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IMPACT OF CERTAIN STRAINS OF YEAST AND FUNGI AS SILAGE INOCULANTS ON CORN SILAGE CHEMICAL COMPOSITION, FERMENTATION CHARACTERISTICS AND *IN VITRO* DIGESTIBILITY

Ali, M.M.[(1)]; Baraghit, G.A.[(1)]; Ahmed, B.M. [(1)]; Elmasry, A.M.[(2)]; Salem, Mai A.[(1)] and Nayel, U.A.[(1)*]

[(1)] Department of Animal Production, Faculty of Agriculture, Menoufia University, Shibin El-Kom 32514, Egypt.

[(2)] Agriculture Microbiology and Biotechnology, Botany Department, Faculty of Agriculture, Menoufia University, Shibin El-Kom 32514, Egypt.

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ABSTRACT: This study was designed to evaluate the effect of certain strains of fungi (*Trichoderma harzianum*) and yeast (*Saccharomyces cerevisiae D-47*) inoculation on silage chemical composition, fermentation characteristics, and in-vitro digestibility. Four treatments were tested i.e., control (C): corn silage without inoculants, Y: corn silage involved saccharomyces cerevisiae, T: corn silage involved Trichoderma harzianum and Y+T: corn silage involved both inoculants. Chopped whole corn was pressed into polyethylene bags (1.5 to 2 kg) using a vacuum sealer, then stored at room temperature for different ensiling times (zero time, 5 h, 10 h, 20 h, and 2, 4, 8, 14, 25, and 35 days). Inoculants had no significant effect on DM and OM, while decreased ($P<0.04$) significantly with ensiling time. In spite corn silage CP and NFE increased ($P< 0.5$) significantly with inoculants and ensiling time than the control (c), the content of CF, NDF, and ADF significantly decreased with the time of ensiling. The values of pH and NH3-N gradually decreased in corn silage with the time of ensiling. The lactic acid concentration increased ($P < 0.001$) with inoculation of Yeast (Y), Trichoderma (T), or both (Y + T) and reached 39.50, 38.99, and 40.77 g/kg DM, respectively. While the acetic and butyric acid followed the opposite trend. Time of ensiling negatively correlated with the concentration of both formic and citric acids while it was positively correlated with the concentration of succinic acids. Silages inoculation increased total bacteria (5.51, 7.69, 7.69, and 7.81 $\log_{10}$ cfu/g DM) for the control, Y, T, and Y+T, respectively. Similarly, lactic acid bacteria significantly increased with inoculation (6.46, 6.89, 6.97 and 7.03 $\log_{10}$ cfu/g DM) for the control, Y, T, and Y+T, respectively. Moreover, yeast count ($\log_{10}$ cfu / g DM) increased ($P<0.05$) significantly with silage inoculation compared to the control silage, and the significantly highest was obtained by Y + T. The inoculation had significantly ($P < 0.05$) increased values of both IVDMD and IVOMD, where the best values appeared with corn silage inoculated with Y+T.
Conclusion, the inoculation of *Saccharomyces cerevisiae D-47* and/or *Trichoderma harzianum* leading to an increase in silage quality compared with the un-inoculated silage.

Key words: Silage, inoculants, yeast, fungi , fermentation characteristics, digestibility

INTRODUCTION

Most silage inoculants have been developed for their ability to promote a restorative fermentation that improves silage quality for ruminant livestock. For these reasons, studies have resorted to adding lactic acid bacteria that produce lactic acid as an end product for fermentation, or additions that increase or improve of performance these bacteria (Jones, 1998 and Davies *et al*., 2005).

This leads to benefiting the energy available in nitrogen presence and improves the true protein content in silage (Haag *et al*., 2015, Ali *et al*., 2015 and Borreani *et al*., 2018). Direct-fed microbes (DFM) can offer benefits to livestock nutrition and health by modifying the

*Corresponding author: usamanayel@yahoo.com

microbial ecology of the digestive tract (Brashears *et al*., 2005 and Nayel *et al*., 2019).

