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Effect of *Ustilago maydis* on the Nutritive Value and Aerobic Deterioration of Maize Silage

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Abstract: The common smut of corn, caused by *Ustilago maydis*, reduces the yield and quality of maize forage. When heavy infestations of corn smut occur, grain yields can be so severely decreased that the most viable economic alternative may be to harvest and ensile the crop. Only a couple of studies have attempted to investigate the influence of aerobic exposure on the nutritive value and aerobic stability of silage, which is prepared from smut-infected maize. In this study, individual whole corn plants were harvested by hand. The plants were distributed into three treatments: 0% infected, 50% infected, and 100% infected. The fresh forage was ensiled in triplicate for a 90-day period. Aerobic exposure lasted for 28 days. Samples were taken on the day of opening and on the 3rd, 7th, 14th, and 28th days. Near infrared spectroscopy (NIRS) calibration equations were used for the prediction of qualitative indicators. Silage prepared from 100% smut-infected maize had comparatively poor quality with dry matter loss, increasing pH and the low amount of starch. It was also distinguished with significant temperature increases from days 15 to 18 of aerobic exposure. Silage prepared from 50% smut-infected maize did not show significant quality changes over the period of the experiment, although it had inferior quality compared to the silage prepared from smut-free maize. While silage prepared from smut-infected maize had an overall worse quality than silage prepared from non-infected maize, it should not have an adverse effect on livestock health or production.

Keywords: maize silage; smut; *Ustilago maydis*; fermentation; aerobic exposure; nutritive value; quality changes

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1. Introduction

Silage preparation and storage are two of the most effective techniques for ensuring animal feed supplies. Lactic acid bacteria (LAB) mainly convert water soluble carbohydrates (WSC) and also a little lignocellulolytic material into organic acids under anaerobic conditions. Due to this acidification, the silage can be stored for a long period of time. Corn is one of the most important forages crops used for ensiling [1,2].

Common smut is a disease caused by *Ustilago maydis* infections, and it is relatively common in corn. It is influenced by unfavourable weather conditions, such as high temperatures and droughts in the period of pollen scattering and filaments spreading. The smut disease has a major impact on corn production globally [3] and can infect corn at all phenological phases. The main symptoms of the disease are chlorosis, necrosis, tumour induction [4], and galls growth [5]. In corn, common smut is mainly found in ears as fungal structures called galls that are filled with black teliospores [6]. Smut galls consist of fungal and host tissues. Young galls are white, firm, and covered with a semi-glossy periderm. As galls begin to mature, interior tissues become semi-fleshy and streaks of black tissues occur as teliospores begin to form. During further maturation, galls become a mass of black powdery teliospores and the periderm ruptures, releasing the spores [4]. There is no direct protection against the pathogen because the use of fungicides does not protect against corn smut infection, and the methods for controlling it are limited primarily to seed dressings [7,8]. The protection against this pathogen is basically based on prevention. Many methods for controlling corn smut have been recommended, such as well-balanced soil fertility that can be obtained possibly based on soil tests. Excessive nitrogen available in soil, accompanied sometimes by low phosphorus levesl, increases the chance for smut infestations. Very dry weather conditions tend to aggravate this further. Furthermore, it is important to avoid mechanical injuries to plants. Implements could cause small cuts and wounds to the leaves and stalks, which then provide entry points for the fungus. The protection against insects is also essential. This can be achieved in the early stage of crop development by using insecticide seed treatments. In addition to these treatments, a frequently mentioned control alternative is host resistance, which provides the most advantageous solution against the pathogen [4].

Smut reduces the yield and quality of maize forage. The yield of infected plants can be reduced by 28.0–61.3%. Losses vary with each growing year, location, and cultivar. Ear development in infected plants may be impaired depending on the number, size, and location of smut galls [9]. Thus, large galls, particularly those above the ear, can cause barren corn plants, and multiple galls per plant also often reduce yields by 100% [4]. Fresh material from smut-infected maize was found to contain smaller quantities of N-free extractives (NFE) and sugars than smut-free fresh material [8]. Richter et al. [10] state that plants infected with maize smut had lower dry matter (DM) content and lower nutrients content, and DM degradability and organic matter did not differ from healthy plants.

When heavy infestations of corn smut occur, grain yields can be so severely decreased that the most viable economic alternative may be to harvest and ensile the crop [11,12]. However, there are very little research studies on the effects of common corn smut on the palatability and nutritive value of corn silage. In particular, there is a paucity of studies examining the changes of smut-infected maize silage quality when the silage is exposed to air for longer than 7 days. The DM content is lower in infected silage as opposed to the silage from healthy maize plants. This confirmed that the infection of maize stands with smut (*Ustilago maydis*) results in lower DM contents ($p < 0.05$) in infected plants. Silage infested with smut tends to have greater fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF), and crude protein (CP) contents but lesser non-structural carbohydrate contents than noninfected silage. The smut infection of maize also influences the change in the microbial composition of silage. This, in accordance with the differing DM content of silage biomasses, affects the quality of fermentation [10]. Some research studies showed that silages made with smut-infected maize did not have a harmful effect on the production and health of cattle [8].

