

# AKADEMİK PERFORMANS DEĞERLENDİRME RAPORU

Yıl: 2025  
Akademisyen Adı Soyadı: MUSTAFA KIZILŞİMŞEK  
Birim: Ziraat Fakültesi Dekanlığı  
Bölüm: Tarla Bitkileri Bölüm Başkanlığı  
Kategori: Diğer  
Faaliyet: Uygulama  
Kapsam: ÇİM Bitkileri ve Yeşil Alan Tesis, Lisans Dersi  
Başlangıç Tarihi: 01.10.2025  
Bitiş Tarihi: 31.12.2025  
Performans: Lisans ders verme  
Puan: 5,00  
Kanıt Dosya Sayısı: 1



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Birim: Ziraat Fakültesi Dekanlığı  
Bölüm: Tarla Bitkileri Bölüm Başkanlığı  
Kategori: Diğer  
Faaliyet: Uygulama  
Kapsam: Mesleki Uygulama, Lisans Dersi  
Başlangıç Tarihi: 01.10.2025  
Bitiş Tarihi: 31.12.2025  
Performans: Lisans ders verme  
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Bölüm:	Tarla Bitkileri Bölüm Başkanlığı
Kategori:	Diğer
Faaliyet:	Uygulama
Kapsam:	Mezuniyet Çalışması
Başlangıç Tarihi:	01.10.2025
Bitiş Tarihi:	31.12.2025
Performans:	Lisans ders verme
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Yıl:	2025
Akademisyen Adı Soyadı:	MUSTAFA KIZILŞİMŞEK
Birim:	Ziraat Fakültesi Dekanlığı
Bölüm:	Tarla Bitkileri Bölüm Başkanlığı
Kategori:	Diğer
Faaliyet:	Uygulama
Kapsam:	Devam Eden YL Öğrencisi
Başlangıç Tarihi:	01.01.2025
Bitiş Tarihi:	31.12.2025
Performans:	Yüksek lisans tez danışmanlığı yapıyor olmak (devam eden her bir öğrenci için)
Puan:	12,00
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Yıl:	2025
Akademisyen Adı Soyadı:	MUSTAFA KIZILŞİMŞEK
Birim:	Ziraat Fakültesi Dekanlığı
Bölüm:	Tarla Bitkileri Bölüm Başkanlığı
Kategori:	Diğer
Faaliyet:	Burslar
Kapsam:	TUBİTAK ÖĞRENCİ BURSU
Başlangıç Tarihi:	01.01.2025
Bitiş Tarihi:	01.07.2025
Performans:	Üniversiteler dışındaki kamu/özel kurumlarla yapılan ve devam eden araştırma projesinde araştırmacı olmak
Puan:	20,00
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Lisans ve Lisansüstü bursiyerlere ilişkin raporlama işlemlerinde Gruba herhangi bir belge göndermeniz gerek yoktur. Ancak, sisteme yükleyeceğiniz İsmail ve ekimci sayılarını saklamaya devam edebilirsiniz. TUBİTAK, gerek gördüğü durumlarda, bu belgeleri ve öğrenmeye ilişkin diğer belgeleri Gruba istemeleri isteyebilir.

Araştırmacı Ekle	Bursiyer Ekle	Doktora Sonrası Bursiyer Ekle	Doktora Sonrası Bursiyer Tanımlı Ekle	Durumları Ekle	Bursiyer Kırılma		
Görevi	Adı		Başlangıç Tarihi	Ayrılış Tarihi	Yerine Geldiği Kişi	Güncelle	P
Yürütücü (PT) Araştır	MUSTAFA KIZILŞİMŞEK KARADAMAMARAS SÜTÇÜ İSMAYİL ZERAT F. DALLA BTK/ELZET B.		20/12/2023				
Araştırmacı/Üstman (PT) Araştır Kıdem: 1458	FATMA AKBAY KILIC MALATYA TURGUT ÖZAL İ. ZERAT F.		20/12/2023				
Araştırmacı/Üstman (PT) Araştır Kıdem: 1458	İSMAYİL GÜVEN KARADAMAMARAS SÜTÇÜ İSMAYİL		20/12/2023				
Bursiyer Bursiyer Yüksek Lisans	ALİ KARACI Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu		01/06/2024				
Bursiyer Bursiyer Yüksek Lisans	SEDA ARICAN Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu		01/06/2024				
Bursiyer Bursiyer Doktora	EYLÜL MEZARCI KIZILŞİMŞEK (Doktora Durumunu - MUSTAFA KIZILŞİMŞEK) Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu		01/10/2024				

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# AKADEMİK PERFORMANS DEĞERLENDİRME RAPORU

Yıl:	2025
Akademisyen Adı Soyadı:	MUSTAFA KIZILŞİMŞEK
Birim:	Ziraat Fakültesi Dekanlığı
Bölüm:	Tarla Bitkileri Bölüm Başkanlığı
Kategori:	Diğer
Faaliyet:	Burslar
Kapsam:	Tübitak Proje Bursu
Başlangıç Tarihi:	01.01.2025
Bitiş Tarihi:	31.12.2025
Performans:	Üniversiteler dışındaki kamu/özel kurumlarla yapılan ve devam eden araştırma projesinde araştırmacı olmak
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Lisans ve Lisansüstü bursiyerlere ilişkin başvuru işlemlerinde Gruba herhangi bir belge göndermeniz gerek yoktur. Ancak, sisteme yüklemenizle ilgili ok talimatları sayfa üzerindeki açıklamaları gerekmektedir. TUBİTAK, gerek gördüğü durumlarda, bu belgeleri ve öğrenmeye ilişkin diğer belgeleri Gruba istemekte isteyebilir.

Araştırmacı Etkisi	Bursiyer Etkisi	Doktora Sonrası Bursiyer Etkisi	Doktora Sonrası Bursiyer Talebi Etkisi	Durumları Etkisi	Bursiyer Kararı		
Görevi	Adı		Başlama Tarihi	Ayrılış Tarihi	Yerine Geldiği Kişi	Görevi	P
Yürütücü (PT) Araştırma	MUSTAFA KIZILŞİMŞEK KARADAMNANARAS SÜTÇÜ İNŞAAT İ. ZEMİN İ. DARLA BİTKİLERİ İ.		20/12/2023				
Araştırmacı/Üstman (PT) Araştırma Kafes Öncesi: 1458	FATMA AKBAŞ KILIC KARADAMNANARAS SÜTÇÜ İNŞAAT İ. ZEMİN İ.		20/12/2023				
Araştırmacı/Üstman (PT) Araştırma Kafes Öncesi: 1458	İNAN GÜVEN KARADAMNANARAS SÜTÇÜ İNŞAAT İ.		20/12/2023				
Bursiyer Bursiyer Yüksek Lisans	ALİ KARACI Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu		01/06/2024				
Bursiyer Bursiyer Yüksek Lisans	SEDA ARKIN Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu		01/06/2024				
Bursiyer Bursiyer Doktora	EYLÜL MEZARCI KIZILŞİMŞEK (Doktora Durumunda - MUSTAFA KIZILŞİMŞEK) Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu		01/10/2024				

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Yıl:	2025
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Birim:	Ziraat Fakültesi Dekanlığı
Bölüm:	Tarla Bitkileri Bölüm Başkanlığı
Kategori:	Diğer
Faaliyet:	Uygulama
Kapsam:	Devam Eden YL Öğrencisi
Başlangıç Tarihi:	01.01.2025
Bitiş Tarihi:	31.12.2025
Performans:	Yüksek lisans tez danışmanlığı yapıyor olmak (devam eden her bir öğrenci için)
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Yıl:	2025
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Bölüm:	Tarla Bitkileri Bölüm Başkanlığı
Kategori:	Diğer
Faaliyet:	Uygulama
Kapsam:	Devam eden doktora
Başlangıç Tarihi:	01.01.2025
Bitiş Tarihi:	31.12.2025
Performans:	Doktora/sanatta yeterlik/tıpta uzmanlık tez danışmanlığı yapıyor olmak (devam eden her bir öğrenci için)
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Bölüm:	Tarla Bitkileri Bölüm Başkanlığı
Kategori:	Diğer
Faaliyet:	Uygulama
Kapsam:	Devam eden doktora danışmanlığı
Başlangıç Tarihi:	01.01.2025
Bitiş Tarihi:	31.12.2025
Performans:	Doktora/sanatta yeterlik/tıpta uzmanlık tez danışmanlığı yapıyor olmak (devam eden her bir öğrenci için)
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Bölüm: Tarla Bitkileri Bölüm Başkanlığı  
Kategori: Diğer  
Faaliyet: Uygulama  
Kapsam: Lisans Danışmanlığı  
Başlangıç Tarihi: 01.01.2025  
Bitiş Tarihi: 31.12.2025  
Performans: Lisans danışmanlığı yapıyor olmak  
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İbrahimpaşa Sütlüce İmam Önder

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Ders İşlemleri

Ders Kayıt İşlemleri

Öğrenci Kayıt İşlemleri

Öğrenci Kayıt İşlemleri

Sınav İşlemleri

Akademik CV

İstatistik İşlemleri

Kayıtlar İşlemleri

Kullanıcı İşlemleri

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Lisans Kütüphaneleri

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Soyad

Program

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Soyad

Öğrenci Tipi

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Sıra

Durumu

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Transkript

Akademik Dersler

Sınav Notları

Mühür Durum

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Diğer İşlemler

Ders Kayıt İşlemleri

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Sınav İşlemleri

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Kayıtlar İşlemleri

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Mühür Durum

Genel Bilgiler

Diğer İşlemler

Ders Kayıt İşlemleri

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İstatistik İşlemleri

Kayıtlar İşlemleri

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Sınav Notları

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Birim: Ziraat Fakültesi Dekanlığı  
Bölüm: Tarla Bitkileri Bölüm Başkanlığı  
Kategori: Diğer  
Faaliyet: Burslar  
Kapsam: Tubitak Proje Bursu Ali Kabak.ı  
Başlangıç Tarihi: 01.01.2025  
Bitiş Tarihi: 31.12.2025  
Performans: Üniversiteler dışındaki kamu/özel kurumlarla yapılan ve devam eden araştırma projesinde araştırmacı olmak  
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Araştırmacı, Başarımlar, Doktora Sonrası Bursiyer okuma/çıkarma taleplerinizi ve Bursiyer Kontenjanlarında boşlukları taleplerinizi sisteme üzerinden gönderirken, talimatla ilgili olarak aşağıdaki formu ve eklerini ilgili Araştırma Destek Grubu'na maillele iletmeye lütfenle gerekmektedir.

Lisans ve Lisansüstü bursiyerlere ilişkin başvuru işlemlerinde Gruba herhangi bir belge göndermemize gerek yoktur. Ancak, sisteme yükleyeceğimiz bazı ek belgeleri aşağıdaki şekilde yüklemenizi gerekmektedir. TUBİTAK, gerek gördüğü durumlarda, bu belgeleri ve diğer ilgili diğer belgeleri Gruba iletmeye isteyecektir.

Araştırmacı Etki	Bursiyer Etki	Doktora Sonrası Bursiyer Etki	Doktora Sonrası Bursiyer Talebi Etki	Durumları Etki	Bursiyer Kararı		
Görevi	Adı		Başlama Tarihi	Ayrılış Tarihi	Yerine Geldiği Kişi	Görevi	P
Yürütücü (PT) Araştırma	MUSTAFA KIZILŞİMŞEK KARADAMAMARAS SÜTÇÜ İNŞAAT İ. ZEMİN İ. DARLA BİTKİLERİ İ.		20/12/2023				
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Bursiyer Bursiyer Yüksek Lisans	SEDA ARICAN Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu		01/06/2024				
Bursiyer Bursiyer Doktora	EYLÜL MEZARCI KIZILŞİMŞEK (Doktora Durumu - MUSTAFA KIZILŞİMŞEK) Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu Bursiyer Bilgi Formunu		01/10/2024				

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Birim:	Ziraat Fakültesi Dekanlığı
Bölüm:	Tarla Bitkileri Bölüm Başkanlığı
Kategori:	Diğer
Faaliyet:	Araştırma
Kapsam:	Araştırma makalesi Q2 dergi
Başlangıç Tarihi:	01.01.2025
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Performans:	SSCI, SCI, SCI-Expanded ve AHCI kapsamındaki dergilerde editöre mektup, özet veya kitap kritiği hariç olmak üzere yayımlanmış makale (Q2)
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## Fermentation and silage quality of sorghum (*Sorghum bicolor* L. Moench) grown with organic fertilise

Fatma Akbay, Mustafa Kizilşimşek & Adem Erol

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RESEARCH ARTICLE



## Fermentation and silage quality of sorghum (*Sorghum bicolor* L. Moench) grown with organic fertiliser

Fatma Akbay<sup>a</sup>, Mustafa Kizilşimşek<sup>b</sup> and Adem Erol<sup>b</sup>

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### ABSTRACT

Sustainable agriculture includes environmentally conscious production practices such as the use of organic fertilisers, green fertilisers, effective water usage techniques, crop rotation systems, and biological control of pests, diseases, and weeds. Organic fertilisers increase crop yields and soil fertility without endangering environmental health. On the other side, ensiling depends on epiphytic microorganism flora, such as lactic acid bacteria, enterobacteria, mold, and yeast. The source of LAB is the epiphytic flora on plant material, and its main source is soil. Therefore, introducing fertilisers to the soil might modify the microflora of silage, thereby altering both the soil and silage quality. A study was done for a period of two years, from 2020 to 2021, to evaluate organic fertilisers such as vermicompost, bovine dung, sheep manure, gyttja, and chicken manure with traditional systems. The results obtained for plant height, number of leaves, and fresh forage production demonstrated that chicken manure was similar to typical nitrogen application in terms of yield. Additionally, the effect of fertiliser on forage sorghum yield was different depending on plant cultivar. The findings indicated the beneficial application of chicken manure in sorghum cultivation, as it improved the production of sorghum plants relative to conventional nitrogen fertilisers.

### ARTICLE HISTORY

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### KEYWORDS

Agricultural sustainability;  
forage quality; silage  
fermentation; organic  
fertiliser; forage yield

## Introduction

The increased use of inorganic fertilisers has both positive and negative effects. While it contributes to food security, it also causes soil acidification and salinisation, water contamination, loss of soil fertility, nutrient imbalances, greenhouse gas emissions, and loss of beneficial soil organisms (Chen et al. 2001). Greenhouse gas emissions have also become an international concern as a result of the increasing use of chemical fertilisers (Islam et al. 2022). Continually high levels of chemical fertilisers are not sustainable, and ecological principles and regulations should be followed in agricultural activities. Therefore, applications such as ‘Ecological Agriculture’ and ‘Sustainable Agriculture’ have been developed to mitigate the adverse effects associated with conventional farming methods (Soyergin 2003).