Moreover, McAllister *et al*. (2011) confirmed that certain DFM enhances the growth rate and milk production and can exclude zoonotic pathogens from the intestinal tract. Although these response mechanisms are still mostly unknown, according to Weinberg *et al.* (2003), several microorganisms used in silage inoculation may improve silage characteristics, remain active in the rumen, and operate synergistically with other bacterial species (Lettat *et al.*, 2012). Therefore, this fourth generation of silage inoculants may change the microbial ecology in ruminants' gastrointestinal tracts to improve their health and/or production efficiency in addition to silage quality, digestibility, and aerobic stability.

Saccharomyces spp., one of the most widely utilised yeasts, has been shown in research by Desnoyers *et al.* (2009) and McAllister *et al.* (2011) to increase feed efficiency, reduce ruminal acidity, and reduce methane emissions. Many fungal strains such as Trichoderma secrete higher levels of active cellulase than bacterial species (Amouri and Gargouri, 2006). *T. harzianum* produces the most effective cellulase for the full hydrolysis of cellulosic substrates into monomeric glucose, a fermentable sugar. Despite the fact that Muck *et al.* (2017) study on yeast concentrated on preventing mould and other harmful silage microorganisms, other yeast studies by Mehrez *et al.* (2008) suggest that might be potential to apply a direct-fed microbial strain capable of surviving during silage and multiply during feed out.

Several studies hypothesized that new microbes could be used as silage inoculants, especially during the silage aerobic phase (Weinberg *et al*., 2003, Mehrez *et al*. 2008 and Lettat *et al*. 2012). The characteristics of the inoculants used should be low nutritional requirements, the ability to convert a complex substrate into a valuable product via their valuable hydrolytic enzymes, and a rapid growth rate (McAllister *et al*. 2011). Additionally, Mehrez *et al*. (2008) recommended that good inoculants characteristics have to be antifungal, non-pathogenic, good tolerance to pH and temperature, non-toxic, used as single cell protein, and good digestibility. On the other hand, Abo-Donia *et al*. (2022) stated that silage inoculations can reduce the aerobic phase, thus leading to decrease aerobic deterioration and improved silage quality.

MATERIALS AND METHODS

This research was conducted in accordance with the ethics of dealing with animals and the approval of the Ethics Committee and dealing with animals used in scientific research of Menoufia University (The Institutional Animal Care and Use Committee- Menoufia University (IACUC)- (Reference No. MUFAG/F/AP/8/22).

The present study was carried out at the Nutrition Laboratory, Department of Animal Production, Faculty of Agricultural, Menoufia University to investigate the effect of fungal (Trichoderma harzianum) and yeast (Saccharomyces cerevisiae D-47) inoculation on silage chemical composition, fermentation characteristics and *in vitro* digestibility.

Collected fresh corn samples from the Experimental Station, Faculty of Agriculture, Menoufia University (Shebin El-Kom) were chopped into 1 to 3 cm in length. The samples were divided into 4 parts, to ensiled into polyethylene bags as follows: control (T1), un-inoculant corn silage, T2: corn silage inoculated by *saccharomyces cerevisiae* (*Saccharomyces cerevisiae D-47*), 10 gm yeast were solved in 30 ml distilled water / 10 kg silage) at a rate of 2.44×10^{11} cfu/g yeast product, T3: corn silage inoculated with *Trichoderma harzianum* (250 ml fungi solution / 10 kg silage) at a rate of 1.4×10^{4} fp/g fresh weight and T4: corn silage inoculated with *Saccharomyces cerevisiae* D-47 plus *Trichoderma harzianum* (5 gm of yeast dissolved plus 125 ml of fungi /10 kg of silage).

Corn ensiled in polyethylene bags sealed (1.5 to 2 kg) using a vacuum sealer, then bags stored at room temperature (25°C). Triplicate silos of each treatment (T1, T2, T3, and T4) at different ensiling times (zero time, 5 h, 10 h, 20 h, and 2, 4, 8, 14, 25 and 35 days) were opened, prepared and analyzed for chemical composition, silage fermentation characteristics and *in-vitro* digestibility. The chemical composition of DM, CP, EE, and ash for experimental corn silage was determined just before ensiling (Table 1) and at different ensiling times according to AOAC (2000). The NDF and ADF were performed as a description by Van Soest *et al*. (1991) with a fiber analysis device.