When the silo is opened for feed outs or after the removal of silage from the silo, silage is exposed to air. At that time, fermentation acids and silage components are oxidized by aerobic bacteria, yeasts, and moulds [13]. This process is characterized by an increase in pH [14]. Silage that is rich in sugar and energy, free of butyric acid, and includes only low concentrations of acetic acid spoil as soon as oxygen enters the material [13]. In general, maize silage is more likely to be spoiled by aerobic deterioration than grass silage because it typically includes higher amounts of lactic acid and lower amounts of acetic acid. Aerobic deterioration is affected by various factors. Biochemical factors affecting aerobic stability include the development of yeasts and moulds during plant growth, field wilting, storage, and the concentration of undissociated acetic acid in silage [13].

While some research studies have been carried out to investigate the chemical composition of silage prepared from smut-infected maize plants, only a couple of studies attempted to investigate the influence of aerobic exposure on the nutritive value and aerobic stability of silage prepared from smut-infected maize [8,10]. The studies concluded that silage obtained from maize plants infected with *Ustilago maydis* generally had worse aerobic stability and quality. However, the studies have been carried out in a short time period (7 days of aerobic deterioration), which may not necessarily reflect the farm's conditions. The aim of the present study was to determine to what extent the presence of smut-infected maize influences the nutritional value of maize silage and the stability of maize silage throughout the aerobic exposure period.

2. Materials and Methods

2.1. General

Individual whole corn (*Zea mays* L.) plants grown in Lithuanian Research Centre for Agriculture and Forestry research fields in 2021 were harvested by hand. All plants were "Duxxbury" (FOA 170) hybrids. This is an early hybrid grown for silage and grains. About 75 thousand plants per hectare were sown. During the pollen scattering and filaments spreading period in July, the average temperature in Lithuania was 22.1 °C (medium perennial air temperature—17.8 °C), and the amount of precipitation was at 58 mm (medium perennial precipitation—84 mm). High temperatures and droughts in the period of pollen scattering and the filament spreading stage influenced the development of *Ustilago maydis* infections.

Approximately 44% of maize were infected in the field. The total damage to the cobs by *Ustilago maydis* was assessed in the field by selecting five random locations in the maize rows where the number of healthy, damaged and undeveloped cobs was counted within a two-metre length [15]. Counts were made of the diseased and healthy cobs on five 2 m lengths of rows selected randomly, and then they were corrected to a "per plot" basis. Stalks were cut at around 15 cm above the soil surface, when corn kernels were in a milk dough stage (R3-R4) and when the milk line was $\frac{1}{2}$ to $\frac{3}{4}$ down relative to the kernel. All corn plants were divided into two groups: maize with an ear that was visibly and severely infected with *Ustilago maydis* (Figure 1A) and maize with an ear that was not visibly infected (Figure 1B).

Figure 1. Corn cobs: (**A**)—ear not visibly infected with corn smut; (**B**)—ear visibly and severely infected with corn smut.

Maize plants were chopped to an average length of 2 cm in a forage chopper. The plants were broken down into three treatments in total: 0% infected, 50% infected (compiled from a homogenic mixture of equal parts of 0% infected and 100% infected plants), and 100% infected. After thorough mixing, the fresh forage was ensiled in triplicate at approximately 1 kg (fresh weight) in polyethylene bags (28 cm × 40 cm, thickness of 100 and 130 μm, Status

Innovations Co., Metlika, Slovenia) and sealed using a vacuum packing machine. After the bags were filled with fresh matter, a temperature sensor (Tempmate. ®-S1 Single-Use USB Data Logger, Heilbronn, Germany) was inserted in the middle of each bag. The data loggers were set to record the temperature data of all sensors every 10 min during the experimental phase, according to the method described by Ferrero et al. [16]. The sealed bags were ensiled for 90 days before initial opening and kept in a lying position in ambient temperatures (20–22 °C) and in darkness.

Before opening the bags, the gas concentration of CH_4, O_2, CO_2, and H_2S was determined in all treatments, using the GFM 400 Series Gas Analyser (UK). The analyser was connected to the sample point by using a gas pipe. The flow of gas through the instrument took place until the reading stabilised.

To start the inflow of oxygen, the bags were opened. The aerobic exposure stage lasted for 28 days. Samples were taken on the day of opening and on the 3rd, 7th, 14th, and 28th days of aerobic deterioration. Three of the sensors were taken out after opening the samples, and the rest were left during the aerobic stage.

