
	Poultry manure	10.12±0.87		
	Cattle manure	10.06±0.29		
	Vermicompost	8.81±0.93		
Stoma length (µm)	Control	bc 40.23±3.13	6.774	0.003*
<b>2</b> <i>j</i>	Synthetic fertilizer/1	abc 41.88±1.87		
	Synthetic fertilizer/2	a 46.76±1.57		
	Poultry manure	ab 43.77±1.63		
	Cattle manure	bc 39.37±1.89		
	Vermicompost	c 37.83±2.44		
1 mm² stoma numbers	Control	ab 71.67±12.58	5.832	0.006*
	Synthetic fertilizer/1	ab 78.33±5.77		
	Synthetic fertilizer/2	b 58.33±2.89		
	Poultry manure	a 86.67±12.58		
	Cattle manure	a 98.33±10.41		
	Vermicompost	ab 83.33±10.41		
1 mm <sup>2</sup> epidermis numbers	Control	b 278.33±32.15	5.972	0.005*
·	Synthetic fertilizer/1	ab 295.00±5.00		
	Synthetic fertilizer/2	b 265.00±26.46		
	Poultry manure	b 275.00±20.00		
	Cattle manure	a 351.67±11.55		
	Vermicompost	ab 311.67±27.54		
Stoma index (SI)	Control	20.66±3.86	1.401	0.292
x ·/	Synthetic fertilizer/1	21.00±1.56		
	Synthetic fertilizer/2	18.24±1.07		
	Poultry manure	23.95±3.63		
	Cattle manure	21.78±2.10		
	Vermicompost	21.34±2.75		

Table 2	Micromorphological	measurements of	f upper leaf of	maize plant	subjected to	different fertilizer treatmen	ts

p<0.05, \*\*Tukey Test. a-f: There is no difference between groups with the same letter.

(Neutral Detergent Insoluble Fiber) is vital for silage quality, ideally below 40%. Synthetic Fertilizer-2 was most effective in reducing the NDF ratio, followed by Synthetic Fertilizer-1. Our findings ranged from 57.31% to 38.9%, with vermicompost showing the highest ratio. Synthetic fertilizers, widely used in cultivated plant cultivation, provide plants with nutrients rapidly. Their chemical content is more

concentrated compared to other organic fertilizers, facilitating high plant uptake (Karaşahin, 2022).

In this study, synthetic fertilizers were observed to enhance plant productivity and quality, particularly in reducing NDF levels. Comparable findings were reported in previous studies examining NDF ratios in corn for silage (Zhao *et al.*, 2022; Koenig *et al.*, 2023; Chayanont *et al.*, 2021; Liu *et al.*, 2021;

		Mean±SD	F	р
Epidermis width (µm)	Control	33.16±1.78	1.171	0.378
	Synthetic fertilizer/1	29.68±2.40		
	Synthetic fertilizer/2	33.28±2.41		
	Poultry manure	31.51±2.83		
	Cattle manure	31.58±1.43		
	Vermicompost	30.42±2.65		
Epidermis length (μm)	Control	ab 108.98±17.88	4.44	0.016*
	Synthetic fertilizer/1	ab 118.29±19.53		
	Synthetic fertilizer/2	a 133.38±8.60		
	Poultry manure	b 93.22±5.12		
	Cattle manure	b 96.30±4.66		
	Vermicompost	ab 111.65±7.68		
Stoma width (µm)	Control	ab 9.48±0.44	3.77	0.028*
	Synthetic fertilizer/1	ab 9.55±0.38		
	Synthetic fertilizer/2	ab 9.60±0.28		
	Poultry manure	b 8.34±0.20		
	Cattle manure	a 9.98±0.68		
	Vermicompost	ab 8.94±0.84		
Stoma length (µm)	Control	a 44.75±1.10	22.05	0.001*
	Synthetic fertilizer/1	ab 42.00±1.59		
	Synthetic fertilizer/2	a 44.54±0.18		
	Poultry manure	b 41.00±0.65		
	Cattle manure	ab 42.60±1.09		
	Vermicompost	c 37.15±0.98		
1 mm² stoma numbers	Control	81.67±10.41	1.457	0.274
	Synthetic fertilizer/1	73.33±20.82		
	Synthetic fertilizer/2	73.33±10.41		
	Poultry manure	91.67±14.43		
	Cattle manure	96.67±14.43		
	Vermicompost	95.00±18.03		
1 mm <sup>2</sup> epidermis numbers	Control	abc 290.00±15.00	5.68	0.006*
	Synthetic fertilizer/1	bc 261.67±27.54		
	Synthetic fertilizer/2	c 241.67±16.07		
	Poultry manure	abc 288.33±30.55		
	Cattle manure	ab 323.33±38.84		
	Vermicompost	a 331.67±10.41		
Stoma index (SI)	Control	21.89±3.00	0.371	0.859
	Synthetic fertilizer/1	21.32±3.60		
	Synthetic fertilizer/2	23.36±3.50		
	Poultry manure	24.07±1.43		
	Cattle manure	22.87±0.70		
	Vermicompost	22.10±3.68		