The production of food and other agricultural products at an environmental cost that does not compromise future generations' access to food and general well-being is known as sustainable agriculture. It also incorporates environmentally friendly production models such as organic and green fertilisers, crop rotation systems, efficient water use techniques, and biological control of pests, diseases, and weeds (Robertson 2015). Organic waste contains mineral nutrients, water, and organic matter, and it's used to increase plant production and prevent them from harmful pests without polluting the environment. Hui et al. (2017), defined that the application of organic fertilisers instead of chemical fertilisers was one of the most eco-friendly. There are many different sources of organic fertiliser. Their use is becoming increasingly widespread around the world. Saltalı (2015) reported that due to its high humic content, gytja manure can be used in agricultural fields. Addition, Oagile and Namasiku (2010) impressed that chicken manure is preferred over other animal wastes due to its high concentration of macronutrients. Xu et al. (2016) expressed that vermicompost could improve plant growth and soil salinity. Khaliq et al. (2006) reported that the influence of organic fertilisers on plant yield is slow and changeable.

Silage sorghum (*Sorghum bicolor* L.) is a forage plant widely used for silage production due to its high biomass yield, allowing for multiple harvests during the summer months; suitability for mechanised agriculture; suitability for second cropping and rotation systems; suitability for livestock feed; and good nutritional composition, such as carbohydrates, proteins, fibre, minerals, and vitamins (Sağlamtimur et al. 1998; Cothren et al. 2000). Addition, valued for its high energy content and digestibility, makes it a valuable feed source for ruminant animals such as cattle, sheep, and goats. Sorghum plants using the C4 photosynthetic pathway have an efficiency advantage, especially in hot and dry environments (Mullet et al. 2014; Tiryaki 2005; von Caemmerer and Furbank 2016). According to Newman et al. (2013), requiring less N fertilisation, the sorghum needs an average of 30% less water than maize to deliver good forage yield. Therefore, in most areas it is considered an alternative to maize plants. Sorghum plants produce more biomass than other cereal plants, which provides and naturally exploits more nutrient elements in the soil. Therefore, excessive amounts of chemical fertilisers are applied to ensure a high yield rate in the query plant. However, adverse effects of chemical fertilisers on the soil and the environment, the use of organic fertiliser is increasingly widespread due to its eliminating effect on the beneficial microorganisms in the ground. On the other hand, ensiling is the important forage source obtained as a result of the natural lactic acid fermentation of rich, fresh forage under anaerobic conditions (Dunière et al. 2013). Ensiling depends on epiphytic microorganism flora, such as lactic acid bacteria (LAB), enterobacteria, mold and yeast. The desired microorganisms in the silage are primarily LABs. The source of LAB is the epiphytic flora on plant material, and its main source is soil (Kızılsimşek et al. 2016). Therefore, applications to the soil can also change the microflora of silage, as it can affect the yield of soil and plants.

The aim of this study was to evaluate the performance of the various fertilisers, such as vermicompost, cattle manure, sheep manure, gytja, chicken manure, and traditional nitrogen application, on silage sorghum.

## Materials and methods

### Experimental locations, climate and soil description

A field experiment was conducted during the period (May–September 2020 and 2021) in the Faculty of Agriculture Engineer, University of Kahramanmaraş Sutcu Imam in Kahramanmaraş, Türkiye. The influence of different fertiliser treatments (vermicompost, cattle manure, sheep manure, gyytja, chicken manure, and traditional nitrogen application) on the growth and forage yield of Nes and Jumbo cultivars. The soil was determined to have a clay loam (70.4) texture, with a pH of 7.54, indicating a slightly alkaline condition. It was found to be non-saline, low in organic matter content, and had good levels of potassium and phosphorus.

Some climatic data in the region are given in Table 1. It has been concluded that the total amount of precipitation is insufficient for sorghum cultivation, and irrigation is necessary. Available relative humidity in 2020 was higher than in 2021. The average temperature was determined to be 27.11°C in 2020 and 27.08°C in 2021.

### Experimental design and treatment

The trial plan was arranged in split plots in a completely randomised block design with 3 replications. Organic fertiliser applications and sorghum cultivars were placed as the main plots and sub-plots, respectively. Each sub-plot consisted of 4 planting rows with a spacing of 70 cm between rows and a length of 5 m. A distance of 2 m was left between the blocks. The experimental area covered an area of 19 m x 37.1 m = 704.9 m<sup>2</sup>. Organic fertilisers applied for the sorghum mixture were applied as 1.6 ton da<sup>-1</sup> gyytja, 0.8 ton da<sup>-1</sup> vermicompost, 1.2 ton da<sup>-1</sup> sheep manure, 1.4 ton da<sup>-1</sup> cattle manure, and 1.0 ton da<sup>-1</sup> chicken manure, which are corresponding to traditional nitrogen application doses of 25 kg da<sup>-1</sup>.

### Forage yield measurement

Plant height: Ten plants were randomly selected from the 2nd and 3rd ridges of each individual plot, and the plant was measured and recorded in (cm). The number of leaves: Ten plants were randomly selected from the 2nd and 3rd ridges of each individual plot, and the number of leaves was determined. Fresh forage yield: When the sorghum plant reached the pulp stage, an area of 5.6 m<sup>2</sup> was cut with a sickle and immediately weighed into da. Hay yield: The green forage of the 5.6 m<sup>2</sup> was left to dry in an oven until a constant weight was reached, then final dry matter yield was calculated in da. When the sorghum reaches the pulping period, all parcels are harvested.

**Table 1.** Climatic data of Kahramanmaraş province for silage sorghum vegetation period.

	Precipitation (mm)			Temperature (°C)			Relative humidity (%)		
	2020	2021	Long years	2020	2021	Long years	2020	2021	Long years
May	23.00	12.00	38.8	21.16	23.15	20.1	54.44	47.76	54.7
June	0.00	0.00	8.6	25.24	26.02	24.9	50.21	48.12	49.2
July	0.00	0.00	2.7	30.75	30.69	28.3	46.43	43.12	44.2
August	0.00	2.60	2.2	29.65	30.25	28.4	40.95	44.82	48.76
September	0.00	2.40	11	28.75	25.28	25	42.86	45.73	45.41
Total/Mean	23.00	17.00	63.3	27.11	27.08	25.34	46.98	45.91	48.45

### Silage fermentation and quality analysis

Plant samples of sorghum were cut separately in a silage chopping machine to approximately 2–4 cm. Afterward, approximately 500 g of the treatment sorghum samples were placed in special plastic silage packages, and the mouth was compressed automatically. Three silage packages were prepared for each treatment. In order to determine the initial dry matter content (DM T0), approximately 100 g of the chopped sample was dried in an air-forced oven at 70 °C for 48 hours. Silages were opened after 60 days and analysed for pH, dry matter (DM), crude protein (CP), crude ash (CA), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). Approximately 100 g samples were taken from each opened silage package and kept in an oven at 70 °C for 48 hours, and DM (T60) was determined. The dried forage samples obtained were ground in a grinding machine with a 1 mm sieve and made ready for analysis. The nitrogen content of the silages was determined using the Kjeldahl method, and then CP content was calculated by multiplying it by 6.25 times. Ash by igniting the samples in a muffle furnace at 525 °C for 8 hours (AOAC 1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) of the silages were determined by the method of Van Soest et al. (1991).

pH measurements 20 g samples were taken from the fresh material from T0 and T60 forages, 180 ml of Ringer solution were added, and mixed with a blender at high speed for one minute. Silage extracts were filtered through Whatman 54 filter paper and microorganism counts were made by making ten-fold dilution series. Lactic acid bacteria numbers were determined by pour plating in MRS agar with double overlaying for anaerobic conditions, and plates were incubated at 36 °C for 48–72 h. Enterobacteria counts were enumerated by pour plating in VRBD agar with a single overlay, and plates were incubated at 36 °C for 18 h. Yeast and mold counts were enumerated by pour planting MEA acidified with lactic acid to pH 4 and with a single overlay, and plates were incubated at 32 °C for 48 h.

### Statistical and correlation analysis

The statistical calculations for the data obtained in this study were performed using a split plot in a randomised complete design with the statistical programme JMP.

## Results

### Forage yield measurement

Sorghum plant length was significantly affected by year ( $p < 0.01$ ), varieties ( $p < 0.01$ ), fertiliser ( $p < 0.01$ ),  $Y \times C$  ( $p < 0.01$ ), and  $F \times C$  ( $p < 0.01$ ) interactions, according to the

**Table 2.** Statistical analysis results of investigated characteristics in the present study.

	Plant length	The number of leaves	Fresh forage yield	Hay yield
Years (Y) LSD (0.05)	5.21**	0.51**	ns	ns
Cultivars(C) LSD (0.05)	5.21**	0.51**	138.49**	ns
$Y \times C$ LSD (0.05)	7.37**	ns	195.86*	ns
Fertilisers(F) LSD (0.05)	9.02**	0.87**	239.92**	76.03**
$Y \times F$ LSD (0.05)	ns	ns	339.25**	107.52**
$F \times C$ LSD (0.05)	12.79**	ns	339.25**	ns
$Y \times C \times F$ LSD (0.05)	ns	ns	479.77**	152.06**

\*\* $P < 0.01$ ; \* $P < 0.05$ ; ns: non-significant.

results of the statistical test (Table 2). Overall, the chicken fertilisation significantly influenced ( $p < 0.01$ ) the plant length and increased this parameter by 16.60% compared to the traditional nitrogen application control. Chicken manure increased plant height from 232.05 cm (traditional application) to 242.57 cm, while sheep manure and vermicompost had no effect on plant height. Sorghum plant length, the number of leaves, and fresh forage yield in Nes were lower than Jumbo (Table 3). According to the  $F \times C$  interaction, the plant height is reduced in varieties compared to the traditional nitrogen application when applying cattle manure and gyttja organic material as fertilisers. In parcels where chicken manure was applied compared to the traditional cultivation system, an increase of 8.7% in plant height was observed in the Jumbo cultivar, while the Nes cultivar showed a 0.8% increase in plant height (Figure 1A).

While the effect of  $Y \times C$ ,  $Y \times F$ ,  $F \times C$ , and  $Y \times F \times C$  interactions on the number of leaves was not significant, the effects of year ( $p < 0.01$ ), cultivars ( $p < 0.01$ ), and fertilisers ( $p < 0.01$ ) were significant (Table 3). The number of leaves of the sorghum forages varied between 10.67 and 12.84 according to fertiliser treatments (Table 3). The highest number of leaves was determined in the traditional nitrogen application (12.84) and chicken manure (12.51) treatment and the lowest in the cattle manure (10.67) treatment. The highest number of leaves was observed in the Jumbo (13.45) cultivar and the lowest in the Nes (10.09).

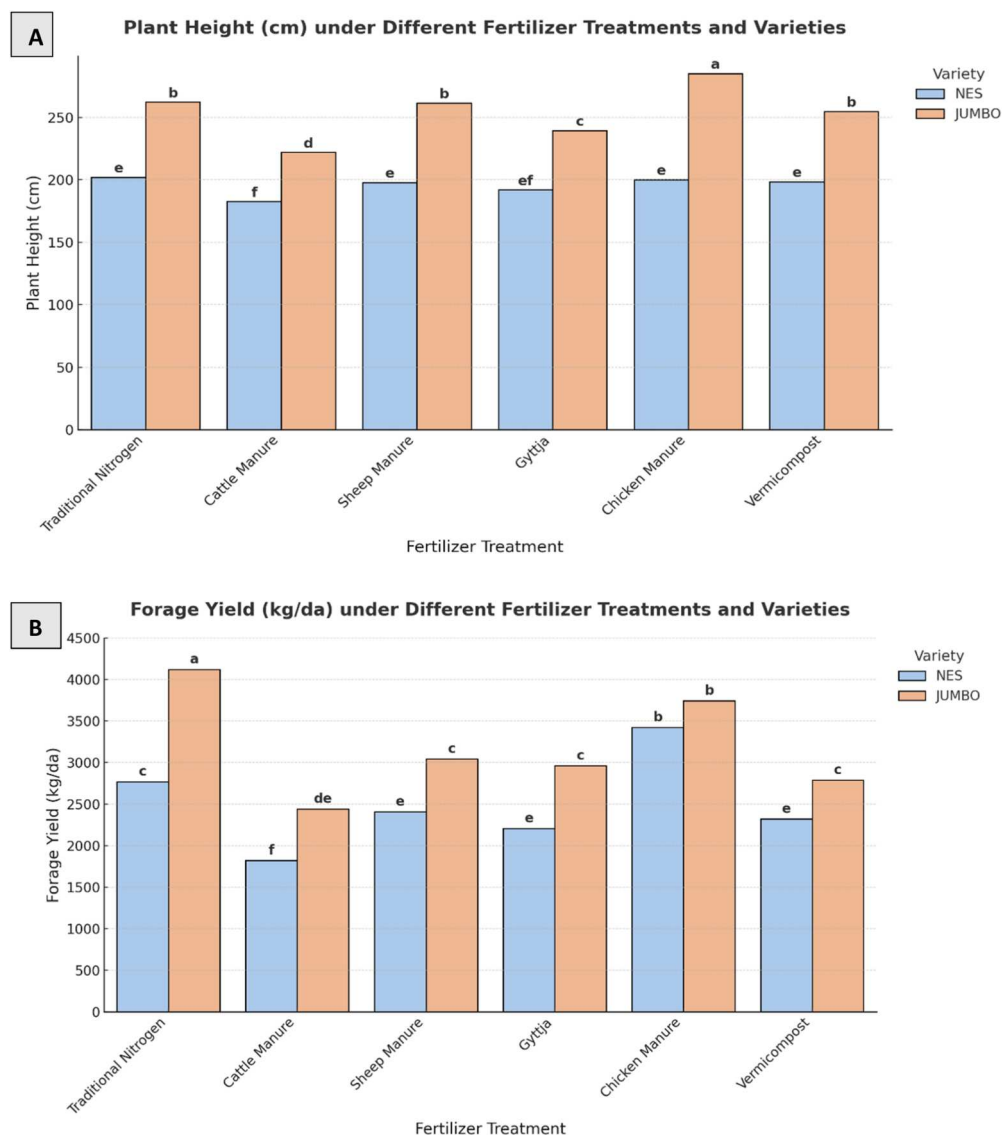
While the effect of year on fresh forage yield was not significant, the effects of cultivars ( $p < 0.01$ ), fertiliser ( $p < 0.01$ ),  $Y \times C$  interaction ( $p < 0.05$ ),  $Y \times F$ ,  $F \times C$ , and  $Y \times F \times C$  interaction ( $p < 0.01$ ) were significant (Table 2). Among the fertiliser treatments, the fresh forage yields varied between 2129.3 kg da<sup>-1</sup> and 3582.6 kg da<sup>-1</sup>. The highest fresh forage yield was obtained from chicken manure and traditional nitrogen application. In addition, sheep manure, gyttja organic material, and vermicompost gave statistically similar results. The lowest fresh forage yield was determined in cattle manure application parcels (Table 3). According to the  $F \times C$  interaction, the highest fresh forage yield was recorded in traditional nitrogen application in the Jumbo cultivar, while the lowest value was in cattle manure in the Nes cultivar (Figure 1B).