2.2. Analysis

Maize silage samples were composited by hand and mixed thoroughly. Two hundred grams from each composited and mixed sample were oven-dried at 65 ± 5 °C temperature to a constant weight and grounded in an ultra-centrifugal mill ZM 200 (Retsch, Germany) to pass a 1 mm screen. Ground samples were analysed in triplicate for absolute DM DM contents by drying maize samples at 105 °C for 48 h in a forced-air oven until the weight of the samples was stable. Near infrared spectroscopy (NIRS) calibration equations (ADAS, UK) were used for the prediction of CP, crude fat (CL), starch, crude fibre (CF), NDF, ADF, metabolizable energy (ME), and net energy lactation (NEL) data by using an NIRS-6500 device with a sample spinning module (Foss-Perstorp, USA) and selecting wavelengths between 400 and 2500 nm in reflectance spectra [17]. Then, dried samples were scanned in three replications using cuvettes, and the acquired spectra were processed with equations used in the device (ADAS, UK). Crude ash (CA) content was determined as the mass left after sample incineration at 550 (±10) °C. The acidity (pH) of fresh (undried) silage samples was measured in water extracts, in accordance with a potentiometric method, using a pH meter (Horiba, UK).

2.3. Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics, version 25. The nutritive value indicators of different silage treatments were presented as mean ± standard deviation (SD) and significant differences between the days of aerobic exposure and between silage with varying levels of smut damage were calculated using one-way ANOVA (Tukey's post hoc test). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Chemical Composition of the Harvested Maize Forage

The chemical composition of corn forages before ensiling is shown in Table 1. The maize forage that was completely infected with smut (smut-infected maize (100%)), harvested at a dough stage, had the lowest amount of DM, CP, starch, and ADF at 308.6, 70.5, 172.4, and 201.4 g kg^{-1}, respectively. It also had the highest amount of CA with 48.5 g kg^{-1} and CF with 243 g kg^{-1}. The intact (smut-free) maize stood out with the highest amounts of DM (333.1 g kg^{-1}), CP (71.3 g kg^{-1}), starch (257 g kg^{-1}), NDF (399.4 g kg^{-1}), and ADF (221.4 g kg^{-1}).

Table 1. DM and chemical composition (DM basis) of freshly chopped whole-plant corn with different *Ustilago maydis* infection rate (before ensiling).

Treatment	DM	CP	CA	Starch	CF	NDF	ADF
	g kg^{-1}						
Smut-free maize	333.1 ± 3.7 ^{b}	71.3 ± 1.8 ^{a}	40.8 ± 0.8 ^{a}	257.4 ± 7.2 ^{c}	216.6 ± 4.4 ^{a}	399.4 ± 8.2 ^{b}	221.4 ± 4.4 ^{b}
Smut-infected maize (50%)	329.1 ± 5.2 ^{b}	71.1 ± 1.9 ^{a}	41.1 ± 1.1 ^{a}	225.4 ± 10.6 ^{b}	217.5 ± 13.7 ^{a}	375.7 ± 9.4 ^{a}	202.3 ± 7.5 ^{a}
Smut-infected maize (100%)	308.6 ± 5.0 ^{a}	70.5 ± 1.7 ^{a}	48.5 ± 2.1 ^{b}	172.4 ± 10.9 ^{a}	243.0 ± 6.7 ^{b}	380.7 ± 10.0 ^{ab}	201.4 ± 2.9 ^{a}

Note. Values with different lowercase letters indicate significant differences among treatments. Values are presented as mean ± SD. Abbreviations: DM—dry matter; CP—crude protein; CA—crude ash; CF—crude fibre; NDF—neutral detergent fibre; ADF—acid detergent fibre.

3.2. Nutritive Value of the Silage at the Opening and during Aerobic Exposure

Changes in the silage quality during the time period of aerobic exposure are presented in Table 2.

Table 2. Effect of *Ustilago maydis* infection rates on fermentation patterns and the nutritive value of the silage at the opening and during aerobic exposure.