Table 1: The chemical composition (% on DM basis) of fresh corn forage before ensiling

Nutrients	Fresh corn forage
Dry matter, DM	32.9
Organic matter, OM	94.1
Crude protein, CP	8.73
Crude fiber, CF	22.35
Nitrogen free extract, NFE	62.22
Neutral detergent fiber, NDF	49.88
Acid detergent fiber, ADF	27.87

Values of silage pH were determined using a pH meter (Model HI 8424). Ammonia-N (NH3-N) concentration was determined according to Preston (1995).

Silage organic acids were determined using HPLC, where the separation was carried out using Eclipse AQ-C18 HP column (4.6 mm x 150 mm i.d., 3 μm), according to Madrid *et al*., (1999).

The total count of bacteria, lactic acid bacteria, and total yeasts were counting according to the microbiological method described by Collin *et al*. (1995) and Awad (2003).

For obtain rumen liquor, three adult Barki rams fitted with rumen fistula with an average body weight of 49 were fed high-quality hay as a basal diet and free water. Rumen liquor collected 4hr post feeding then filtered through 4 layers of cheesecloth and mixed with the buffered mineral solution at a ratio of 1:3 (rumen fluid to buffer, v/v). *In vitro* dry matter (IVDMD) and organic matter (IVOMD) degradability were estimated using the two-stage technique of Tilley and Terry (1963) as modified by Marten and Barnes (1979).

The obtained results were statistically analyzed using Statistical Analytical System (SAS, 2002), Version, 9.3.1, according to the following model:

$$Y_{ijk} = \mu + T_i + S_j + TS_{ij} + e_{ijk}$$

Where:

Y_{ijk} = the observation;

μ = Overall mean;

T_i = the fixed effect of the treatments

S_j= the fixed effect of the ensiling time

TS_{ij}= the interaction between treatments and ensiling time

e_{ijk} = Random error component assumed to be normally distributed.

Duncan's multiple range tests (Duncan, 1955) was performed to detect the significant differences among means.

RESULTS AND DISCUSSION

Effect of yeast and fungal inoculants on corn silage chemical composition and fiber content

Effect on chemical composition

Data in Table (2) present the effect of corn silage inoculants at different ensiling times on DM, OM, CP, CF, NFE, NDF and ADF content. No significant effect on the DM content of corn silage by inoculants was found, while it was significantly ($P < 0.04$) affected by the ensiling time. The oxygen contained in the packed forage enables biological and chemical processes that consume nutrients and energy, producing water, carbon dioxide, heat, and free ammonia before the active fermentation phase can start. This activity raises the temperature of the silage, which has a negative impact on the silage DM and quality losses (McAllister and Hristov, 2000; Holmes, 2006).

A decrease in DM content and quality losses throughout the ensiling process was observed by Borreani *et al*. (2018). Lower dry matter losses in corn silages made with additions comprising hetero- and homo-fermenting microorganisms compared to silages made without additives

(Rabelo *et al*., 2012 and Silva *et al*., 2014). According to a number of studies by Borreani *et al.* (2007), Bernardes *et al*. (2012), and Lattamae *et al*. (2012), silage with mould counts larger than 6 log10 cfu/g had DM losses of more than 20%. While Lima *et al*. (2017) and Borreani and Tabacco (2012, 2014) noted that losses could approach 40% of the initially ensiled DM. No interaction was observed between inoculant and ensiling time as shown in Table (2).

A similar trend was observed for OM, where inoculations had no significant effect on OM change in silage, while ensiling time led to a significant ($P < 0.05$) decrease in OM content from 94.12% to 93.23% at 35 days of vanishing. Borreani *et al*. (2018) noted a reduction in OM and quality losses as resulting of ensiling process. Rabelo *et al*. (2012) and Silva *et al*. (2014) attribute lower losses of organic and dry matter for corn silages to the use of additives containing hetero fermentative and homo fermentative microorganisms in relation to silages without additives. Kim *et al*. (2021) observed that LAB inoculants improve silage quality and reduce DM and OM losses under long-term storage. No interaction was observed between inoculant and ensiling time as shown in Table (2).