While the effect of year, cultivar,  $Y \times C$  interaction, and  $F \times C$  interaction on hay yield was not significant, the effects of fertiliser ( $p < 0.01$ ),  $Y \times F$  interaction ( $p < 0.05$ ), and  $Y \times$

**Table 3.** Plant length, stem thickness, the number of leaves, the leaf ratio in fresh forage, the stalk ratio in fresh forage value of years, cultivar and fertilisers.

	Plant length (cm)	The number of leaves	Fresh forage yield	Hay yield
Years				
2020	251.01a	12.75a	2914.09	758.69
2021	198.59b	10.79b	2756.85	709.31
Cultivars				
Nes	195.46b	10.09b	2756.85b	750.61
Jumbo	254.14a	13.45a	2914.09a	717.39
Fertilisers				
Traditional Nitrogen	232.05b	12.84a	3442.2a	898.03a
Cattle Manure	202.31d	10.67d	2129.3c	545.87c
Sheep Manure	229.66b	11.98ab	2723.0b	705.89b
Gyttja	215.64c	11.08cd	2581.6b	666.81b
Chicken Manure	242.57a	12.51a	3582.6a	940.35a
Vermicompost	226.60b	11.54bc	2554.2b	647.07b

Note: <sup>a-d</sup>Mean values with different superscripts have significant differences.



**Figure 1. A,** Plant height changes of Cultivar  $\times$  Fertiliser interactions; **B,** Fresh forage yield changes of Cultivar  $\times$  Fertiliser interactions.

F  $\times$  C interaction ( $p < 0.01$ ) were significant (Table 2). In the fertiliser treatment, the highest hay yield was obtained from chicken manure with  $940.35 \text{ kg da}^{-1}$  and from the traditional nitrogen application with  $898.03 \text{ kg da}^{-1}$ , which were statistically considered in the same group. Additionally, sheep manure, vermicompost manure, and gyttja organic fertiliser were statistically the same group. The lowest hay yield of  $545.87 \text{ kg da}^{-1}$  was obtained from cattle manure (Table 3). Fertiliser treatment among them showed that chicken manure showed related results to the traditional nitrogen application, while other organic fertilisers were statistically grouped among themselves. It was also observed that cattle manure resulted in the lowest hay yield.

### ***Silage fermentation and quality analysis***

According to the results of the statistical test (Table 4), there were significant effects of cultivars and  $Y \times C \times F$  interactions on DM ( $T_0$ ) ( $p < 0.01$ ). Among the cultivars, the highest DM ( $T_0$ ) value was obtained from Nes (%30.20). The DM ( $T_{60}$ ) value from 2021 is higher than the 2020 value. In addition, significant differences were found among cultivars with respect to DM content in the resulting silage ( $T_{60}$ ).

The effects of year, cultivars, and the  $Y \times C$  interaction on the pH of initial silages were significant (Table 4). Results showed that organic fertiliser applications do not affect the initial pH of silage, and the silages presented similar pH values. While the effect of  $Y \times C$ ,  $Y \times F$ ,  $F \times C$ , and  $Y \times C \times F$  on pH ( $T_{60}$ ) is insignificant, the effects of year ( $p < 0.01$ ), cultivars ( $p < 0.01$ ), and fertilisers ( $p < 0.01$ ) were significant (Table 4). The highest pH was obtained from the Jumbo cultivar with 4.33, while the lowest was found from the Nes cultivar with 4.07.

The highest lactic acid bacteria count was obtained from the Jumbo cultivar with 4.71 ( $\log_{10}$  cfu/g resulting silage), while the lowest was found from the Nes cultivar with 3.52 ( $\log_{10}$  cfu/g resulting silage). The count of lactic acid bacteria was similar among organic fertiliser treatments in resulting silage. In addition, a higher number of LAB in 2021 may be associated with a higher DM content. In  $F \times C$  interactions, the highest lactic acid bacteria count was recorded in traditional nitrogen, gytja, and vermicompost in the Jumbo cultivar, while the lowest value was in vermicompost manure in the Nes cultivar (Figure 2A).

While the effect of year, cultivar,  $Y \times C$  interaction, fertilisers,  $F \times C$ , and  $Y \times F \times C$  interaction on enterobacteria count was significant, the effects of  $Y \times F$  interaction were not significant (Table 4). In the fertiliser treatment, the highest enterobacteria count was obtained from chicken manure and traditional nitrogen application, which were statistically considered in the same group. The lowest enterobacteria count was obtained from cattle manure (Table 5). On the other hand, other organic fertilisers have been found to reduce the count of enterobacteria found in the natural flora of plants. Among the cultivars, the highest enterobacteria count ( $T_0$ ) was obtained from Jumbo (6.79  $\log_{10}$  cfu/g fresh material). In  $Y \times C$  interaction, the highest enterobacteria count was obtained in Jumbo in 2020, and the lowest enterobacteria count was obtained in Nes in 2021 (Figure 2B). Enterobacteria were not detected in the end of fermentation.

It was determined to be 4.17 ( $\log_{10}$  cfu/g fresh material) in the Nes variety and 4.27 ( $\log_{10}$  cfu/g fresh material) in the Jumbo variety. In the fertiliser treatment, the highest mold count was obtained from chicken manure, and the lowest count of mold was determined in parcels used for cattle manure and sheep manure, which were statistically considered in the same group. In the  $F \times C$  interaction, the highest mold count was recorded in chicken manure in the Jumbo cultivar, while the lowest value was in cattle manure in the Nes cultivar (Figure 3A).

Mold were not detected in end of fermentation. While the effect of year and cultivar on yeast count were significant, the effects of fertilizers,  $Y \times C$ ,  $Y \times F$ ,  $F \times C$  and  $Y \times C \times F$  interaction were not significant (Table 4). According to the varieties, the number of yeast in silages varied between 2.38 and 3.16 ( $\log_{10}$  cfu/g resulting silage), and the highest number was obtained from the Jumbo variety. In Figure 3B, the highest yeast count was recorded in traditional nitrogen, cattle manure and vermicompost in Jumbo cultivar, while the lowest value was in chicken manure in Nes cultivar.

The chemical composition of the resulting silage are given in Table 6, Table 7. The percentage of CP increased ( $p < 0.05$ ) with organic fertilisers, ranging from 4.15% to

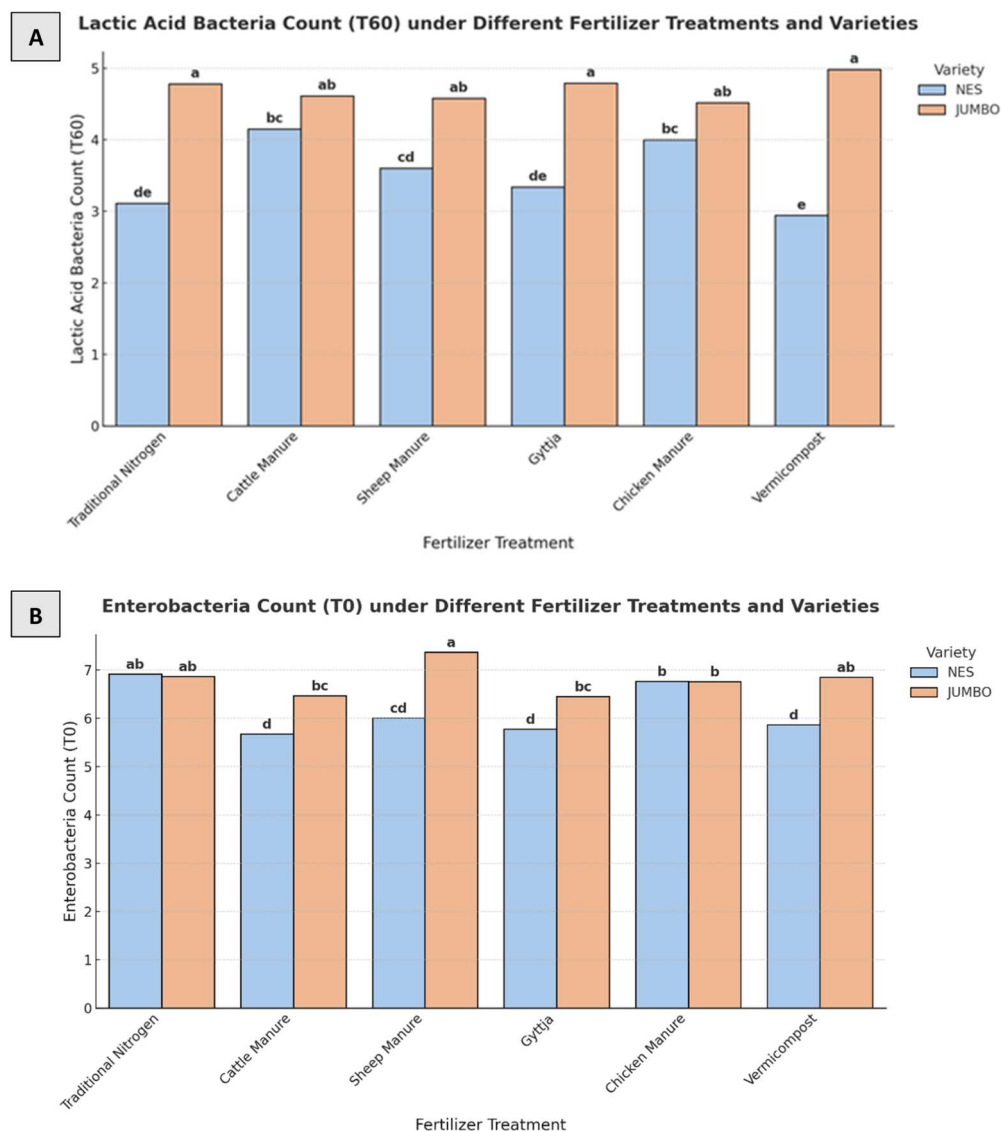


**Table 4.** Statistical analysis results of investigated characteristics in the present study.

	DM ( $T_0$ )	DM ( $T_{60}$ )	pH ( $T_0$ )	pH ( $T_{60}$ )	Lactic acid bacteria ( $T_0$ )	Lactic acid bacteria ( $T_{60}$ )	Enterobacteria ( $T_0$ )	Mold ( $T_0$ )	Yeast ( $T_0$ )	Yeast ( $T_{60}$ )
Years (Y) LSD (0.05)	ns	1.01*	0.04**	0.08**	ns	0.24*	0.22*	0.24*	0.20**	ns
Cultivars (C) LSD (0.05)	**	1.01**	0.02**	0.08**	ns	0.24**	0.22**	ns	0.20*	0.25*
Y $\times$ C LSD (0.05)	ns	ns	0.06**	ns	ns	ns	0.30*	ns	ns	ns
Fertilisers (F) LSD (0.05)	ns	ns	ns	0.14**	ns	ns	0.38**	0.42*	ns	ns
Y $\times$ F LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
F $\times$ C LSD (0.05)	ns	ns	ns	ns	ns	0.61**	0.53**	0.61*	ns	0.61**
Y $\times$ C $\times$ F LSD(0.05)	2.90**	3.49**	ns	ns	ns	ns	0.75**	ns	ns	ns

\*\* $P < 0.01$ ; \* $P < 0.05$ ; ns: non-significant.





**Figure 2.** **A**, Lactic acid bacteria (T<sub>60</sub>) count of Cultivar × Fertilisers interactions, **B**, Enterobacteria count of (T<sub>60</sub>) Cultivar × Fertilisers interactions.

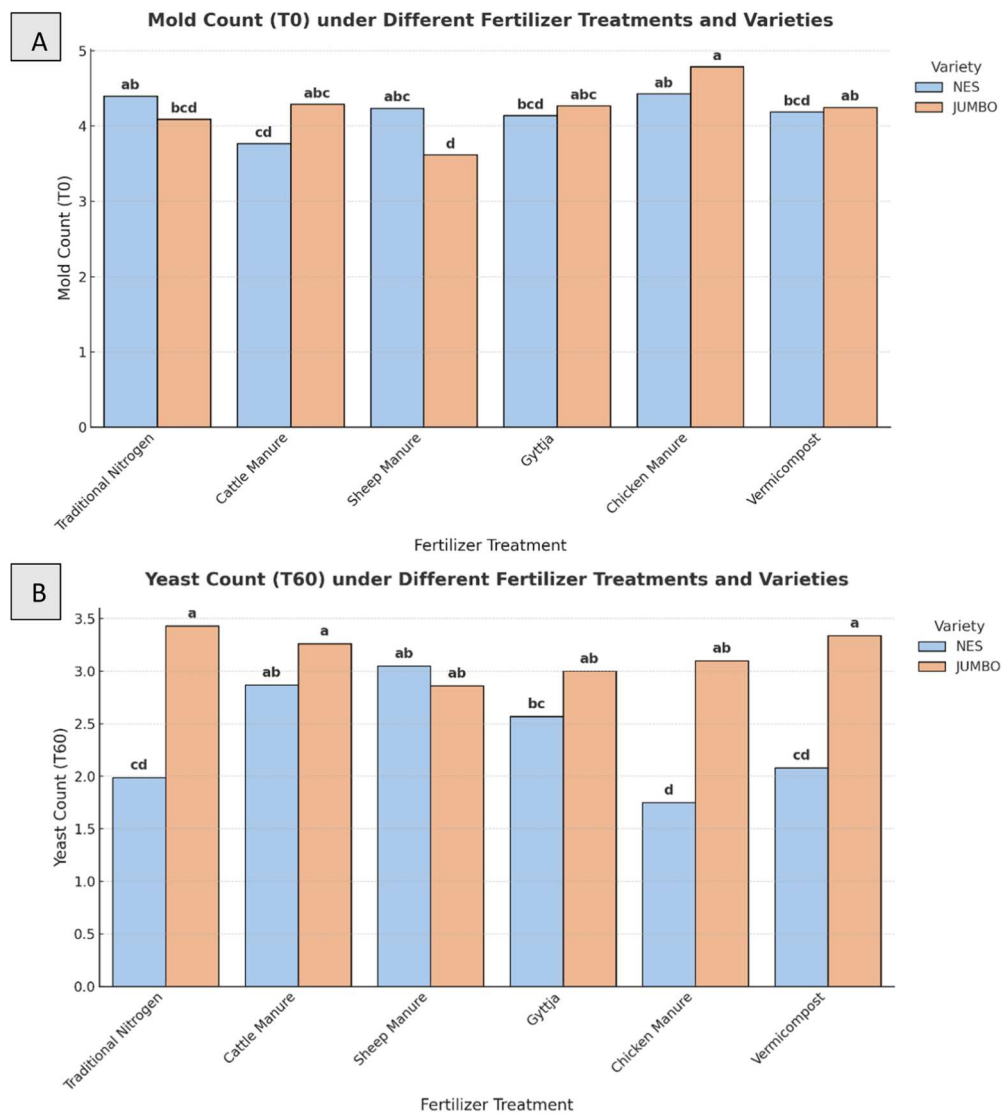
6.55% (Table 7). This linearly increasing CP content in the silage is explained by the fact that chicken manure. In addition, was no difference between the CP values of the sorghum varieties, but there is a statistically significant difference between OM and ash content (Table 7).