Table 3: Micromorphological measurements of lower leaf of maize plant subjected to different fertilizer treatments.
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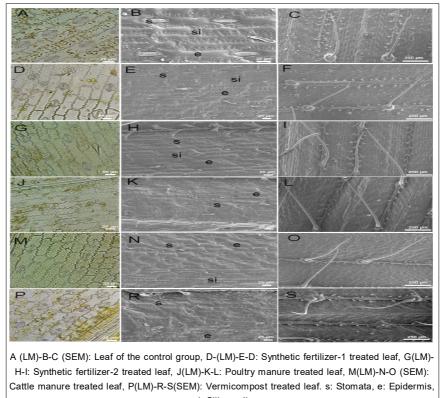
\*p<0.05, \*\*Tukey Test, a-f: No. difference between groups with the same letter.

Karydogianni *et al.*, 2022). However, some studies reported higher NDF ratios (Behrouzi *et al.*, 2022; Amasaib *et al.*, 2022; Cai *et al.*, 2020), likely due to variations in fertilizer content.

For quality forage, ADF ratios should ideally be around 30% or lower (Keleş and Çıbık, 2014). Synthetic Fertilizer-1 yielded the best quality results in our study, followed by Synthetic Fertilizer-2, indicating their superior effectiveness compared to organic fertilizers. Vermicompost exhibited the poorest silage quality, with the highest ADF ratio, while cattle and poultry manure resulted in the lowest quality silage. Our results ranged from 30.55% to 20.11%, aligning with some previous studies (Zhao *et al.*, 2022; Ma *et al.*, 2023; Koenig *et al.*, 2023; Chayanont *et al.*, 2021; Liu *et al.*, 2021), but differing



Fig 1: Experiment setup and harvest.



si: Silica cell.

Fig 2: Upper surface light microscopy (LM) and scanning electron microscopy (SEM) images of Zea mays leaves.

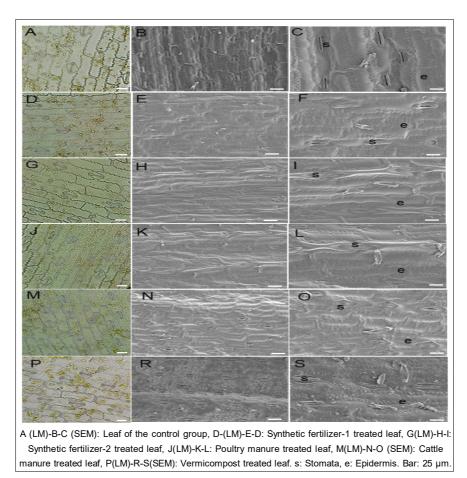


Fig 3: Lower surface light microscopy (LM) and scanning electron microscopy (SEM) images of Zea mays leaves.

from others (Behrouzi *et al*., 2022; Amasaib *et al*., 2022; Cai *et al*., 2020).