Treatments had a highly significant ($P< 0.005$) effect on CP. Crude protein content was 8.11, 9.32, 9.15, and 9.08% for control, Y, T, and both Y+T, respectively. No difference was found between Y and T and both. Ensiling time significantly ($P< 0.001$) increased CP content from 8.65% up to 9.33% at 35d of ensiling. Generally, the treated silages increased CP which means that the ensiling environment was good and silage quality was better. No interaction was observed between inoculant and ensiling time as shown in Table (2).

Aragon *et al*., (2012) reported that the high-quality silage is rich in energy and protein. Most silage inoculants have the ability to promote a beneficial fermentation that maximizes the nutritive value of the silage for ruminant livestock. The silage inoculants have improved the readily available energy and true protein content of silages (Jones, 1998; Davies *et al*., 2005; Wee *et al*., 2006; Haag *et al*., 2015 and Borreani *et al*., 2018). Crude fiber significantly ($P< 0.001$) decreased from 21.62 in the control (without inoculant) to 19.41, 19.52 and 19.37% in Y, T, and Y+T inoculants, respectively. Along with the time of ensiling CF decreased significantly ($P< 0.001$) while no interaction was observed between inoculant and time. According to Vieira *et al*. (2013), high-nutritional value corn silages have between 7 and 9% CP, 48 and 58% NDF, and 23 and 30% ADF. Sun *et al.* (2021), demonstrated that the fundamental goal of silage conservation is to keep nutritional value, particularly fiber, non-structural carbohydrates, and protein as closely as possible to the nutrients in the fresh plants before to ensiling.

Treatments had a significant ($P< 0.001$) increase from 61.36% in control up to 63.65, 63.48, and 63.45% for inoculates Y, T, and Y+T, respectively. Ensiling time shows a significant ($P< 0.01$) fluctuation effect on NFE with average value of 62.98%. Sun *et al*. (2021) reported that maintaining nutritional value, mainly fiber, non-structural carbohydrates (NFE), and protein as closely as feasible to the nutrients in the fresh plants before ensiling is the fundamental goal of silage conservation. Water-soluble carbohydrates (WSC) in the crop are fermented by epiphytic lactic acid bacteria into lactic acid and, to a lesser amount, acetic acid, which decrease NFE (Jalc *et al*., 2010; Rodrigues *et al*., 2015; Zurac *et al*., 2018 and Zhang *et al*., 2019).

Table 2: Effect of corn silage inoculants at different ensiling times on chemical composition and fiber content (%).

Item	Proximate analysis (%)					Fiber content (%)	
	DM	OM	CP	CF	NFE	NDF	ADF
Experimental silages							
C	32.31	90.41	8.11^{b}	21.62^{b}	61.36^{b}	47.64^{c}	25.63^{c}
Y	32.18	90.38	9.32^{a}	19.41^{a}	63.65^{a}	46.63^{b}	24.66^{b}
T	32.09	90.30	9.15^{a}	19.52^{a}	63.48^{a}	46.86^{b}	24.89^{b}
Y+T	31.88	90.32	9.08^{a}	19.37^{a}	63.45^{a}	45.68^{a}	23.64^{a}
Ensiling time							
0 hr.	32.78^{a}	94.12^{a}	8.65^{cd}	22.41^{a}	62.25^{a}	49.86^{f}	27.81^{e}
5 hr.	32.40^{ab}	92.72^{b}	8.36^{d}	21.31^{b}	62.69^{ab}	49.69^{f}	27.72^{e}
10 hr.	32.27^{ab}	90.60^{c}	8.87^{abcd}	20.45^{c}	63.13^{bc}	49.60^{f}	27.59^{e}
20 hr.	31.94^{b}	87.27^{e}	8.84^{abcd}	20.33^{c}	63.02^{bc}	48.30^{e}	26.40^{d}
2 d.	31.98^{b}	85.31^{f}	8.69^{cd}	20.12^{c}	62.93^{bc}	46.83^{d}	24.83^{c}
4 d.	32.12^{ab}	84.45^{g}	8.75^{bcd}	20.23^{c}	62.83^{abc}	46.17^{c}	24.44^{c}
8 d.	31.92^{b}	89.33^{d}	9.04^{abc}	19.98^{c}	62.65^{ab}	45.03^{b}	23.07^{b}
14 d.	31.97^{b}	93.29^{b}	9.29^{ab}	18.87^{d}	63.19^{bcd}	43.98^{a}	21.59^{a}
25 d.	31.85^{b}	93.18^{b}	9.35^{a}	18.06^{d}	63.44^{cd}	43.73^{a}	21.80^{a}
35 d.	31.91^{b}	93.23^{b}	9.33^{a}	18.04^{d}	63.73^{d}	43.82^{a}	21.78^{a}
SEM	0.07	0.32	0.07	0.17	0.12	0.24	0.24
P.value							
S	0.225	0.957	0.001	0.001	0.001	0.001	0.001
T	0.040	0.001	0.001	0.001	0.001	0.001	0.001
S*T	1.000	1.000	0.577	0.124	0.003	0.001	0.001