The lowest NDF and ADF content has been detected in the Nes cultivar. While the effect of year, cultivar and  $Y \times C \times F$  interaction on CT content were significant, the effects of fertilisers,  $Y \times C$ ,  $Y \times F$  and  $F \times C$  were not significant (Table 6). According to the varieties, condense tannin content varied between 0.64% and 1.31%, and the highest CT content was obtained from the Nes variety.

**Table 5.** DM content ( $T_0$ ,  $T_{60}$ ), pH value ( $T_0$ ,  $T_{60}$ ), LAB count ( $T_0$ ,  $T_{60}$ ), enterobacteria count ( $T_0$ ,  $T_{60}$ ), Mold count ( $T_0$ ) and yeast count ( $T_0$ ,  $T_{60}$ ) value of years, cultivar and fertilisers.

	DM ( $T_0$ )	DM ( $T_{60}$ )	pH ( $T_0$ )	pH ( $T_{60}$ )	LAB ( $T_0$ )	LAB ( $T_{60}$ )	Enterobacteria ( $T_0$ )	Mold ( $T_0$ )	Yeast ( $T_0$ )	Yeast ( $T_{60}$ )
Years										
2020	26.63	23.58b	5.56a	4.33a	4.08	3.98b	6.73a	4.10b	4.79b	2.72
2021	26.16	25.63a	5.63b	4.07b	3.98	4.25a	6.24b	4.34a	5.11a	2.82
Cultivars										
Nes	30.20a	27.81a	5.56a	4.10b	4.00	3.52b	6.17b	4.17	4.90b	2.38b
Jumbo	22.50b	21.40b	5.53b	4.29a	4.06	4.71a	6.79a	4.27	5.00a	3.16a
Fertilisers										
TraditionalNitrogen	26.95	26.15	5.57	4.17b	4.09	3.95	6.90a	4.25ab	5.16	2.71
Cattle Manure	26.16	23.71	5.56	4.21b	3.95	4.38	6.08c	4.03b	4.81	3.06
Sheep Manure	26.46	24.10	5.55	4.37a	4.14	4.09	6.69ab	3.93b	5.14	2.96
Gyttja	26.32	24.43	5.52	4.14b	4.00	4.06	6.11c	4.21ab	4.87	2.78
ChickenManure	26.60	25.22	5.54	4.18b	4.03	4.26	6.74a	4.61a	4.78	2.42
Vermicompost	25.90	24.02	5.53	4.13b	3.99	3.96	6.36bc	4.32ab	4.94	2.71

\*\* $P < 0.01$ . <sup>a,b,c</sup>Means within a row with different letters differ by LSD's test. DM ( $T_0$ ): Dry matter in fresh material, DM ( $T_{60}$ ): dry matter in maturing silage, LAB( $T_0$ ): lactic acid bacteria count in fresh material, LAB( $T_{60}$ ): lactic acid bacteria count in maturing silage.



**Figure 3.** A, Mold count (T<sub>0</sub>) of Cultivar × Fertilisers interactions, B, Yeast count of (T<sub>60</sub>) Cultivar × Fertilisers interactions.

**Table 6.** Statistical analysis results of investigated forage nutrition composition in the present study.

	CP	OM	Ash	NDF	ADF	CT
Years (Y) LSD (0.05)	ns	0.41**	0.40**	8.97 **	ns	8.97**
Cultivars (C) LSD (0.05)	ns	0.41**	0.40**	63.05**	2.61**	63.05**
Y × C LSD (0.05)	0.77**	ns	ns	1.33**	ns	ns
Fertilisers (F) LSD (0.05)	0.63**	ns	ns	ns	ns	ns
Y × F LSD (0.05)	ns	ns	ns	ns	ns	ns
F × C LSD (0.05)	1.09**	ns	ns	ns	ns	ns
Y × C × F LSD (0.05)	ns	ns	ns	ns	ns	3.07*

\*\* $P < 0.01$ . <sup>a,b,c</sup> Means within a row with different letters differ by LSD's test. ns: non-significant CP: crude protein ratio, OM: organic matter ratio, Ash: crude ash ratio, NDF: neutral detergent fibre, ADF: acid detergent fibre, CT: condensed tannin.

**Table 7.** CP, OM, Ash, NDF, ADF, CT (%) content of silage sorghum ( $T_{60}$ ).

	CP	OM	Ash	NDF	ADF	CT
Years						
2020	5.08	90.25b	9.75a	60.43b	35.22	1.09a
2021	4.89	91.29b	8.71b	65.72a	37.24	0.85b
Cultivars						
Nes	4.82	92.13a	7.87b	59.20b	32.67b	1.31a
Jumbo	5.19	89.41b	10.59a	66.94a	39.78a	0.64b
Fertilisers						
Traditional Nitrogen	5.06b	91.02	8.99	63.72	35.69	1.08
Cattle Manure	4.53bc	90.84	9.17	63.96	36.26	1.14
Sheep Manure	4.55bc	90.36	9.64	62.40	34.97	0.83
Gyttja	5.06bc	90.72	9.28	62.76	36.36	1.15
Chicken Manure	6.55a	91.02	8.98	62.53	37.19	0.86
Vermicompost	4.15c	90.66	9.34	62.97	36.92	0.79

Notes: CP: crude protein ratio, OM: organic matter ratio, Ash: crude ash ratio, NDF: neutral detergent fibre, ADF: acid detergent fibre, CT: condense tannin.

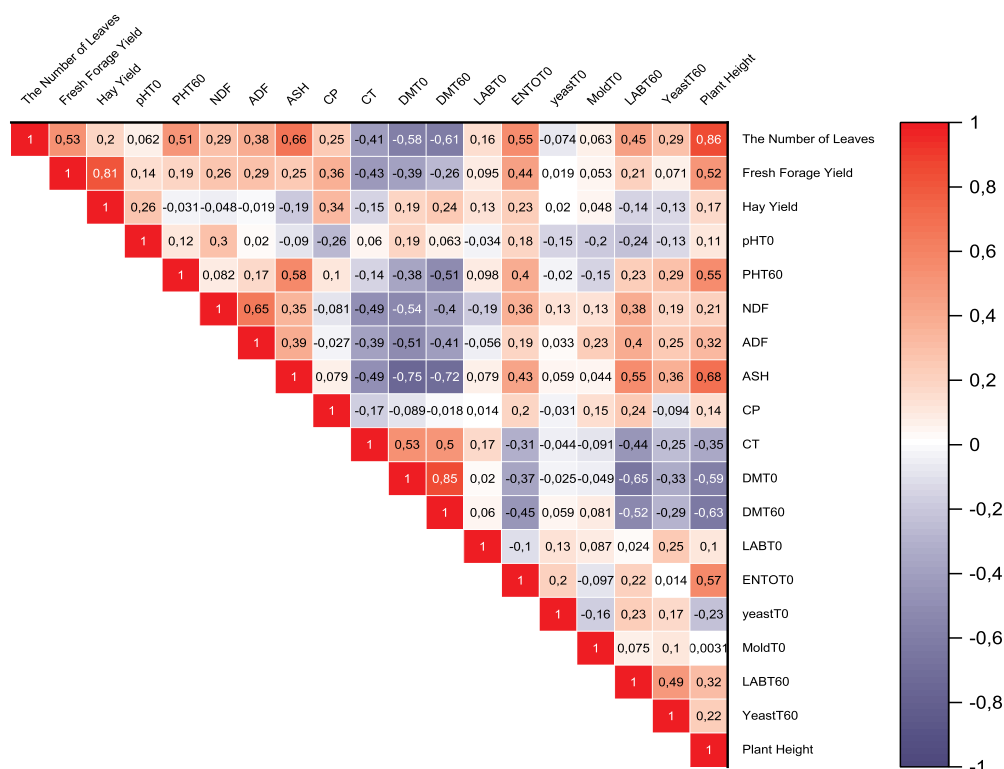
### Correlation relationship between character

The plant height variable has a direct, positive effect with the same correlation sign with 5 different variables, such as the number of leaves, fresh forage yield, pH ( $T_{60}$ ), crude ash content, and enterobacteria count ( $T_0$ ). There was a positive and strong correlation between the number of leaves and fresh forage yield, hay yield, pH ( $T_{60}$ ), crude ash count, enterobacteria count, and lactic acid bacteria count ( $T_{60}$ ). In addition, there was a positive correlation between the number of leaves and NDF, ADF, crude protein ratio, yeast count ( $T_{60}$ ), and LAB count ( $T_0$ ). At the same time, there is a negative and strong relationship between the number of leaves and dry matter rates.

Explanatory variables are the main determinants of changes in the main variable. As a result, it is possible to estimate the efficiency of indirect selection. As a result, it is possible to estimate the efficiency of indirect selection. In this case, the number of leaves and the number of LAB with DM stand out as the most associated variables. These variables are of great importance when it is desired to get answers related to LAB. In other words, while there was a positive relationship between DM ( $T_0$ ) and lactic acid bacteria count ( $T_0$ ), a negative relationship was observed between enterobacteria count ( $T_0$ ), yeast count, and mold count. Therefore, increasing dry matter content will increase the number of desired microorganisms in silage and decrease the number of undesired microorganisms. There is a negative and strong relationship between the pH variable and DM; therefore, a decrease in pH value will be observed when the dry matter is high. It was determined that there was a negative relationship between the pH of fresh material and the number of microorganisms (Figure 4).

### Discussion

Chicken manure increased plant height from 232.05 cm (traditional application) to 242.57 cm, while sheep manure and vermicompost had no effect on plant height. Similarly, Khaliq et al. (2006) reported that the application of a mixture of chicken manure and urea showed the best performance of all treatments applied. Furthermore, Agbede et al. (2008) reported that the application of chicken manure significantly increased plant height. Through the utilisation of gyttja and cattle manure, a decrease in plant



**Figure 4.** Correlation relationship between character. DM(T<sub>0</sub>): Dry matter of fresh material, DM(T<sub>60</sub>): dry matter of mature silages, Ash: ash ratio, CP: crude protein ratio, LAB: lactic acid bacteria count, Ento: enterobacteria count, NDF: neutral detergent fibre, ADF: acid detergent fibre, CT: condense tannin.

height has been observed. Particularly, the lowest plant height was attained through the application of cattle manure. Plant height is a significant trait that exerts an influential impact on yield in forage plants cultivated with the aim of achieving optimal productivity and facilitating silage production (Geren and Kavut 2009). It has been reported by some researchers that it may vary according to varieties (Güney et al. 2010; Karadağ and Özkurt 2014). Acar et al. (2002) determined the plant height of the Jumbo cultivar as 231 cm in conditions of Konya. One of the main reasons for this difference may be the different sorghum plant varieties used in the study. Additionally, cultural and environmental factors such as ecological conditions, nitrogen doses, and irrigation factors can be associated with the variations observed between the studies.

In the study, it was found that chicken manure is more beneficial for sorghum and the sorghum-sudangrass hybrid, and it provides a similar yield in terms of leaf number compared to traditional nitrogen applications. Similarly, Amujoyegbe et al. (2007) and Agbede et al. (2008) reported that application of chicken manure increases the leaf area and improves morphological plant characteristics of forage sorghum. Although significant main effects of year, cultivar, and fertiliser application on leaf number were observed, their interactions were not statistically significant. This may be attributed to the inherent genetic stability of the sorghum cultivars (NES and Jumbo), which likely

limited their responsiveness to variations in environmental conditions and fertiliser treatments. Moreover, the relatively consistent environmental conditions during the study period may have further minimised interaction effects.

The highest fresh forage yield was obtained from the Jumbo cultivar and applied with chicken manure and traditional nitrogen fertilisers. Similarly, Delate and Combardella (2000) reported that the forage yield obtained from organic fertilisers grown maize plants is higher compared to the traditional nitrogen application. Khaliq et al. (2000) found that the application of chicken manure and urea mixture showed the best performance among all fertiliser (cattle manure, chicken manure, and urea mixture) applications. Spargo et al. (2016) reported that the application of chicken litter fertilisers provides significant economic benefits. In the fertiliser treatment, the highest hay yield was obtained from chicken manure and from the traditional nitrogen application. Additionally, sheep manure, vermicompost manure, and gyttja organic fertiliser were statistically the same group. The reason for obtaining it from chicken manure can be attributed to the pellet structure of chicken manure, which requires a longer time for its dissolution compared to other organic fertilisers. The higher hay and forage yields observed under chicken manure application can be explained by the slow release of essential nutrients, notably nitrogen, throughout the growing season, supporting prolonged vegetative growth. Furthermore, the positive effects of chicken manure on soil structure, organic matter content, and microbial biomass likely enhanced nutrient availability and water retention, leading to improved biomass accumulation compared to conventional nitrogen fertiliser. Similarly, Nazlı (2011) reported that organic fertilisers are slow-release fertilisers, and approximately 50% of the nitrogen in the waste can become available to the plant in the first season. Therefore, it should be noted that the remaining portion will become suitable for the next crop and should not be overlooked. However, it has been determined that there is an approximate loss of  $395 \text{ kg da}^{-1}$  in hay yield in the area where cattle manure is applied instead of chicken manure. This loss is significant both for livestock enterprises and operational costs. It was also observed that cattle manure resulted in the lowest hay yield. Similarly, Khan et al. (2008) noted that in maize cultivation, the use of three times more cattle manure yielded lower productivity compared to  $10 \text{ t ha}^{-1}$  poultry manure. Basso et al. (2017) stated that instead of NPK usage, pig compost and chicken manure can be used as alternative organic fertilisers. However, Lim et al. (2010) determined that the highest dry matter yield in sorghum  $\times$  sudangrass plants was obtained through chemical fertiliser application. Arslan found that high yields cannot be achieved with organic fertilisers. The cultivar of plants, the sowing rate, the fertiliser dosage, and the fertiliser source can be associated with the differences observed among the findings.