Item	Treatment	Days of Aerobic Exposure 0	3	7	14	28
DM g kg^{-1}	Smut-free maize silage	301.2 ± 3.8 ^{aA}	335.1 ± 5.1 ^{b}	351.0 ± 5.7 ^{bC}	404.0 ± 3.7 ^{cC}	496.0 ± 13.1 ^{dC}
	Smut-infected maize silage (50%)	314.0 ± 7.9 ^{aAB}	327.7 ± 8.6 ^{ab}	328.0 ± 6.7 ^{abB}	317.0 ± 6.1 ^{aB}	344.0 ± 5.6 ^{bB}
	Smut-infected maize silage (100%)	324.8 ± 5.9 ^{bB}	328.0 ± 7.0 ^{b}	306.0 ± 5.4 ^{aA}	301.0 ± 8.5 ^{aA}	289.0 ± 6.2 ^{aA}
pH	Smut-free maize silage	4.10 ± 0.04 ^{A}	4.00 ± 0.05 ^{A}	4.10 ± 0.08 ^{A}	4.02 ± 0.05 ^{A}	4.10 ± 0.05 ^{A}
	Smut-infected maize silage (50%)	4.10 ± 0.04 ^{A}	4.10 ± 0.04 ^{A}	4.12 ± 0.05 ^{A}	4.12 ± 0.05 ^{A}	4.20 ± 0.05 ^{A}
	Smut-infected maize silage (100%)	4.30 ± 0.04 ^{aB}	4.30 ± 0.03 ^{aB}	4.30 ± 0.04 ^{aB}	4.25 ± 0.04 ^{aB}	5.30 ± 0.04 ^{bB}
CP g kg^{-1}	Smut-free maize silage	93.8 ± 3.2 ^{aB}	93.7 ± 1.0 ^{a}	88.8 ± 1.2 ^{a}	92.4 ± 0.4 ^{a}	99.8 ± 3.1 ^{bA}
	Smut-infected maize silage (50%)	93.5 ± 0.3 ^{B}	89.3 ± 4.5	92.8 ± 0.8	91.6 ± 1.1	90.0 ± 2.7 ^{A}
	Smut-infected maize silage (100%)	87.2 ± 2.6 ^{aA}	94.8 ± 0.9 ^{a}	91.4 ± 3.1 ^{a}	90.5 ± 1.8 ^{a}	121.7 ± 6.9 ^{bB}
CL g kg^{-1}	Smut-free maize silage	24.9 ± 0.4 ^{A}	24.5 ± 0.3 ^{A}	23.3 ± 2.5 ^{A}	22.7 ± 0.3 ^{A}	23.1 ± 0.9
	Smut-infected maize silage (50%)	26.8 ± 0.8 ^{bB}	24.8 ± 0.3 ^{abA}	25.4 ± 0.6 ^{abA}	25.6 ± 1.2 ^{abB}	24.7 ± 0.7 ^{a}
	Smut-infected maize silage (100%)	30.7 ± 0.5 ^{bC}	27.7 ± 0.7 ^{bB}	35.2 ± 1.6 ^{cB}	30.2 ± 0.8 ^{bC}	23.5 ± 2.1 ^{a}
CA g kg^{-1}	Smut-free maize silage	54.1 ± 5.7	52.9 ± 1.5	55.7 ± 2.8	55.3 ± 1.9	55.0 ± 1.3 ^{B}
	Smut-infected maize silage (50%)	54.1 ± 1.2	50.3 ± 2.1	52.9 ± 0.7	53.1 ± 4.8	48.4 ± 3.4 ^{A}
	Smut-infected maize silage (100%)	46.8 ± 2.8 ^{ab}	53.0 ± 3.7 ^{b}	53.5 ± 2.6 ^{b}	49.8 ± 4.6 ^{ab}	44.2 ± 1.3 ^{aA}
Starch g kg^{-1}	Smut-free maize silage	245.9 ± 7.9 ^{bB}	202.3 ± 15.8 ^{aB}	222.7 ± 11.7 ^{abC}	216.9 ± 1.5 ^{aB}	217.9 ± 10.7 ^{abB}
	Smut-infected maize silage (50%)	233.4 ± 9.9 ^{bB}	181.4 ± 9.1 ^{aB}	188.7 ± 1.2 ^{aB}	196.2 ± 14.0 ^{aB}	200.1 ± 20.2 ^{abAB}
	Smut-infected maize silage (100%)	162.5 ± 18.2 ^{bA}	117.3 ± 8.4 ^{aA}	139.8 ± 2.7 ^{abA}	159.1 ± 8.0 ^{bA}	166.7 ± 15.3 ^{bA}
CF g kg^{-1}	Smut-free maize silage	188.0 ± 5.6	200.3 ± 8.4 ^{B}	194.1 ± 5.0	197.0 ± 10.3	193.4 ± 4.6 ^{B}
	Smut-infected maize silage (50%)	192.9 ± 6.7 ^{ab}	181.2 ± 7.1 ^{aA}	198.1 ± 3.4 ^{b}	199.3 ± 1.9 ^{b}	186.0 ± 6.8 ^{abB}
	Smut-infected maize silage (100%)	199.8 ± 6.8 ^{bc}	211.8 ± 5.1 ^{cB}	205.4 ± 5.2 ^{c}	189.9 ± 4.8 ^{b}	138.0 ± 1.2 ^{aA}
NDF g kg^{-1}	Smut-free maize silage	390.0 ± 14.1 ^{A}	409.2 ± 5.2 ^{A}	397.4 ± 7.8 ^{A}	400.3 ± 8.4 ^{A}	396.0 ± 3.7 ^{A}
	Smut-infected maize silage (50%)	403.2 ± 9.6 ^{A}	395.0 ± 14.4 ^{A}	416.5 ± 5.8 ^{B}	406.3 ± 5.1 ^{A}	402.5 ± 4.2 ^{A}
	Smut-infected maize silage (100%)	435.3 ± 7.4 ^{B}	466.2 ± 4.5 ^{B}	448.4 ± 4.4 ^{C}	430.5 ± 8.9 ^{B}	466.4 ± 12.1 ^{B}
ADF g kg^{-1}	Smut-free maize silage	210.2 ± 7.0	222.4 ± 9.4 ^{B}	217.2 ± 5.7	216.2 ± 12.4 ^{B}	212.9 ± 4.4
	Smut-infected maize silage (50%)	202.2 ± 9.4	190.4 ± 9.1 ^{A}	209.7 ± 5.4	210.6 ± 2.1 ^{AB}	193.3 ± 8.9
	Smut-infected maize silage (100%)	204.2 ± 15.1 ^{bc}	227.6 ± 6.9 ^{cB}	217.9 ± 4.2 ^{c}	192.9 ± 9.4 ^{bA}	160.1 ± 6.0 ^{aA}
ME MJ/kg	Smut-free maize silage	10.87 ± 0.14	10.71 ± 0.11 ^{A}	10.77 ± 0.06 ^{B}	10.74 ± 0.16	10.79 ± 0.07 ^{A}
	Smut-infected maize silage (50%)	10.88 ± 0.07 ^{abc}	11.01 ± 0.12 ^{cB}	10.74 ± 0.05 ^{abB}	10.73 ± 0.08 ^{a}	10.96 ± 0.08 ^{bcB}
	Smut-infected maize silage (100%)	10.71 ± 0.09 ^{ab}	10.50 ± 0.10 ^{aA}	10.61 ± 0.03 ^{aA}	10.89 ± 0.11 ^{b}	11.67 ± 0.02 ^{cB}
NEL MJ/kg	Smut-free maize silage	6.57 ± 0.08	6.45 ± 0.08 ^{AB}	6.50 ± 0.05 ^{B}	6.47 ± 0.12	6.51 ± 0.05 ^{A}
	Smut-infected maize silage (50%)	6.55 ± 0.06	6.63 ± 0.13 ^{B}	6.47 ± 0.04 ^{B}	6.46 ±0.04	6.61 ± 0.07 ^{A}
	Smut-infected maize silage (100%)	6.45 ± 0.07 ^{bc}	6.27 ± 0.06 ^{aA}	6.37 ± 0.02 ^{abA}	6.57 ± 0.07 ^{c}	7.15 ± 0.02 ^{dB}