C: corn silage applied without inoculants, Y:corn silage applied with *yeast (Saccharomyces cerevisiae D-47)* .

T, corn silage applied with *Trichoderma harzianum*, Y+T: corn silage applied with *Saccharomyces cerevisiae D-47* plus *Trichoderma harzianum*, SEM, standard error of means, S: Silage treatment, T: Time, and S*T: interaction

^{a,b,c}means within each column with different superscript differ significantly.

Effect on fiber content

Neutral detergent fiber significantly (P< 0.001) decreased from 47.64 in the control (without inoculant) to 46.63, 46.86and 45.68% in Y, T and Y+T inoculants, respectively. Along with time of ensiling NDF gradually decreased from 49.86% at zero time to 43.82%; differences were highly significantly (P< 0.001). Significant interaction was observed between inoculant and time regarding NDF (Table 2). The data revealed that NDF content was linearly decreased with time of ensiling. Vieira *et al.* (2013) reported that corn silages of high-nutritional value have between 48 and 58% NDF, and 23 and 30% ADF. Sun *et al.* (2021) reported that the major aim of silage conservation is to maintain nutritional value, mainly fiber, non- structural carbohydrates, and protein as much as comparable to the nutrients in the fresh plants before ensiling as is humanly possible. Adesogan *et al*. (2010) illustrated that corn silage produced in warm climes often has

higher concentrations of NDF and less starch than corn silage produced in temperate settings. Additionally, plants grown in places with warm climates have decreased NDF digestibility (NDFD) (Cone and Engels, 1990; Adesogan *et al*., 2010).

The effect of silage inoculates and time of ensiling on ADF followed the same pattern of NDF. Acid detergent fiber significantly (P< 0.001) decreased from 25.63 in the control (without inoculant) to 24.66, 24.89 and 23.64% in Y, T and Y+T inoculants, respectively. Along with time of ensiling ADF gradually decreased from 27.81% at zero time to 21.78%; differences were highly significantly (P< 0.001); significant interaction was observed between inoculant and time regarding ADF (Table 2). The data revealed that ADF content was linearly decreased with time of ensiling. Vieira *et al.* (2013) reported that corn silages of high-nutritional value have between 48 and 58% NDF, and 23 and 30% ADF.

Sun *et al.* (2021) reported that the major aim of silage conservation is to maintain nutritional value, mainly fiber, non- structural carbohydrates, and protein as much as possible similar to the nutrients in the fresh plants before ensiling.

Effect of yeast and fungal inoculants on corn silage fermentation characteristics

Effect on pH

Values of silage pH (Table 3) revealed that inoculants decreased pH significantly (P< 0.001) from 5.09 in control to 4.64, 4.69 and 4.54 for Y, T and Y+T, respectively. Differences, however, between Y and T and both were not significant. Values of pH along with time of ensiling ADF gradually decreased from 6.01 at zero time to 3.9; differences were highly significantly (P< 0.001). Interaction was not observed between inoculant and time regarding pH values. The decrease in pH in silage was related to the concentration of lactic acid as illustration in Figure (1).