The DM important value is for forage productivity. McDonald et al. (1991) reported that the high moisture content of fresh material is considered to increase fermentation of undesirable microorganisms, especially clostridium, which would result in the nutrition loss of effluent and spoilage during the ensiling process. Açıkgöz (1995) impressed that a high-quality silage should have a dry material ratio of 23.5%. Although the DM content of Nes silage sorghum was at the desired level, it was determined that the DM content of Jumbo silage  $\times$  sudanotu hybrid was low. The DM content related to the vegetation period. When sorghum and maize plants are harvested in early periods of vegetation, it is known that the plants have a high water content, low carbohydrate and DM content,

and with the progress of harvest time, the rate of DM is also expected to increase (Şahan, 2017). The highest DM ( $T_0$ ,  $T_{60}$ ) value was obtained from the Nes cultivator, which implies that silage can be made easier. Akbay et al. (2023) reported that the content of DM can affect the initial pH and resulting pH values of silages. Researchers impress that the initial pH increased with the increased content of DM, but high DM content contributed to the achievement of a lower pH value as a result of fermentation. On the other hand, maybe Nes cultivator has a high content of water-soluble carbohydrates (WSC), resulting in contributing to low pH. Results showed that organic fertiliser applications do not affect the initial pH of silage, and the silages presented similar pH values. However, the differences between the pH values of the sorghum varieties are statistically significant. Similarly, it has been determined that the initial pH of the Nes (5.56) species with a high DM is higher compared to the Jumbo (5.53) variety (Akbay et al., 2023). The highest pH was obtained from the Jumbo cultivar with 4.33, while the lowest was found from the Nes cultivar with 4.07. The pH ( $T_{60}$ ) value differed between fertiliser treatments and was generally lower for manure treatment than for the sheep manure and inorganic fertiliser treatments; this may also have affected the microorganism count. The lactic acid bacteria (LAB) are responsible for lowering the pH in silages and preserving the nutrient content for a long time, so LAB sees its key task for silage (Ávila and Carvalho 2020). LAB is found in epiphytic flora on plant material. However, its main source is soil (Kızılışımşek et al. 2016), so any application to the soil can change the density of the microorganism. This can also directly affect the direction of fermentation, the number of microorganisms, and microorganism variety. Pahlow et al. (2003) reported that the plant type of the amount of lactic acid bacteria and that the plant may change according to the state of maturity, and were determined corn and maize between  $10^1$  and  $10^7$  cfu/g fresh material. The count of lactic acid bacteria was similar among organic fertiliser treatments in resulting silage. In addition, a higher number of LAB in 2021 may be associated with a higher DM content. da Silva et al. (2017) reported that advancing crop maturity results in increases in DM, carbohydrates, and LAB populations, as well as microorganism numbers. Özduven et al. (2009) found that the LAB count varied between 4.63 and 5.44  $\log_{10}$  cfu/g, resulting in silage of different maize varieties. Kaya and Polat (2010) reported that the LAB density of the maize varieties varied between 2.9-4.1  $\log_{10}$  cfu/g resulting in silage. At the beginning of the silage period, the highest number of yeast in the Nes variety is noticed. This condition is a bonding of the species Nes, so that the content of WSC is higher and provides the environment for the formation of lactic acid bacteria. This reduces the number of undesirable microorganisms. In addition, Müller (2009) impressed that above 40% DM, reduction in water activity could reduce the growth of yeast. Yeasts convert sugars and lactic acid basically to ethanol, resulting in losses of DM and nutrients. Moreover, a smaller amount of yeasts lowers the risks of aerobic spoilage, because these microorganisms have been shown to be initiators of this process (McDonald et al. 1991). Kızılışımşek et al. (2016) found that according to the opening time and isolations of silage, the number of yeast varied between 1.79-5.99  $\log_{10}$  cfu/g. Koç et al. (2018) informed us that the number of yeasts in corn silages varied between 4.29 and 5.60  $\log_{10}$  cfu/g. The difference between the results of silage construction, technique, and the plant used from their species.

This linearly increasing CP content in the silage is explained by the fact that chicken manure. In addition, there was no difference between the CP values of the sorghum

varieties, but there is a statistically significant difference between OM and ash content. Jumbo cultivars presented higher ash content, probably because of the higher ratio of leaf to stem. Akbay et al. (2020) reported that changes in ash content may also be due to the change in proportion of leaf to stem ratio. Researchers also reported that ash content decreased with increasing maturity. Therefore, the decrease in the amount of ash in 2021 can be associated with the DM content. The lowest NDF and ADF content has been detected in the Nes cultivar. DM has a significant effect on the digestibility of silage nutrients, especially non-volatile nitrogen compounds and raw fibre (Podkowska 1995). In fact, increased DM content has also been shown in many studies to reduce digestibility. Podkowska et al. (2001) found that the highest digestibility in the plant occurs when the percentage of DM reaches 30%, and the digestibility decreases due to the delay in harvesting. Despite the fact that the DM content of the Nes is higher than that of the Jumbo, it has been determined that the NDF and ADF content is lower than the Nes. Decreasing NDF and ADF in the Nes sorghum variety can be associated with swing bonding. Similarly, Filya (2004) reported that NDF content decreased with maturity in the whole crop maize, which was associated with an increase in ADL and a decrease in CP contents. Filya (2001) showed that lower NDF led to higher DM and OM degradability. Because of these characteristics, it can be said that the Nes variety is advantageous.

According to the varieties, condensed tannin content varied between 0.64% and 1.31%, and the highest CT content was obtained from the Nes variety. Tannins might be associated with adverse effects as an anti-nutritional factor, causing lower dry matter intake and reduced digestion of protein and fibre. The low amount of tannins has an inhibitory effect on swelling in animals, so feed sources containing condensed tannins and tannins can be prevented from the economic losses caused by parasites by participating in the diet in certain proportions. Therefore, it is stated that the TMR to be included in the ration should be known as the contents (Kamalak et al. 2005). It is known that between 2% and 3% of the CT in the forage prevents swelling in ruminant animals, while the level of CT above 5% has a toxic effect, which reduces the consumption of feed and, consequently, the digestibility of protein (Barry and Blaney 1987). Nes and Jumbo silage sorghum varieties can be said to have CT content within acceptable limits and can be used in ruminant animal nutrition.

## Conclusions

According to the research results, the highest plant height, fresh forage yield, and hay yield were obtained from the application of chicken manure. Similar results were obtained between organic fertilisers and traditional nitrogen application (NPK) in terms of stem ratio. However, when it comes to leaf ratio, lower values were obtained with the use of organic fertilisers compared to the traditional nitrogen application (NPK). It has been determined that chicken manure stands out more than other organic fertiliser treatments for silage sorghum cultivation, but it cannot provide sufficient nitrogen for the cluster ratio of the plant. It has been determined that there are significant differences in the varieties in terms of microflora numbers, fermentation properties, and silage quality, and the Nes variety stands out with its high dry matter content and low pH value. Additionally, it was determined that there was no difference



between the CP values of the varieties, but the Nes variety had lower NDF and ADF values. For this reason, it was determined that the Nes variety was more prominent in terms of silage fermentation and silage quality.

In this context, increasing doses of chicken manure should be investigated for silage sorghum plants. Additionally, a combination of chicken manure and chemical fertiliser can be applied, or chicken manure doses can be split and applied according to the growth stages of the plant. According to the obtained results, it has been determined that the Jumbo cultivar is more productive than the Nes cultivar. The Jumbo cultivar is recommended for Kahramanmaraş province and similar ecological conditions. Future studies should be conducted under varying climatic and soil conditions, including economic feasibility assessments, to validate and expand upon the current findings and support region-specific fertiliser recommendations for sorghum cultivation.

## Disclosure statement

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

## References

- Acar R, Akbudak MA, Sade B. 2002. The determination of yield and some yield components of sorghum-sudangrass hybrid cultivars for silage production in Konya ecological conditions. *Journal of Agriculture Faculty of Selçuk University*. 16(29):88–95.
- Açıkgöz E. 1995. Forage crops. Bursa (TR): Uludağ University, Faculty of Agriculture Publications.
- Agbede TM, Ojeniyi SO, Adeyemo AJ. 2008. Effect of poultry manure on soil physical and chemical properties, growth and grain yield of sorghum in Southwest, Nigeria. *American-Eurasian Journal of Sustainable Agriculture*. 2(1):72–77.
- Akbay F, Günaydin T, Arıkan S, Kızılsımsek M. 2023. Performance of new lactic acid bacteria strains as inoculants on the microorganism composition during fermentation of alfalfa silage containing different dry matter content. *Black Sea Journal of Agriculture*. 6(4):402–410.
- Akbay F, Kamalak A, Erol A. 2020. The effect of vegetative periods of faselya (*Phacelia tanacetifolia* Benth) on hay yield, nutrient content and methane production. *KSU Journal of Agriculture and Nature*. 23(3):981–985.
- Amujoyegbe BJ, Opabode JT, Olayinka A. 2007. Effect of organic and inorganic fertilizer on yield and chlorophyll content of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench). *African Journal of Biotechnology*. 6(16):1869–1873. doi:10.5897/AJB2007.000-2278.
- AOAC. 1990. Official method of analysis, 15th ed. Washington, DC, USA: Association of Official Analytical Chemists.
- Ávila CLS, Carvalho BF. 2020. Silage fermentation—updates focusing on the performance of microorganisms. *Journal of Applied Microbiology*. 128:966–984.
- Barry TN, Blaney BJ. 1987. Secondary metabolites of forage legumes. In: Hacker J.B., editor. Nutritional limits to animal production from pastures. St Lucia (QLD): Commonwealth Scientific and Industrial Research Organization (CSIRO); p. 91–120.
- Basso CJ, Muraro DS, Girotto E, da Silva DRO, da Silva N. 2017. Poultry litter and swine compost as nutrients sources in millet. *Agricultural science*. 33(2):288–296.
- Chen SK, Edwards CA, Subtler S. 2001. Effects of the fungicides benaomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. *Soil Biology and Biochemistry*. 33(14):1971–1980.
- Cothren JT, Matocha JE, Clark LE. 2000. Integrated crop management for sorghum. In: Smith C.W., R.A. Frederiksen, editors. *Sorghum: origin, history, technology, and production*. New York (NY): John Wiley and Sons; p. 409–441.

- da Silva TC, da Silva LD, Santos EM, Oliveira JS, Perazzo AF. 2017. Importance of the fermentation to produce high-quality silage. In: Faustino JAF, editor. *Fermentation Processes*. Rijeka (HR): InTechOpen; p. 1–20.
- Delate K, Combardella C. 2000. Comparison of organic and conventional crops at the Nelly-Kinyon Long-Term Agroecological Research. Lova University. *Armstrong Research and Demonstration Farm Bulletin*. 7:208–210.
- Dunière L, Sindou J, Chaucheyras-Durand F, Chevallier I, Thévenot-Sergentet D. 2013. Silage processing and strategies to prevent persistence of undesirable microorganisms. *Animal Feed Science and Technology*. 182(1-4):1–15. doi:10.1016/j.anifeedsci.2013.04.006.
- Filya I. 2001. Silage fermentation. ARES Technical Bulletin I. Hakan Publications, Izmir, Turkey (in Turkish).
- Filya I. 2004. Nutritive value and aerobic stability of whole crop maize silage harvested at four stages of maturity. *Animal Feed Science and Technology*. 116(1-2):141–150. doi:10.1016/j.anifeedsci.2004.06.003.
- Geren H, Kavut YT. 2009. An investigation on comparison of Sorghum (*Sorghum* sp.) species with corn (*Zea mays* L.) grown under second crop production. *Journal of Agriculture Faculty of Ege University*. 46(1):9–16.
- Güney E, Tan M, Gül ZD, Gül İ. 2010. Determination of yield and silage quality of some maize cultivars in Erzurum conditions. *Journal of Agriculture Faculty of Atatürk University*. 41(2):105–111.
- Hui LI, Feng WT, He XH, Ping ZHU, Gao HJ, Nan SUN, XU MG. 2017. Chemical fertilizers could be completely replaced by manure to maintain high maize yield and soil organic carbon (SOC) when SOC reaches a threshold in the Northeast China Plain. *Journal of Integrative Agriculture*. 16(4):937–946. doi:10.1016/S2095-3119(16)61559-9.
- Islam SM, Gaihre YK, Islam MR, Ahmed MN, Akter M, Singh U, Sander BO. 2022. Mitigating greenhouse gas emissions from irrigated rice cultivation through improved fertilizer and water management. *Journal of Environmental Management*. 307:114520.
- Kamalak A, Canbolat Ö, Gürbüz Y, Özay O, Erer M, Özkan ÇÖ. 2005. A Study of the effect of condensed tannin on ruminant animals. *KSU Journal of Science and Engineering*. 8(1):132–137.
- Karadağ Y, Özkurt M. 2014. Effect of different row spacings on the yield and quality of silage sorghum (*Sorghum Bicolor* (L.) Moench) cultivars to be second crop grown. *Journal of Agriculture Faculty of Gaziosmanpaşa University*. 2014(1):19–24.
- Kaya Ö, Polat C. 2010. Determination of silage fermentation characteristics and feed value of some corn varieties cultivars as first and second crop in Tekirdag Ecological Conditions. *Journal of Tekirdag Agricultural Faculty*. 7(3):129–136.
- Khaliq A, Abbasi MK, Hussain T. 2006. Effects of integrated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan. *Bioresource Technology*. 97(8):967–972. doi:10.1016/j.biortech.2005.05.002.
- Khaliq T, Mahmood T, Kamal J, Masood A. 2000. Effectiveness of farmyard manure, poultry manure and nitrogen on corn (*Zea mays* L.) productivity. Faisalabad (PK): Department of Agronomy, University of Agriculture.
- Khan HZ, Malik MA, Saleem MF. 2008. Effect of rate and source of organic material on the production potential of spring maize (*Zea mays* L.). *Pakistan Journal of Agricultural Sciences*. 45(1):40–43.
- Kızılsimşek M, Öztürk Ç, Küsek M, Mokhtari NEP, Ertem P. 2016. Isolated and selected from Turkey's flora on fermentation profile and aerobic stability of corn silage. *Journal of Central Research Institute for Field Crops*. 25(2):278–284.
- Koç F, Özduven ML, Demirci AŞ, Şamlı HE. 2018. Evaluation of the changes in microbial composition of corn silage under farm conditions during aerobic stability using thermal camera imaging technique. *KSU Journal of Agriculture and Nature*. 21(2):167–174.
- Lim SS, Lee SM, Lee SH, Choi WJ. 2010. Dry matter yield and nutrients uptake of sorghum × sudangrass hybrid grown with different rates of livestock manure compost. *Korean Journal of Soil Science and Fertilizer*. 43(4):458–465.