Note. Values with different uppercase letters indicate significant differences among treatments on the same day during aerobic exposure. Values with different lowercase letters indicate significant differences among days after opening for the same treatments. Values with no letters did not show significant differences among treatments or days ($p < 0.05$). Values are presented as mean ± SD. Abbreviations: SEM—standard error of means; DM—dry matter; CP—crude protein; CL—crude fat; CA—crude ash; CF—crude fibre; NDF—neutral detergent fibre; ADF—acid detergent fibre; ME—metabolizable energy; NEL—neto energy lactation.

It was observed that the amount of DM decreased in the silage prepared from 100% smut-infected maize. When comparing the silos of different infection levels, it was observed that on day 0, the DM content of the silage prepared from 100% infected material was significantly higher than that of the non-infected silage ($p < 0.05$), while the content of the silage prepared from 50% infected silage was not significantly different from that of the two silos ($p > 0.05$). In the 100% smut-infected maize silos, the pH increased significantly ($p < 0.05$). When comparing the silos of different infection levels, it was observed that the pH was slightly higher in the 50% damaged silos than in the undamaged silos throughout the entire period, but it was not statistically significant ($p > 0.05$). In the silos prepared from the 100% damaged maize, the pH was significantly higher on all days compared to the other treatments ($p < 0.05$).

The CP content of 100% infected silage increased significantly by 39.5% on day 28 ($p < 0.05$) The CP content of the 100% infected silage was significantly lower at day 0 ($p < 0.05$ compared to both silages).

In the silos prepared from 50% infected maize, the CL content decreased by 7.8% at day 28 compared to day 0 ($p < 0.05$). In the silos made from 100% infected maize, the CL content was the lowest on day 28 over the entire study period compared to a 0–14 days period. When comparing the silos with different infection levels, it was observed that during the entire study period (except for day 28), the CL content was significantly higher in the 100% smut-infected silos than in the non-infected ($p < 0.05$) and 50% infected ($p < 0.05$) silos.

In the silos, prepared from 50% infected maize, a significant decrease in starch content was observed on days 3, 7, and 14 ($p < 0.05$) compared to day 0, but it did not differ from day 28 ($p > 0.05$). The 100% damaged silage had a significantly lower starch content on all days from day 0 to day 14 compared to the undamaged silage and the 50% damaged silage ($p < 0.05$).

On day 14, a significant decrease was observed in the CF content in the 100% infected silos compared to days 3 and 7 ($p < 0.05$), and on day 28, the fibre content was the lowest during the entire study period and it decreased by 31% from day 0 ($p < 0.05$). When comparing silos with different infection levels, it was observed that on day 3 the CF content was significantly lower in the 50% infected silage than in the non-infected ($p < 0.05$) and the 100% smut-infected ($p < 0.05$) silages and on day 28 the CF content was lower by 28% in the silos prepared from 100% infected maize than in the silos prepared from non-infected maize, and it was 25% lower compared to the 50% infected maize silage ($p < 0.05$).

The NDF content of both intact and infected silos did not change statistically significantly over the study period ($p < 0.05$), although the NDF content of the 100% infected silage was significantly higher on all days compared to the 50% infected and non-infected silage ($p < 0.05$). The ADF content was lower in 50% smut-infected silage than that of the other two silages on day 3 ($p < 0.05$), and on day 28, it was significantly lower than that of the undamaged silage only ($p < 0.05$). The ADF content of the 100% infected silos was significantly lower than that of the undamaged silage on day 14 ($p < 0.05$), and on day 28, it was significantly lower than that of the two silages ($p < 0.05$).