For silage quality, parameters like ADF (Acid Detergent Insoluble Fiber), NDF (Neutral Detergent Insoluble Fiber) and ADL (Acid Detergent Insoluble Lignin) are crucial, as they are digestible by animals. Lignin, providing rigidity to cell walls, increases as plants age and is indigestible by animal enzymes. Hence, a low ADL value is desirable in silage materials (Meltem et al., 2013). Our study yielded ADL values ranging from 3.35% to 2.06%. Synthetic Fertilizer-2 performed best in ADL ratio, followed by Synthetic Fertilizer-1, while the lowest result was from Cattle Manure. These results were consistent with other quality parameters (NDF and ADF), indicating synthetic fertilizers' superior efficiency over control and organic fertilizers. Similar studies showed ADL results of 3.99-3.39% (Koenig et al., 2023), 3.60-2.92% (Chayanont et al., 2021), 3.14% (Liu et al., 2021) and 3.49±0.54% (Cai et al., 2020), supporting our findings.

Silage dry matter rate is another critical factor in silage maize. Vermicompost, followed by cattle and poultry manure, were the most effective fertilizers on silage dry matter, outperforming synthetic fertilizers and control treatment. Organic fertilizers, containing fewer chemicals, are generally less harmful to living tissue (Korkmaz and Akıncı, 2023). This suggests organic fertilizer varieties' effectiveness over synthetic ones, especially in increasing dry matter and its accumulation in the plant. In our study, the silage dry matter rate ranged from 25.61% to 24.11%, lower than results from similar studies such as 29.37% (Liu *et al.*, 2021) and 28.14% (Korkmaz *et al.*, 2019).

In several Zea mays studies, leaf epidermis, silica cell structure and stomatal characters were explored (Driscoll *et al.*, 2006; Gao *et al.*, 2006; Suriyaprabha *et al.*, 2012). Light and scanning electron microscopy showed smaller stomatal dimensions in vermicompost-treated Zea mays leaves. The highest stomatal index occurred in Poultry Manure-treated leaves. Many small stomata are associated with higher photosynthesis rates (Suriyaprabha *et al.*, 2012; Drake *et al.*, 2012). Poultry and vermicompost treatments promoted plant growth. Fertilizer applications affected cuticle thickness, epidermis and stomatal properties. Stomata play crucial roles in photosynthesis and gas exchange. Stomatal size varies by environmental conditions (Pekşen *et al.*, 2006). Stomatal size differs among species and cultivars (Budaklı and Çelik, 2013).

## CONCLUSION

The effects of organic and synthetic fertilizers on silage maize were different, with synthetic fertilizers being more effective in terms of silage quality. However, the analysis of microdata and micromorphological structure revealed that organic fertilizers were generally more effective than synthetic fertilizers. Based on this result, organomineral fertilization studies combining both types of fertilizers are recommended for future research. To reduce the overuse of synthetic fertilizers, it is suggested that more sensitive studies should be established and organic fertilizers can be used as a supplement to synthetic fertilizer applications, which will be more effective in terms of both micromorphological and silage quality. In terms of animal health, it is recommended to improve the content of organic fertilizers or to evaluate organomineral fertilizers in such studies.

## Author contribution statements

Gözde Hafize Yıldırım: Investigation, Project administration, Funding acquisition; Nuri Yılmaz: Investigation, Writingreview and editing; Ayşe Özge Şimşek Soysal: Interpretation of the study in terms of silage quality characteristics; Şükran Öztürk: Examination and interpretation of the study in terms of micromorphological characteristics; Öznur Ergen Akçin: Investigation, Writing- review and editing; Ebru Batı Ay: Writing-review and editing.

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No funding was received for this study.

## Data availability statement

The data are available in the form of supplementary information.

## Informing

This study was presented in abstract form at the Congress of Food, Agriculture and Veterinary Sciences (2023).

## **Conflict of interest**

No potential conflict of interest was reported by the author(s).

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