- McDonald P, Henderson AR, Heron SJE. 1991. The biochemistry of silage, 2nd ed. Canterbury, UK: Chalcombe Publ.
- Müller CE. 2009. Influence of harvest date of primary growth on microbial flora of grass herbage and haylage, and on fermentation and aerobic stability of haylage conserved in laboratory silos. *Grass and Forage Science*. 64(3):328–338. doi:10.1111/j.1365-2494.2009.00695.x.
- Mullet J, Morishige D, McCormick R, Truong S, Hilley J, McKinley B, Rooney W. 2014. Energy Sorghum—a genetic model for the design of C4 grass bioenergy crops. *Journal of Experimental Botany*. 65(13):3479–3489. doi:10.1093/jxb/eru229.
- Nazlı Rİ. 2011. Use of possibilities of some organic residues in sorghum × sudangrass hybrid (*Sorghum bicolor* × *Sorghum bicolor* var. *sudanense*) cultivation [master's thesis]. Adana (TR): Çukurova University, Institute of Science and Technology; p. 84.
- Newman Y, Erickson J, Vermerris W, Wright D. 2013. Forage sorghum (*Sorghum bicolor*): overview and management. Gainesville (FL): University of Florida, Institute of Food and Agricultural Sciences (IFAS). EDis Publication No.: AG343. [accessed 2025 May 10]. <https://edis.ifas.ufl.edu/publication/AG343>.
- Oagile D, Namasiku M. 2010. Chicken manure-enhanced soil fertility and productivity: Effects of application rates. *Journal of Soil Science and Environmental Management*. 1(3):46–54.
- Özdüven ML, Koç F, Başkavak S, Polat C, Şamlı HE, Çoşkuntuna L. 2009. Effects of fermentation characteristics and feed value of some maize cultivars ensiled at different stages of maturity. *Journal of Tekirdag Agricultural Faculty*. 6(2):121–129.
- Pahlow G, Muck RE, Driehuis F, Elferink O, Spoelstra SJWH, F S. 2003. Microbiology of ensiling. Madison, WI: Silage Science and Technology. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.
- Podkówka Z. 1995. The effect of maize forage fragmentation for nutritive value. *Kukurydza*. 2:19. (in Polish).
- Podkówka Z, Čermák B, Podkówka L. 2001. The influence of harvest time of silage maize on digestibility of organic mass. *Annals of Warsaw Agricultural University – SGGW, Animal Science*. 39:20–23.
- Robertson GP. 2015. A sustainable agriculture? *Daedalus*. 144(4):76–89. doi:10.1162/DAED\_a\_00355.
- Sağlamtimur T, Tansı V, Baytekin H. 1998. Yem Bitkileri Yetiştirme. Çukurova Üniversitesi Ziraat Fakültesi Ders Kitabı No:74. Adana.
- Saltalı K. 2015. Use of gyttja to improve soil quality in agriculture. Proceedings of the Natural Nutrition and Lifelong Health Summit; 2015 May 20-23; Bilecik, Turkey. Abstract Book.
- Soyergin S. 2003. Preservation of soil fertility, fertilizers and organic soil amendments in organic farming. Yalova: Atatürk Central Horticultural Research Institute.
- Spargo JT, Cavigelli MA, Mirsky SB, Meisinger JJ, Ackroyd VJ. 2016. Organic supplemental nitrogen sources for field corn production after a hairy vetch cover crop. *Agronomy Journal*. 108(5):1992–2002. doi:10.2134/agronj2015.0485.
- Tiryaki İ. 2005. Sorghum: genetic origin, utilization, cultivation techniques and biotechnological advances. *KSU Journal of Science and Engineering*. 8(1):84–90.
- Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.
- von Caemmerer S, Furbank RT. 2016. Strategies for improving C4 photosynthesis. *Current Opinion in Plant Biology*. 31:125–134. doi:10.1016/j.pbi.2016.04.003.
- Xu L, Yan D, Ren X, Wei Y, Zhou J, Zhao H, Liang M. 2016. Vermicompost improves the physiological and biochemical responses of blessed thistle (*Silybum marianum* Gaertn.) and peppermint (*Mentha haplocalyx* Briq) to salinity stress. *Industrial Crops and Products*. 94:574–585. doi:10.1016/j.indcrop.2016.09.023.

# AKADEMİK PERFORMANS DEĞERLENDİRME RAPORU

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# Effect of inoculations with different lactic acid bacteria on the fermentation profile and quality of high-moisture fodder pea (*Pisum sativum* L.) silage

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**ABSTRACT.** One of the efficient techniques for preserving high moisture forages is ensiling. However, the successful ensiling progress of legumes largely depends on the epiphytic microbial flora, buffering capacity, and the water soluble carbohydrate content of the ensiled forage. In this study, three selected strains of lactic acid bacteria (LAB), were used as microbial additives (at  $10^6$  CFU/g fresh matter) to fodder pea (*Pisum sativum* L.). These strains included *Lactobacillus bifermentans* (LS-65-2-2) and *Lactobacillus plantarum* (LS-72-2), both isolated from rangelands in Türkiye, along with *Bacillus subtilis*, which is already applied for these purposes. The aim was to assess the effects of these strains on microbial composition and the quality of the resulting silage. Silage opening was conducted at five time points (on days 0, 2, 5, 7 and 45) with three replicates. The effects of LAB inoculations were determined to be statistically different ( $P < 0.001$ ). The study results demonstrated the following values of the parameters tested: pH (4.52–4.86), lactic acid bacteria ( $5.51\text{--}8.46 \log_{10}$  CFU/g silage), enterobacteria ( $2.24\text{--}3.61 \log_{10}$  CFU/g silage), yeasts ( $6.20\text{--}7.03 \log_{10}$  CFU/g silage), neutral detergent fibre (38.85–41.93%), acid detergent fibre (ADF, 32.91–35.75%), and relative feed value (RFV, 135.90–151.73). LAB inoculations caused a significant decrease in pH and an increase in dry matter (DM) recovery ( $P < 0.001$ ) in fodder pea silage compared to the control. The abundance of LAB in the silages increased significantly ( $P < 0.001$ ), while the content of enterobacteria ( $P < 0.001$ ), pH,  $\text{NH}_3\text{-N}$  ( $P < 0.01$ ) and ADF ( $P < 0.05$ ) in inoculated silages decreased. The RFV significantly improved following inoculation with the *L. bifermentans* strain. Overall, the addition of LAB strains improved the fermentation process and silage quality compared to *B. subtilis*, as well as enhanced DM recovery and reduced silage pH.

## Introduction

Silage is an important method of preserving highmoisture forage crops, significantly reducing quality and nutrient losses compared to haymaking. Silage quality largely depends on the flora of epiphytic microorganisms present on the forage, such as lactic acid bacteria, enterobacteria, moulds,

and yeasts. Lactic acid bacteria convert water-soluble carbohydrates (WSC) into lactic acid, which facilitates a rapid decrease in silage pH. Fodder pea (*Pisum sativum* L.) is valued as a forage legume for its high protein content compared to many other forage crops (Blagojević et al., 2017). However, it is difficult to ensile due to its relatively low WSC content (Canpolat et al., 2019), high buffering

capacity (Fraser et al., 2001), and low DM content at harvest. The use of microbial inoculants has the potential to improve silage quality prepared from fodder pea plant. These inoculants can alter many silage quality parameters, although the magnitude of their effects on fermentation profiles depends heavily on the characteristics of the strains used (Ertekin and Kizilsimsek, 2020; Günaydin et al., 2023; Akbay et al., 2023a,b). Lactic acid bacteria (LAB) strains have been classified into homofermentative and heterofermentative based on their physiological characteristics. Homo-LAB strains, such as *Lactobacillus*, *Enterococcus* and *Pediococcus* are widely used as silage inoculants due to their rapid and efficient production of lactic acid (2 mol) from glucose (1 mol) (Weinberg and Muck, 1996; Muck, 2010; Ellis et al., 2016). *Bacillus subtilis*, traditionally used as a direct feed supplement (Zhang et al., 2016), or as a bacterial inoculant in biological feeds for ruminants, has been classified as a fourth-generation strain silage inoculant (Bai et al., 2022) due to its potential to enhance animal performance (Zhang et al., 2016) and improve fermentation quality (Bai et al., 2021). The aim of this study was to determine the impact of selected LAB strains – *Lactobacillus bifermentans* and *Lactobacillus plantarum*, isolated from grassland flora – on the fermentation process and silage quality of high-moisture fodder pea and compare them to the currently utilised *B. subtilis*.

## Material and methods

### Silage raw material and LAB strains

The Taskent fodder pea (*Pisum sativum* L.) cultivar was grown in 2022 at the Experimental Farm of the University of Kahramanmaraş Sutcu Imam University in Southern Türkiye under rainfed growing conditions. The plants were harvested in the early morning hours on 25 May during the bottom pod formation stage. The *B. subtilis* KUEN 1581 inoculant, with a density of  $2 \times 10^9$  CFU/g, was obtained from SIM Silage (Kahramanmaraş, Türkiye). *L. bifermentans* and *L. plantarum* isolated from Türkiye grassland flora under a project supported by the Türkiye Scientific and Technical Research Organization (TUBITAK) were used as microbial inoculants. *L. bifermentans* (LS-65-2-2) and *L. plantarum* (LS-72-2) were regenerated in MRS (De Man, Rogosa ve Sharpe) broth in 400 ml bottles by incubation at 37 °C for 48 h. Cell densities were determined by cultivation on MRS agar medium.

### Silage preparation and microbial and chemical analyses

Each *Lactobacillus* strains was added to 4000 g of fresh fodder pea plant material at a theoretical concentration of  $10^6$  CFU/g ensuring thorough mixing by hand in sterile gloves. All inoculants were diluted with 10 ml of distilled water, and for the control silages, 10 ml of deionised water was used in place of inoculants. The plant material was chopped into 2–4 cm fragments and ensiled in vacuum-sealed plastic bags. Approximately 400 g of fresh forage material was placed into each bag. A total of 60 vacuumed silage packages were prepared, representing four treatment groups (Control, *L. bifermentans*, *L. plantarum* and *B. subtilis*), five silage opening time points ( $T_{0\text{day}}$ ,  $T_{2\text{day}}$ ,  $T_{5\text{day}}$ ,  $T_{7\text{day}}$  and  $T_{45\text{day}}$ ), and three replicates. The silages were maintained in a cool, shaded area under laboratory conditions. Homogenised samples (20 g) were collected from the silage material at each opening time point ( $T_0$ ,  $T_2$ ,  $T_5$ ,  $T_7$  and  $T_{45}$ ). The samples were mixed with 180 ml of Ringer solution and blended at high speed for one minute. The pH of the silage extracts was immediately measured after filtration through Whatman 54 filter paper (Whatman, Florham, NJ). Microbial counts were conducted using ten-fold serial dilutions. The number of lactic acid bacteria was determined by pour-plating on MRS agar with a double overlay for anaerobic conditions, followed by incubation at 36 °C for 48 to 72 h. The number of enterobacteria was determined by pour-plating on violet red bile glucose agar (VRBD) with a single overlay, and the plates were incubated at 36 °C for 18 h. Yeast and mould counts were enumerated by pourplanting on malt extract agar (MEA) acidified with lactic acid to pH 4, with a single overlay, and the plates were incubated at 32 °C for 48 h. The DM content of the fresh forage ( $T_0$ ) and the resulting silage ( $T_{45}$ ) was determined by drying samples at 70 °C in a forced-air oven for 48 h. The silages were opened after 45 days of ensiling and analysed for pH,  $\text{NH}_3\text{-N}$ , neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP), and crude ash (CA) contents. Ash content was determined by incinerating the dry samples in a muffle furnace at 525 °C for 8 h. Nitrogen (N) content was measured using the Kjeldhal method, and crude protein content was calculated as  $\text{N} \times 6.25$ . Ether extract were analysed using the method of AOAC (1990). Cell wall fibre components, including NDF and ADF were analysed according to the method described by Van Soest et al. (1991). To assess feed quality, the relative feed value (RFV) was calculated using the following formula:

$$\text{DDM} = 88.9 - (0.779 \times \text{ADF}\%);$$

$$\text{DMI} = 120/(\text{NDF}\%);$$

$$\text{RFV} = (\text{DDM} \times \text{DMI})/1.29;$$

where: DDM – digestible dry matter; ADF – acid detergent fibre; DMI – dry matter intake; NDF – neutral detergent fibre; RFV – relative feed value.

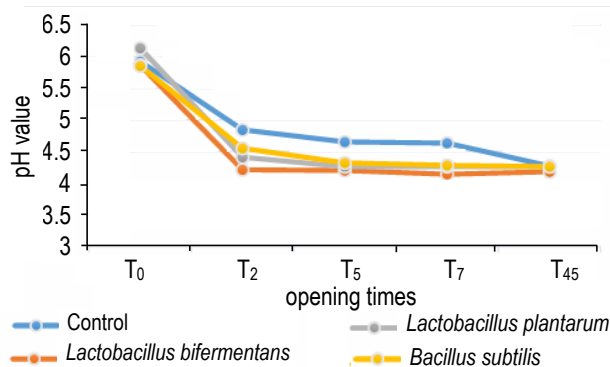
Dry matter recovery (DMR), which indicates how much DM was retained in the silage compared to its initial content, was calculated using the formula:

$$\text{DMR} (\%) = \frac{\text{DM of } T_{60} \text{ silage}}{\text{DM of } T_0 \text{ silage}} \times 100.$$

Statistical analysis of the data was performed using JMP statistical software (SAS Institute, Cary, NC), and treatment groups were compared using the least significant difference (LSD) test.

## Results

The pH of the control treatment was statistically higher compared to all inoculated treatments, reaching a value of 4.86. The pH values of samples inoculated with *L. plantarum* and *B. subtilis* were comparable. The *L. bifermentans* strain was particularly effective in sharply reducing pH ( $P < 0.001$ ) of the silage from the beginning of fermentation compared to other microbial inoculants. This rapid and pronounced reduction in pH provides a significant advantage by preventing proteolysis in legume silages (Table 1). Interactions between opening time points and LAB strains is presented in Figure 1, where it is evident that the pH of fresh material ( $T_0$ ) was higher compared to silage samples taken at subsequent time points ( $T_2$ ,  $T_5$ ,  $T_7$  and  $T_{45}$ ). After 7 days, the pH in the *L. bifermentans* inoculated silage



**Figure 1.** Effect of interaction between lactic acid bacteria strains and silage opening time points on pH value;

$T$  – silage opening time points ( $T_0$ ,  $T_2$ ,  $T_5$ ,  $T_7$ ,  $T_{45}$  – openings at days 0, 2, 5, 7, 45, respectively)

decreased to 4.14 ( $P < 0.001$ ), i.e. it was significantly lower than in the untreated silage. The pH values of both *L. plantarum* and *L. bifermentans*-inoculated silages decreased rapidly during the first 2 days of ensiling; however, after this period, the pH in the *L. plantarum*-inoculated silage stabilised at this level ( $P < 0.001$ ), while it continued to decrease in *L. bifermentans*-inoculated silage throughout the fermentation process.