The ME did not change significantly in silos during aerobic exposure. When comparing silos with different infection levels, it was observed that on day 3, the ME content was significantly higher in the 50% damaged silos than in the undamaged and 100% damaged silos ($p < 0.05$). On day 28, the ME content was significantly different in all silos (the lowest being in the non-infected silos and the highest in the 100% infected silos) ($p < 0.05$). The NEL content increased significantly on day 14 compared to days 3 and 7 ($p < 0.05$) in the 100% infected silage, and it was the highest on day 28 compared to the entire study period ($p < 0.05$).

3.3. Temperature of the Silage at the Opening and during Aerobic Exposure

The temperature development represented in Figure 2 shows the course of reheating. In the healthy silos, the temperature did not change significantly during the first 18 days of the study, but it increased significantly on days 21 (compared to days 0 and 3, $p < 0.05$; compared to days 6, 9, 12, and 18, $p < 0.05$; compared to day 15, $p < 0.05$) and 24 (compared to all days 0 to 18 $p < 0.05$), and on day 28, it was the highest during the entire study period (compared to days 0 to 18, $p < 0.05$; compared to day 21, $p < 0.05$; compared to day 24, $p > 0.05$).

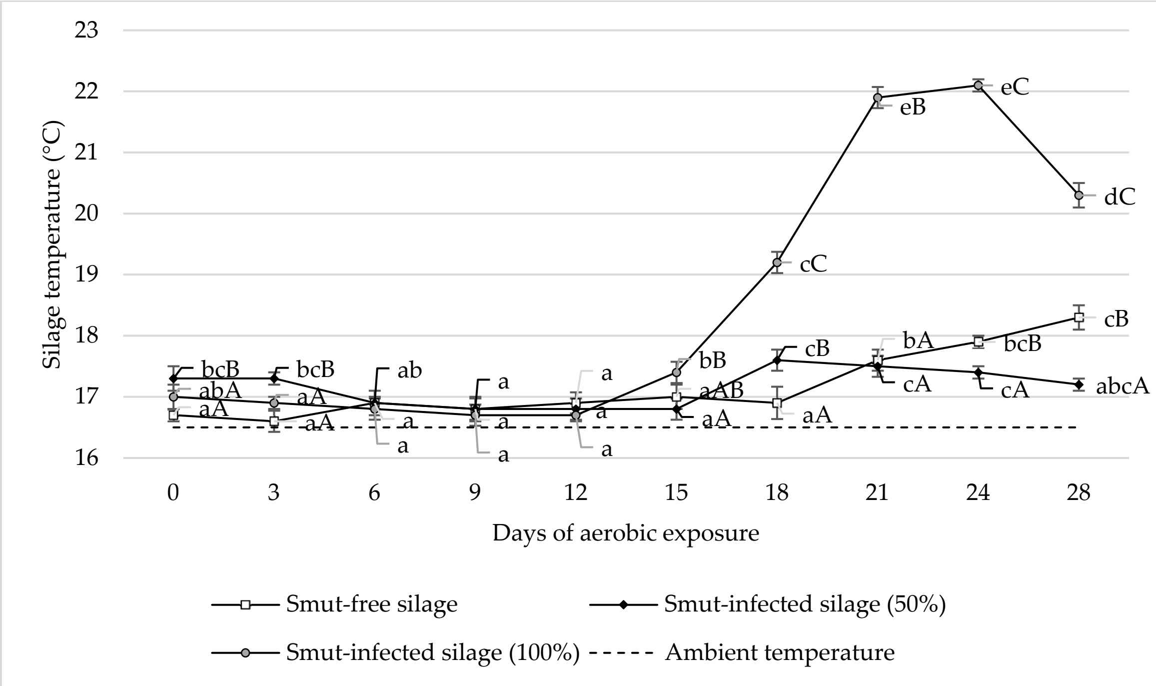

Figure 2. Change in temperatures measured in silage during aerobic exposure while silage was affected with different *Ustilago maydis* infection rates. Error bars represent SD. Note. Values with different uppercase letters indicate significant differences among treatments on the same day during aerobic exposure. Values with different lowercase letters indicate significant differences among days after opening for the same treatments. Values with no letters did not show significant differences among treatments or days ($p < 0.05$).

In the 50% damaged silos, on days 9, 12, and 15, the temperature decreased statistically significantly (compared to days 0 and 3, $p < 0.05$); on day 18, the temperature was the highest (compared to days 9, 12 and 15, $p < 0.05$, and compared to day, 6 $p < 0.05$); on days 21 and 24, the temperature decreased, but it was still significantly higher (compared to days 6, 9, 12, and 15, $p < 0.01$); on day 28, it decreased and was not statistically different relative to the temperature for the entire period.