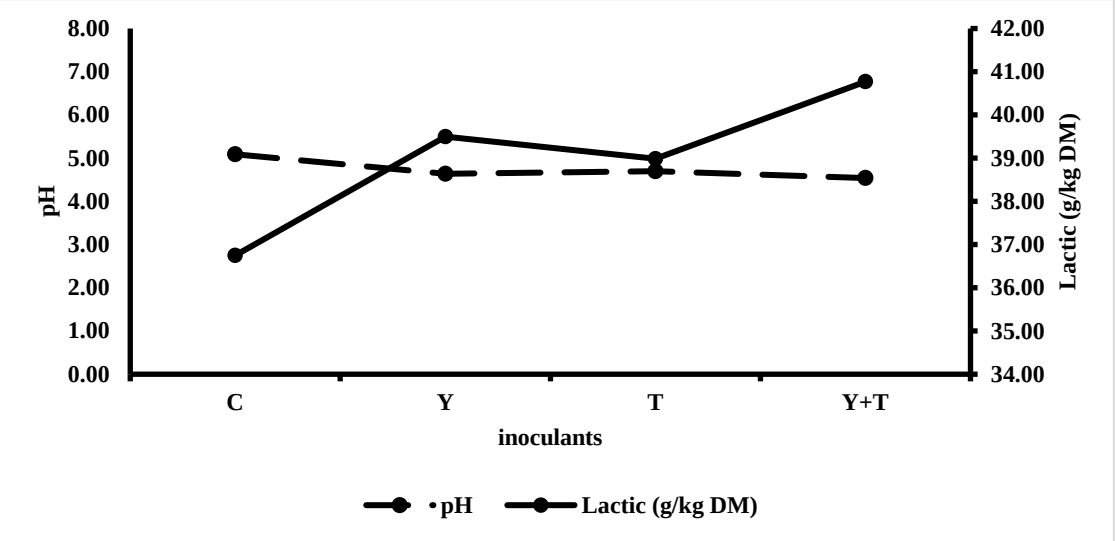

Fig. (1): The relationship between lactic acid concentration and pH shifting in inoculated silage compared with un-inoculated silage.

Effect on Ammonia-N

Table (3) presents the effect of corn silage inoculants at different ensiling times on corn silage ammonia-N (g/kg total N). Ammonia-N decreased from 39.06 in control comparing to 37.95, 38.44 and 37.0 g/kg total N. Differences were significant (P< 0.001). Ammonia nitrogen gradually increased with the time of ensiling in a curve-linear way. Differences were highly significant (P< 0.001). Kristensen *et al.* (2010) reported that *L. buchneri* inoculation increased silage pH and contents of ammonia and decreased lactic acid content in silages. Neither of the inoculation treatments affected milk production under field conditions compared with the control. Nkosi *et al.* (2011) noted that inoculant treatments enhanced intake, digestibility, and N retention of maize silage diets. They also had a favourable impact on the fermentation of maize silage. In maize silage, *Lactococcus lactis* raised the lactic acid content while lowering the ammonia N content.

Effect on organic acids

Results summarized in Table (3) indicated that lactic acid concentration in silages of control was 36.75 g/kg DM. Treating silages with inoculant yeast (Y), trichoderma (T) led to an increase (P< 0.001) in lactic acid concentration being 39.5 and 38.99 g/kg DM, respectively. The best value was that of Y+T treatment (40.77g/kg DM). Lactic acid concentration was not detected up to 20h, and concentration thereafter increased (P< 0.001) in a curve linear manner to reach the peak at 14d from ensiling. Ensiling is a technique for preserving forage that relies on anaerobic spontaneous lactic acid fermentation. Water-soluble carbohydrates (WSC) in the crop are fermented by epiphytic lactic acid bacteria into lactic acid and, to a lesser extent, acetic acid (Weinberg and Muck, 1996; Merry *et al.,* 1997; Jalc *et al.,* 2010; Rodrigues *et al*., 2015; Zurac *et al*., 2018 and Zhang *et al*., 2019).