Table 2 presents the variation in the abundance of LAB, enterobacteria, yeasts, and moulds at individual silage opening time points. Silage inoculated with *L. plantarum* showed higher counts of LAB compared to the untreated silage and other inoculations. The number of LAB in the fresh material was determined at 4.00 log<sub>10</sub> CFU/g, which increased during the early fermentation period, reaching 11.98 log<sub>10</sub> CFU/g at time point  $T_7$ . However, this count dropped to 3.16 log<sub>10</sub> CFU/g by the end of the fermentation process, indicating that the silage had stabilised and fermentation was almost complete. During the fermentation period, the count of LAB in both treated and untreated silages exhibited significant variability across opening time points, which suggested the presence of an interaction between opening time and the abundance of LAB (Figure 2a). For example, the number of *L. plantarum* was lower than *B. subtilis* at  $T_2$ , equal at  $T_5$ , and higher at  $T_7$  and  $T_{45}$ . Similarly, *L. bifermentans* counts were higher at  $T_2$ ,  $T_5$ , and  $T_{45}$  in comparison to *B. subtilis* abundance, but the values were opposite at  $T_7$ . After day 7, both *L. bifermentans* and *B. subtilis* counts decreased, falling below the levels observed in the fresh material ( $T_0$ ), indicating that these strains were particularly aggressive during fermentation. By day 45 of fermentation, LAB counts decreased by 92.93% for *B. subtilis* compared to  $T_7$ , while these values for the control silage,

**Table 1.** Effects of different bacterial inoculants on the pH of silages at different opening time points

Bacteria inoculant	$T_0$	$T_2$	$T_5$	$T_7$	$T_{45}$	Mean
Control	5.92 <sup>b</sup>	4.84 <sup>a</sup>	4.65 <sup>a</sup>	4.63 <sup>a</sup>	4.27 <sup>m</sup>	4.86 <sup>A</sup>
<i>Lactobacillus bifermentans</i>	5.86 <sup>c</sup>	4.21 <sup>jk</sup>	4.20 <sup>k</sup>	4.14 <sup>l</sup>	4.18 <sup>kl</sup>	4.52 <sup>C</sup>
<i>Lactobacillus plantarum</i>	6.13 <sup>a</sup>	4.41 <sup>g</sup>	4.26 <sup>ij</sup>	4.26 <sup>ij</sup>	4.26 <sup>ij</sup>	4.66 <sup>B</sup>
Commercial ( <i>Bacillus subtilis</i> )	5.85 <sup>c</sup>	4.55 <sup>s</sup>	4.32 <sup>h</sup>	4.28 <sup>hi</sup>	4.26 <sup>ij</sup>	4.65 <sup>B</sup>
Mean	5.94 <sup>A</sup>	4.50 <sup>B</sup>	4.36 <sup>C</sup>	4.33 <sup>D</sup>	4.24 <sup>E</sup>	
P-value	T:0.0001 LAB:0.0001 TXLAB:0.0001					
LSD	T:0.03** LAB:0.25** TXLAB: 0.06**					
CV	0.73					

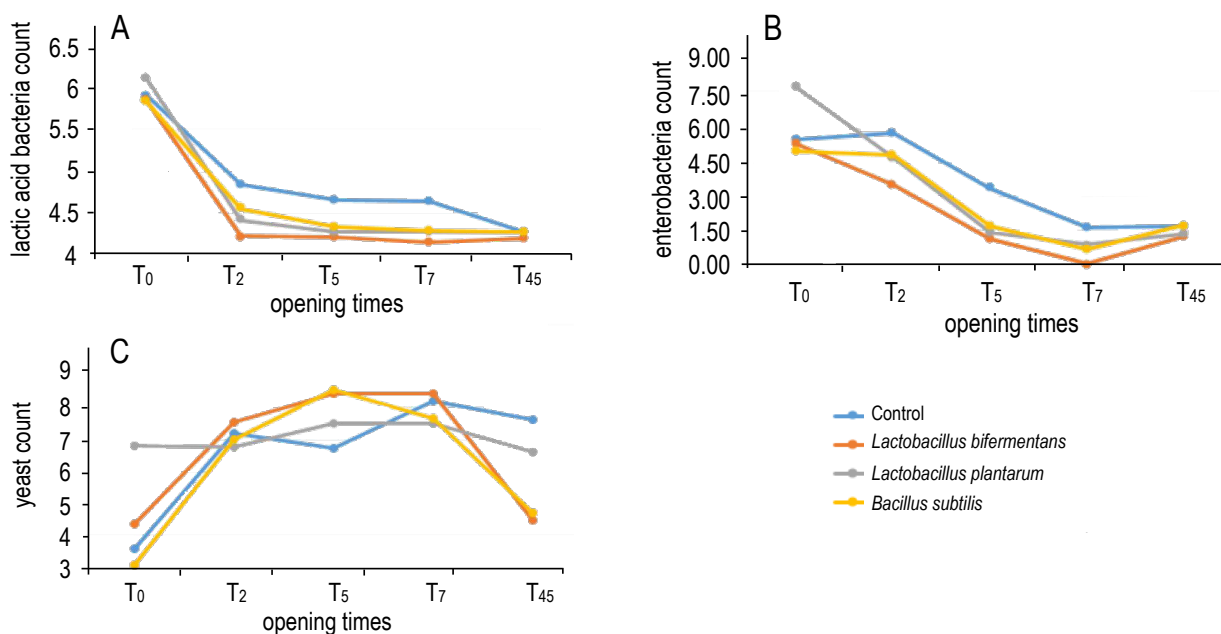
$T$  – silage opening time points ( $T_0$ ,  $T_2$ ,  $T_5$ ,  $T_7$ ,  $T_{45}$  – openings at days 0, 2, 5, 7, 45, respectively), LSD – least significant difference, CV – coefficient of variation, LAB – lactic acid bacteria, TXLAB – effect of interaction between silage opening time points and LAB \*\* $P < 0.001$ ; a, k, ABC – different letters indicate significant differences between mean values



**Table 2.** Effects of different bacterial inoculants on the number of lactic acid bacteria, enterobacteria and yeasts in silages at different opening time points

Bacteria inoculant	T <sub>0</sub>	T <sub>2</sub>	T <sub>5</sub>	T <sub>7</sub>	T <sub>45</sub>	Mean
<b>Lactic acid bacteria</b>						
Control	2.91 <sup>l</sup>	7.26 <sup>gh</sup>	7.13 <sup>gh</sup>	8.91 <sup>d</sup>	1.32 <sup>m</sup>	5.51 <sup>D</sup>
<i>Lactobacillus bifementans</i>	4.17 <sup>l</sup>	7.62 <sup>ef</sup>	8.70 <sup>d</sup>	11.31 <sup>c</sup>	3.59 <sup>k</sup>	7.08 <sup>B</sup>
<i>Lactobacillus plantarum</i>	5.75 <sup>a</sup>	7.05 <sup>gh</sup>	8.00 <sup>e</sup>	14.69 <sup>a</sup>	6.82 <sup>h</sup>	8.46 <sup>A</sup>
<i>Bacillus subtilis</i>	3.19 <sup>kl</sup>	7.50 <sup>efg</sup>	8.00 <sup>e</sup>	13.02 <sup>b</sup>	0.92 <sup>m</sup>	6.53 <sup>C</sup>
Mean	4.00 <sup>D</sup>	7.36 <sup>C</sup>	7.96 <sup>B</sup>	11.98 <sup>A</sup>	3.16 <sup>E</sup>	
P-value	T:0.0001	LAB:0.0001	TXLAB:0.0001			
LSD	T:0.27**	LAB:0.24**	TXLAB: 0.53**			
CV, %	4.67					
<b>Enterobacteria</b>						
Control	5.55 <sup>bc</sup>	5.83 <sup>b</sup>	3.37 <sup>f</sup>	1.61 <sup>gh</sup>	1.68 <sup>g</sup>	3.61 <sup>A</sup>
<i>Lactobacillus bifementans</i>	5.33 <sup>cd</sup>	3.54 <sup>f</sup>	1.10 <sup>ijk</sup>	nd <sup>l</sup>	1.22 <sup>hij</sup>	2.24 <sup>D</sup>
<i>Lactobacillus plantarum</i>	7.89 <sup>a</sup>	4.71 <sup>e</sup>	1.40 <sup>ghi</sup>	0.85 <sup>k</sup>	1.32 <sup>ghn</sup>	3.23 <sup>B</sup>
<i>Bacillus subtilis</i>	5.00 <sup>de</sup>	4.82 <sup>e</sup>	1.69 <sup>gh</sup>	0.66 <sup>k</sup>	1.72 <sup>g</sup>	2.78 <sup>C</sup>
Mean	5.94 <sup>A</sup>	4.72 <sup>B</sup>	1.89 <sup>C</sup>	0.78 <sup>E</sup>	1.48 <sup>D</sup>	
P-value	nd	T:0.0001	LAB:0.0001	TXLAB:0.0001		
LSD	T:0.23***	LAB:0.21***	TXLAB: 0.46***			
CV, %	9.43					
<b>Yeast</b>						
Control	3.59 <sup>h</sup>	7.20 <sup>ode</sup>	6.72 <sup>ef</sup>	8.21 <sup>ab</sup>	7.62 <sup>c</sup>	6.67 <sup>B</sup>
<i>Lactobacillus bifementans</i>	4.36 <sup>g</sup>	7.52 <sup>cd</sup>	8.41 <sup>a</sup>	8.43 <sup>a</sup>	4.47 <sup>g</sup>	6.64 <sup>B</sup>
<i>Lactobacillus plantarum</i>	6.79 <sup>ef</sup>	6.77 <sup>ef</sup>	7.48 <sup>cd</sup>	7.49 <sup>cd</sup>	6.59 <sup>f</sup>	7.03 <sup>A</sup>
<i>Bacillus subtilis</i>	3.10 <sup>h</sup>	7.01 <sup>def</sup>	8.54 <sup>a</sup>	7.66 <sup>bc</sup>	4.70 <sup>g</sup>	6.20 <sup>C</sup>
Mean	4.46 <sup>D</sup>	7.13 <sup>B</sup>	7.79 <sup>A</sup>	7.95 <sup>A</sup>	5.84 <sup>C</sup>	
P value	T:0.0001	LAB:0.0001	TXLAB:0.0001			
LSD	T:0.28**	LAB:0.25**	TXLAB: 0.56**			
CV, %	5.32					

T – silage opening time points (T<sub>0</sub>, T<sub>2</sub>, T<sub>5</sub>, T<sub>7</sub>, T<sub>45</sub> – openings at days 0, 2, 5, 7, 45, respectively), LSD – least significant difference, CV – coefficient of variation, LAB – lactic acid bacteria, TXLAB – effect of interaction between silage opening time points and LAB; ns – non-significant, \*\*P < 0.001; a-k, ABC – different letters indicate significant differences between mean values

**Figure 2.** A. Effect of interaction between lactic acid bacteria strains and silage opening time points on the count of lactic acid bacteria; B. Effect of interaction between lactic acid bacteria strains and silage opening times points on the count of enterobacteria; C. Effect of interaction between lactic acid bacteria strains and silage opening time points on the count on yeast count

T – silage opening time points (T<sub>0</sub>, T<sub>2</sub>, T<sub>5</sub>, T<sub>7</sub>, T<sub>45</sub> – openings at days 0, 2, 5, 7, 45, respectively)



and silages supplemented with *L. bifermantans*, and *L. plantarum* strains were 85.19, 68.26 and 53.57%, respectively.

The count of enterobacteria in the untreated silages was  $3.61 \log_{10}$  CFU/g silage, while this value in silages inoculated with *L. bifermantans* significantly decreased to  $2.24 \log_{10}$  CFU/g, showing that this strain was more effective than *L. plantarum* and *B. subtilis* ( $P < 0.001$ ). The abundance of enterobacteria decreased during the fermentation period and a slight increase was observed on day 45 of ensiling ( $P < 0.001$ ). However, even at this stage, enterobacteria levels remained much lower than those recorded at  $T_0$ ,  $T_2$  and  $T_5$  (Table 2). Figure 2b shows that the number of enterobacteria was low in silages inoculated with *L. bifermantans* ( $0.00 \log_{10}$  CFU/g fresh material) at  $T_7$  ( $P < 0.001$ ). At all sampling points, untreated silages had consistently higher enterobacteria counts compared to inoculated silages.

The yeast count (Figure 2c) increased from the beginning of the ensiling process to day 7 but then decreased by day 45. The yeast count in untreated silages was  $6.67 \log_{10}$  CFU/g silage, while this value significantly decreased to  $6.20 \log_{10}$  CFU/g in silages treated with *B. subtilis*, showing that *B. subtilis* was more effective at restricting yeast growth than both *L. plantarum* and *L. bifermantans* ( $P < 0.001$ ). Although the highest number of yeasts in the early fermentation period was found in *L. bifermantans*-inoculated silages, their abundance decreased at the end of ensiling ( $T_{45}$ ) in all treated silages compared to the control treatment.

Treatment with LAB strains did not statistically alter the DM content of the fresh material ( $T_0$ ). However, a statistically significant difference in DM content was observed in the resulting silage ( $T_{45}$ ), indicating that the DMR of the silage was improved by inoculation. Fodder pea silages inoculated with

both *L. plantarum* (25.81%) and *L. bifermantans* (24.76%) had higher DM values compared to the untreated silages (24.15%) and those treated with *B. subtilis* (23.36%) ( $P < 0.05$ ). Although LAB inoculation did not significantly affect the overall DMR, the highest recovery values were found in silages treated with *L. plantarum* (99.05%), followed by *L. bifermantans* (98.85%), *B. subtilis* (96.23%), and the control (92.03%) (Table 3).

The mean values of  $\text{NH}_3\text{-N}$ , CP, CA, NDF, ADF, and RFV content of fodder pea silages at  $T_{45}$  are given in Table 4. The  $\text{NH}_3\text{-N}$  concentration in the mature silage, which reflects the extent of proteolysis in the silage, was significantly lower in the silage treated with LAB, with *L. bifermantans* showing the greatest effect (15.25 g/50 ml) ( $P < 0.01$ ). The CP content ranged from 16.32 to 17.84%, with *L. plantarum* yielding the highest protein levels.