Reheating was observed in silage prepared from 100% smut-infected silos. On day 15, the temperature increased significantly compared to days 3, 6, and 9, and on day 18, it increased again ($p < 0.05$ compared to the days before). On days 21 and 24, the temperature was the highest ($p < 0.05$ compared to the days before), and on day 28, the temperature dropped significantly compared to days 21 and 24 ($p < 0.05$); however, it was still high compared to the other days ($p < 0.05$).

When comparing silos with various infection levels, the temperature was significantly higher on day 0 in the silos made from 50% infected maize compared to the healthy silos ($p < 0.05$), although it was not significantly different from the 100% damaged silos ($p > 0.05$). On day 3, the temperature in the 50% damaged silos continued to be significantly higher than in the smut-free maize silage ($p < 0.05$). On day 15, the temperature was significantly higher in the 100% infected silos compared to the 50% damaged silos ($p < 0.05$), but it was not significantly different from the healthy silos ($p > 0.05$). On day 18, the temperature in the 50% infected silos was significantly higher compared to the healthy silos ($p < 0.05$), while the temperature in the 100% damaged silos was the highest compared to the other silos ($p < 0.005$).

On day 21, the temperature in the intact silage was not different from the 50% damaged silos, while the temperature in the 100% damaged silos continued to be significantly higher compared to the other silos ($p < 0.05$). On days 21 and 24, the 100% damaged silos had the highest temperature, and it was approximately +4 °C higher compared to the other silos ($p < 0.05$).

3.4. Gas Concentration Measurements after the Opening of Samples

While comparing silage treatments with different smut infection levels, there were no statistically significant differences found between CH_4, O_2, CO_2, and H_2S gas concentrations ($p > 0.05$) (Table 3). The maximum values of CH_4 and H_2S were found in smut-free silage, while the maximum value of CO_2 was found in silage prepared from 100% smut-infected maize (1.75 and the amount was 1.23 times higher; the maximum values were then found in smut-free and 50% smut-infected silage, respectively). There was no oxygen found in any of the treatments.

Table 3. Concentrations of gases in silage affected with different *Ustilago maydis* infection rates (measured after 90 days of ensiling).

Treatment	CH_4	O_2	CO_2	H_2S
	ppm			
Smut-free silage	3.6 ± 2.7	0	303.2 ± 121.5	14 ± 5.5
Smut-infected silage (50%)	1.8 ± 2.6	0	430.8 ± 181.5	8 ± 4.5
Smut-infected silage (100%)	2.6 ± 2.5	0	531.0 ± 173.6	8 ± 4.5

Note. Values are presented as mean ± SD. Abbreviations: CH_4—methane; O_2—oxygen; CO_2—carbon dioxide; H_2S—hydrogen sulphide.

4. Discussion

Maize smut (*Ustilago maydis*) is a fungal disease that, under unfavourable conditions, can damage a large number of maize plants in the field, especially during droughts followed by heatwaves. The disease affects all above-ground parts of the plant and produces a large number of spores (teliospores), causing new infections. Several generations of these teliospores can develop during the growing season. Maize smut reduces the yield and quality of maize forage [8].

In the present study, an attempt was made to determine to what extent the presence of smut-infected maize influences the nutritional value of maize silage and the stability of maize silage throughout the aerobic deterioration stage.

4.1. Chemical Composition of the Harvested Maize Forage

The raw material of freshly harvested 100% smut-infected maize forage had significantly lower amounts of DM and starch compared to other treatments. Other researchers also obtained similar results of the intact maize forage [11,18]. The DM amount might have been influenced by the fact that maize kernels did not achieve maturity due to *Ustilago maydis* infection damage on corn cobs. This led to decreased grain yields and thus lower ratios of grain to cob + stalk + leaf in the infected plants than in the non-infected plants [11]. Many studies report yield losses due to smut infection. A study by Potkański and Weiermüller [8] stated that *Ustilago maydis* is able to metabolize all provided types of sugar, and it is generally acknowledged that the maize fodder is depleted of carbohydrates due to smut infections [8,19–21].

4.2. Nutritive Value of the Silage at the Opening and during Aerobic Exposure

In the current experiment, there was a trend of DM decreasing in the silage prepared from 100 % smut-infected maize. Doleza et al. [10] and Richter et al. [22] also stated lower DM content in smut-infected silage as opposed to the silage from healthy maize plants. This confirmed that the infection of maize with *Ustilago maydis* results in lower DM content of infected plant silage. Intact silage and silage prepared from 50% smut-infected maize DM was increased by aerobic exposure due to evaporation of moisture and volatile organic compounds as silage was not covered [23].

It is obvious from the results, as shown in Table 2, that the smut infection of maize plants also influenced the change in silage pH. After the initial opening, all silage samples had pH values right above the threshold of 4 (Table 2). The pH increased significantly in the silage prepared from 100% smut-infected maize, reflecting the faulty aerobic stability influenced by poor fermentation qualities in the early stages of ensiling. However, the pH was stable in the intact silage as well as in silage prepared from 50% smut-infected maize, which indicated adequate fermentation. However, the observed difference between the intact silage and silage prepared from 100% smut-infected maize pH levels differs from the findings presented by Potkański et al. [8], in which a silage stability test showed increasing pH values in all samples (smut-infected and non-infected), with the exception of those with the chemical additive. This might be influenced by the fact that the raw material of 100% smut-infected maize stood out with a low amount of starch, as starch contents along with the activity of epiphytic LAB determine the rate of decline in pH during the early stages of ensiling, which is important for producing stable silage [24].