**Table 4.** Chemical compositions of *Pisum sativum* L. silages at day 45 ( $T_{45}$ )

Bacteria inoculant	$\text{NH}_3\text{-N}$	CP	CA	NDF	ADF	RFV
Control	20.55 <sup>a</sup>	16.32 <sup>c</sup>	7.43	41.83	35.75 <sup>a</sup>	135.90 <sup>b</sup>
<i>Lactobacillus bifermantans</i>	15.25 <sup>c</sup>	17.01 <sup>b</sup>	8.02	38.85	32.91 <sup>b</sup>	151.73 <sup>a</sup>
<i>Lactobacillus plantarum</i>	18.53 <sup>b</sup>	17.84 <sup>a</sup>	7.37	41.93	35.53 <sup>a</sup>	136.13 <sup>b</sup>
<i>Bacillus subtilis</i>	19.88 <sup>ab</sup>	16.95 <sup>b</sup>	6.99	40.00	32.79 <sup>b</sup>	147.30 <sup>ab</sup>
Mean	18.55	17.03	7.45	40.65	34.24	142.77
P-value	0.002 <sup>6</sup>	0.0017	0.6251	0.0764	0.0183	0.0502
LSD	1.99**	0.49**	ns	ns	2.03*	12.70*
CV	5.39	1.41	12.54	3.25	2.97	4.45

LSD – least significant difference, CV – coefficient of variation, CP – crude protein, CA – crude ash, NDF – neutral detergent fibre, ADF – acid detergent fibre, RFV – relative feed value, \*\* $P < 0.01$ ; \* $P < 0.05$ ; ns – non-significant; <sup>abc</sup> – different letters indicate significant differences between mean values

**Table 3.** Effects of different bacterial inoculants on the number of dry matter ratio of silages at different opening time points

Bacteria inoculant	DM ( $T_0$ )	DM ( $T_{45}$ )	DMR
Control	26.39	24.15 <sup>B</sup>	92.03
<i>Lactobacillus bifermantans</i>	25.04	24.76 <sup>AB</sup>	98.85
<i>Lactobacillus plantarum</i>	26.06	25.81 <sup>A</sup>	99.05
<i>Bacillus subtilis</i>	24.03	23.36 <sup>B</sup>	96.23
Mean	25.10	24.52	97.94
P-value	0.3807	0.0256	0.3775
LSD	ns	1.40*	ns
CV, %	5.89	2.86	5.28

DM – dry matter, DMR – dry matter recovery, T – silage opening time points ( $T_0$ ,  $T_{45}$  – openings at days 0, 45, respectively), LSD – least significant difference, CV – coefficient of variation; \* $P < 0.05$ ; ns – non-significant; <sup>AB</sup> – different letters indicate significant differences between mean values

Silages treated with *L. bifermantans* and *B. subtilis* contained similar protein levels, while the lowest protein content was determined in the untreated silages ( $P < 0.01$ ). The CA content ranged from 6.99 to 8.02%, and differences between the treatments were not statistically significant. The NDF content varied between 38.85 and 41.93%, and the differences in NDF values were also not statistically significant. ADF values ranged from 32.79% in *L. plantarum*-inoculated silages to 35.75% in the control silages. *L. bifermantans* and *B. subtilis* were more effective in reducing the ADF value than *L. plantarum* ( $P < 0.05$ ). LAB inoculation significantly increased ( $P < 0.05$ ) the RFV content, with the highest RFV value obtained in the *L. bifermantans*-inoculated silage (151.73), and the lowest (153.90) in the control treatment.

## Discussion

The number of epiphytic LAB in the microbial composition of the ensiling material is one of the important factors determining the direction of silage fermentation. Silage is generally well preserved if the number of epiphytic LAB exceeds the value of  $10^5$  CFU/g fresh material (Cai et al., 1999). According to our results, the number of LAB in the fresh material of *Pisum sativum* L. was very low, and their abundance increased following LAB inoculations before ensiling. The inoculants improved the microbial profile, notably increasing lactic acid bacteria and reducing yeast and mould counts in the resulting fodder pea silage. Muck (1988) has observed that silage fermentation is largely influenced by the number and type of epiphytic microorganisms on the plants, and a higher ratio of LAB in the silage can lead to more efficient fermentation, resulting in a lower pH and the inhibition of growth of undesirable microorganisms. Higher yeast counts were detected in the forage pea plants inoculated with *L. plantarum* compared to the control, both in the fresh material and at the end of fermentation. LAB-inoculated silages had a lower pH than the untreated (control) silages, and *L. bifermentans* strain (a homofermentative LAB) was the most effective strain in reducing the pH value. *L. bifermentans* sharply lowered silage pH from the beginning of fermentation compared to *B. subtilis* and *L. plantarum* inoculants. These results are consistent with those of Fraser et al. (2001), who obtained a relatively low pH at the end of fermentation using homofermentative LAB strains. Moreover, under anaerobic conditions, LAB were shown to cause a rapid drop in the pH during ensiling (Muck, 2013). Similarly, in the present study, the fastest pH reduction throughout fermentation was observed in the silage treated with *L. bifermentans*. It is well established that the final pH of silage is a key indicator of fermentation quality (Wang et al., 2019; Peng et al., 2021). Silage with a pH value of 4.20 or lower is typically considered to be well-fermented (Kung et al., 2018). The pH of the ensiled mixture is affected by various factors, such as anaerobic conditions, WSC concentration, microorganisms in the epiphytic flora, DM content, and the buffering capacity of forage crops (Muck, 1988). In legume plants, it is particularly difficult to obtain a pH of 4.20 or below due to their high buffering capacity, low WSC content, and lower DM at harvest. On the other hand, Scherer et al. (2019) suggested that amino acid deamination and

decarboxylation, indicated by  $\text{NH}_3\text{-N}$  levels, could decrease the nutritional quality of silage. In the current study, the control silage showed signs of deterioration, including an increase in pH and  $\text{NH}_3\text{-N}$  concentration, as well as a reduction in DM by day 45. However, inoculation with *L. bifermentans* helped prevent spoilage to some extent, as indicated by relatively lower pH and  $\text{NH}_3\text{-N}$  levels. This suggested that a lower silage pH could inhibit the hydrolysis of protein fractions in fodder pea silage. Similar findings were reported in highmoisture alfalfa silage by Yang et al. (2020). The present results demonstrated that the  $\text{NH}_3\text{-N}$  concentrations in all inoculant treatments were significantly lower compared to the control, suggesting that the inoculants were effective in preserving protein. In particular, *L. plantarum* was the most effective strain, as evidenced by the highest CP content in the resulting silage.

In this experiment, LAB inoculants had no significant effect on the NDF content of the fodder pea silage; however, in some studies, the addition of these bacteria caused a decrease in the ADF content of silage (Okuyucu et al., 2018). Additionally, some studies, such as those by Koç et al. (2017), reported a reduction in the NDF content following LAB inoculation. These discrepancies observed between studies may be primarily attributed to differences in the DM content of the plants. In the present study, the DM content was initially determined to be 25.10%, and during the fermentation process, it decreased to 24.52%, indicating some DM losses. DM recovery was low in the control silages compared to those treated with inoculants. Our results align with the findings of Kizilsimsek et al. (2020) and Ren et al. (2021). In addition, DM content may be better preserved through inoculation with homofermentative LAB, as Bai et al. (2021) noted that such inoculants are particularly advantageous for legume silages. Homofermentative LAB can produce higher levels of lactic acid, thereby minimising DM losses. Reduced DM is undesirable because it signifies the depletion of valuable nutrients that could otherwise be utilised by animals (Robinson et al., 2016). Inoculant application appears to have a significant inhibitory effect on the growth and activity of unwanted microorganisms, which helps minimise nutrient losses during silage fermentation.

## Conclusions

*Pisum sativum* L. is a valuable legume forage for ruminants and is widely cultivated worldwide. However, lower concentrations of water-soluble

carbohydrates, reduced dry matter content at harvest, and high buffering capacity in legumes present challenges in producing high-quality silage. The inoculation of lactic acid bacteria (LAB) can have a significant impact on the composition of microbial communities during the ensiling process of *Pisum sativum* L. The present study demonstrated that the use of LAB inoculants could improve silage fermentation, preserve forage nutrients, and enhance animal performance. Notably, inoculations with *Lactobacillus bifermentans* reduced pH and  $\text{NH}_3\text{-H}$  levels while inhibiting the growth of enterobacteria.

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## Conflict of interest

The Authors declare that there is no conflict of interest.

## References

- Akbay F., Günaydin T., Arıkan S., Kızılsımsek M., 2023a. Performance of new lactic acid bacteria strains as inoculants on the microorganism composition during fermentation of alfalfa silage containing different dry matter content. *Black Sea J. Agri.* 6, 402–410, <https://doi.org/10.47115/bsagriculture.1303220>
- Akbay F., Günaydin T., Arıkan S., Kızılsımsek M., 2023b. Usage opportunity of asparagus officinalis L. inoculated with lactic acid bacteria as silage feed. *Kahramanmaraş Sutçu Imam Univ. J. Agri. Nature* 26, 1199–1208, <https://doi.org/10.18016/ksutarimdog.vi.1225806>
- AOAC, 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15<sup>th</sup> edition. The Association Official Analytical Chemists, Arlington, VA (USA)
- Bai J., Ding Z., Ke W., Xu D., Wang M., Huang W., Zhang Y., Liu F., Guo X., 2021. Different lactic acid bacteria and their combinations regulated the fermentation process of ensiled alfalfa: ensiling characteristics, dynamics of bacterial community and their functional shifts. *Microb. Biotechnol.* 14, 1171, <https://doi.org/10.1111/1751-7915.13785>
- Bai J., Franco M., Ding Z., Hao L., Ke W., Wang M., Guo X., 2022. Effect of *Bacillus amyloliquefaciens* and *Bacillus subtilis* on fermentation, dynamics of bacterial community and their functional shifts of whole-plant corn silage. *J. Anim. Sci. Biotech.* 13, 1–14, <https://doi.org/10.1186/s40104-021-00649-0>
- Blagojević M., Đorđević N., Bora D., Marković J., Vasić T., Milenković J., Petrović M., 2017. Determination of green forage and silage protein degradability of some pea (*Pisum sativum* L.) + oat (*Avena sativa* L.) mixtures grown in Serbia. *J. Agri. Sci.* 23, 415–422, <https://doi.org/10.15832/ankutbd.385865>
- Cai Y., Benno Y., Ogawa M., Kumai S., 1999. Effect of applying lactic acid bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. *J. Dairy Sci.* 82, 520–526, [https://doi.org/10.3168/jds.S0022-0302\(99\)75263-X](https://doi.org/10.3168/jds.S0022-0302(99)75263-X)
- Canbolat Ö., Akbay K.C., Kamalak A., 2019. Possibilities of use of molasses as carbohydrate source in pea silages. *Kahramanmaraş Sutçu Imam Univ. J. Agri. Nature* 22, 122–130, <https://doi.org/10.18016/ksutarimdog.vi.455713>
- Ellis J. L., Hindrichsen I.K., Klop G., Kinley R.D., Milora N., Bannink A., Dijkstra J., 2016. Effects of lactic acid bacteria silage inoculation on methane emission and productivity of Holstein Friesian dairy cattle. *J. Dairy Sci.* 99, 7159–7174, <https://doi.org/10.3168/jds.2015-10754>
- Ertekin İ., Kızılsımsek M., 2020. Effects of lactic acid bacteria inoculation in pre-harvesting period on fermentation and feed quality properties of alfalfa silage. *Asian-Australas. J. Anim. Sci.* 33, 245, <https://doi.org/10.5713/ajas.18.0801>
- Fraser M.D., Fychan R., Jones R., 2001. The effect of harvest date and inoculation on the yield, fermentation characteristics and feeding value of forage pea and field bean silages. *Grass Forage Sci.* 56, 218–230, <https://doi.org/10.1046/j.1365-2494.2001.00268.x>
- Günaydin T., Akbay F., Arıkan S., Kızılsımsek M., 2023. Effects of different lactic acid bacteria inoculants on alfalfa silage fermentation and quality. *J. Agri. Sci.* 29, 555–560, <https://doi.org/10.15832/ankutbd.1136844>
- Kızılsımsek M., Keklik K., Günaydin T., 2020. Using possibilities of new lactic acid bacteria isolates as microbial inoculant on different dry matter containing alfalfa (*Medicago sativa* L.) silage. *Kahramanmaraş Sutçu Imam Univ. J. Agri. Nature* 23, 1331–1339, <https://doi.org/10.18016/ksutarimdog.vi.691853>
- Koç F., Aksoy S.O., Okur A.A., Celikyurt G., Korucu D., Özduven M.L., 2017. Effect of pre-fermented juice, *Lactobacillus plantarum* and *Lactobacillus buchneri* on the fermentation characteristics and aerobic stability of high dry matter alfalfa bale silage. *J. Anim. Plant Sci.* 27, 1766–1773
- Kung Jr. L., Shaver R.D., Grant R.J., Schmidt R.J., 2018. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. *J. Dairy Sci.* 101, 4020–4033, <https://doi.org/10.3168/jds.2017-13909>
- Muck R., 2013. Recent advances in silage microbiology. *Agri. Food Sci.* 22, 3–15, <https://doi.org/10.23986/afsci.6718>
- Muck R.E., 1988. Factors influencing silage quality and their implications for management. *J. Dairy Sci.* 71, 2992–3002, [https://doi.org/10.3168/jds.S0022-0302\(88\)79897-5](https://doi.org/10.3168/jds.S0022-0302(88)79897-5)
- Muck R.E., 2010. Silage microbiology and its control through additives. *Rev. Bras. de Zootecnia* 39, 183–191, <https://doi.org/10.1590/S1516-35982010001300021>
- Okuyucu B., Koç F., Özduven M.L., 2018. The effects of different doses lactic acid bacteria and enzyme mixture inoculants on the fermentation, aerobic stability and feed value of alfalfa silage. 2<sup>nd</sup> International Animal Nutrition Congress, November 1-4, Antalya/TURKIYE 193–198
- Peng C., Sun W., Dong X., Zhao L., Hao J., 2021. Isolation, identification and utilization of lactic acid bacteria from silage in a warm and humid climate area. *Sci. Rep.* 11, 12586, <https://doi.org/10.1038/s41598-021-92034-0>
- Ren H., Wang L., Sun Y., Zhao Q., Sun Y., Li J., Zhang B., 2021. Enhancing the Co-ensiling performance of corn stover and cabbage waste via the addition of cellulase. *Biores.* 16, 6342–6362, <https://doi.org/10.15376/biores.16.3.6342-6362>

- Robinson P.H., Swanepoel N., Heguy J.M., Price P., Meyer D.M., 2016. Total 'shrink' losses, and where they occur, in commercially sized silage piles constructed from immature and mature cereal crops. *Sci. Total Environ.* 559, 45–52 <https://doi.org/10.1016/j.scitotenv.2016.03.103>
- Scherer R., Gerlach K., Taubert J., Adolph S., Weiß K., Südekum K.H., 2019. Impact of forage species and ensiling conditions on silage composition and quality and the feed choice behaviour of goats. 74, 47, <https://doi.org/10.1111/gfs.12414>
- Van Soest P.V., Robertson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597, [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Wang Y., He L., Xing Y., Zhou W., Pian R., Yang F., Zhang Q., 2019. Bacterial diversity and fermentation quality of *Moringa oleifera* leaves silage prepared with lactic acid bacteria inoculants and stored at different temperatures. *Biores. Technol.* 284, 349–358, <https://doi.org/10.1016/j.biortech.2019.03.139>
- Weinberg Z.G., Muck R.E., 1996. New trends and opportunities in the development and use of inoculants for silage. *FEMS Microbiol. Rev.* 19, 53–63, <https://doi.org/10.1111/j.1574-6976.1996.tb00253.x>
- Yang F., Wang Y., Zhao S., Wang Y., 2020. *Lactobacillus plantarum* inoculants delay spoilage of high moisture alfalfa silages by regulating bacterial community composition. *Front. Microbiol.* 11, 1989, <https://doi.org/10.3389/fmicb.2020.01989>
- Zhang L., Ma Q., Ma S., Zhang J., Jia R., Ji C., Zhao L., 2016. Ameliorating effects of *Bacillus subtilis* ANSB060 on growth performance, antioxidant functions, and aflatoxin residues in ducks fed diets contaminated with aflatoxins. *Toxins* 9, 1, <https://doi.org/10.3390/toxins9010001>