The amount of CP kept increasing during aerobic exposure in the silage prepared from 100% smut-infected maize. In comparison, the CP was stable in the intact silage and silage prepared from 50% smut-infected maize. The amount of CP tended to increase on a DM basis as some easily oxidizable constituents of forage were quickly depleted [18,25]. Potkansi et al.'s [8] experiment showed very similar results for CP changes after the stability test was performed, where it remained stable in smut-free silage (69.19 g kg^{-1} DM before the stability test and 69.31 g kg^{-1} DM after) and increased in smut-infected silage (75.87 g kg^{-1} DM before and 87.35 g kg^{-1} DM after).

The CL had a moderate decrease in all treatments and can be explained by oxidation processes in fatty acids, which caused a substantial amount of ME [23]. The concentration of *Ustilago maydis* infection in maize did not show effects on CA content as it remained stable in all treatments during the period of aerobic exposure. The starch content remained unchanged when silages were exposed to the air, which seems to be consistent with another research study [23].

The NDF content of the 100% infected silage was significantly higher on all days compared to the 50% infected and non-infected silage. According to Filya and Sucu [26] in the 90 days of ensiling, no significant difference was observed in the NDF of maize silages ($p > 0.05$). This finding suggests that lowering the quantity of NDF in the silage prepared from 100% smut-infected maize during aerobic exposure was a result of smut infection in maize as other research shows only a small increase in NDF in the intact maize silage [18,23]. ADF is partly reduced at the process of ensiling as hemicellulose is sensitive to lower pH and is partially hydrolyzed under acidic conditions [27]. However, the content of ADF decreased more prominently in the silage prepared from 100% smut-infected maize. These results are likely to be related to the scale of the maize smut infection of the plant. Previous research did not show significant changes in the ADF amount of the healthy silage [18].

The amount of ME and NEL did not change significantly during the time of aerobic exposure in the intact silage and silage prepared from infected plants. These results are in line with those of previous studies [18,23], which were obtained by investigating infection-free maize. As for maize silage infected with *Ustilago maydis*, there are insufficient data reflecting the changes in energy indicators.

Some of the changes noted in the chemical composition of smut-infected silage are similar to those noted in drought-stressed corn silage. Drought stress decreases the grain content and thereby causes increased NDF and decreased non-structural carbohydrate content [11]. In the present study, maize plants were not partitioned into grain, cob, stalk, and leaves. However, visual observation and laboratory analyses (NDF, ADF, and starch) suggest that smut-infected corn had a lesser grain content than noninfected corn. This would be expected because of the effects of smut on grain yields.

4.3. Temperature of the Silage at the Opening and during Aerobic Exposure

An increase in silage temperature is seen as a convenient indicator for the extent and intensity of aerobic deterioration [18]. The energy released by the aerobic degradation of WSC in plant respiration is responsible for the elevation in temperature [23]. In this experiment, the temperature of the silage, prepared from 100% smut-infected maize, increased drastically after 15 days of aerobic exposure due to the fact that common smut gall tissue can be colonized by mycotoxigenic fungi and contaminated with mycotoxins [6]. The aerobic exposure of silage can induce the growth of mould and mycotoxin formation [28–30]. It has to be considered that the ambient temperature was 17–20 °C during this trial, which provides good conditions to spoilage organisms such as aerobic yeasts mostly being active at 20–30 °C [31].

Temperature development also expresses DM losses because microbial respiration is an exothermic process [32]. These results further support the idea that *Ustilago maydis* infections influenced the significant decrease in DM in silage prepared from 100% smut-infected maize.

Gas concentration measurements did not show any statistically significant differences.

5. Conclusions

The results of this investigation identified that silage prepared from 100% smut-infected maize is distinguished by a decrease in dry matter and crude fibre content and an increase in pH over the time of aerobic exposure. It also had a lower amount of starch than other treatments and experienced significant reheating after opening. While it had an overall worse quality than silage prepared from non-infected maize, it should not have an adverse effect on livestock health or production. Silage prepared from 50% smut-infected maize did not show significant quality changes over the period of the aerobic exposure; however, it had inferior quality compared to the silage prepared from smut-free maize, which shows that even a moderate infestation with smut can aggravate the quality of the silage. These findings suggest that, in general, increasing maize contagion levels with *Ustilago maydis* will negatively impact the quality and aerobic stability of maize silage. This study enhanced our understanding of the effects of *Ustilago maydis* infection on maize silage quality relative to aerobic deterioration as the primary concern, paying particular attention to a long period of aerobic exposure. However, further research is needed to gain a better understanding of maize smut effects on maize silage nutrition and fermentation quality.

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