Organic acid concentration in silages (formic, citric and succinic acids) of control, Y, T and Y+T was 92.52, 92.39, 92.36 and 92.32mg/kg DM, respectively for formic acid; the respective values for citric acid were 84.95, 84.68, 84.62 and 84.49mg/kg DM and that for succinic acid were 4.49, 5.66, 5.61, 6.26mg/kg DM. Differences between inoculant groups were not significant for formic and citric acids but significant (P< 0.001) for succinic acid. Treating silages with inoculant yeast (Y), *trichoderma* (T) led to an increase (P< 0.001) in succinic acid concentration. Davies *et al*., (2007) demonstrated that a variety of products can accumulate in silage organic acids as a result of the fermentation, which may be carried out by a number of both facultative and strictly anaerobic bacteria that enter the silo on the forage. Concentration of acetic and butyric acid concentration; it was not detected up to 20h, and concentration thereafter increased (P< 0.001) in a curve linear manner to reach the peak at 4d from ensiling for acetic acid and 8d for butyric acid. Results summarized in Table (3) indicated that acetic acid concentration in silages of control was 27.28g/kg DM. Treating silages with inoculant yeast (Y), *trichoderma* (T) or both (Y+T) led to a decrease (P< 0.001) in acetic acid concentration being 26.42, 26.78 and 26.69g/kg DM, respectively. Results of acetic acid took almost the same trend as lactic acid concentration.

The stability of silage can be increased by formic, acetic, propionic, and butyric acids as well as more volatile fatty acids as valeric and caproic. (Ohyama and McDonald, 1975; Ohyama *et al*., 1975; Woolford, 1975; Woolford, 1978; Ashbell and Lisker, 1988; Detmer *et al.,* 1999; Meeske *et al.,* 2002; Kung *et al.,* 2000 and Nkosi *et al*., 2011). Ensiling fermentation under anaerobic conditions makes epiphytic lactic acid bacteria ferment the water-soluble carbohydrates (WSC) in the crop to lactic acid, and to a lesser extent to acetic acid. (Weinberg and Muck, 1996; Merry *et al.,* 1997; Jalc *et al.,* 2010; Rodrigues *et al*., 2015; Zurac *et al*., 2018 and Zhang *et al*., 2019). Ranjit and Kung (2000) suggested that there is a relationship between the amount of yeast in silage and its aerobic stability. A drop in overall lactic acid concentrations and an increase in acetic acid concentrations will happen at the high inoculation rate. The silage VFA profile had no impact at the modest rate of inoculation. The amount of yeast was significantly decreased and the concentration of acetate was doubled at the high rate, nevertheless.

Table (3): Effect of yeast and fungal inoculants at different ensiling times on corn silage fermentation characteristics

Item	Measurements							
	pH	NH_3-H g/kg total N	Lactic (g/kg DM)	Formic (mg/kg DM)	Citric (mg/kg DM)	Succinic (mg/kg DM)	Acetic (g/kg DM)	Butyric (g/kg DM)
Experimental Silage								
C	5.09^{b}	39.06^{d}	36.75^{d}	92.52	84.95^{b}	4.49^{c}	27.28^{b}	0.286^{b}
Y	4.64^{a}	37.95^{c}	39.50^{b}	92.39	84.68^{ab}	5.66^{b}	26.42^{a}	0.251^{a}
T	4.70^{a}	38.44^{b}	38.99^{c}	92.36	84.62^{ab}	5.61^{b}	26.78^{a}	0.250^{a}
Y+T	4.54^{a}	37.00^{a}	40.77^{a}	92.32	84.49^{a}	6.26^{a}	26.69^{a}	0.225^{a}
Ensiling times								
0hr.	6.01^{e}	20.32^{a}	ND	93.09^{a}	85.09^{a}	4.13^{a}	ND	ND
5hr.	5.89^{e}	20.44^{a}	ND	92.72^{ab}	84.90^{ab}	4.88^{b}	ND	ND
10hr	5.85^{e}	27.22^{b}	ND	92.52^{ab}	84.87^{ab}	4.97^{b}	ND	ND
20 hr.	4.79^{c}	33.90^{c}	ND	92.22^{b}	84.76^{ab}	5.57^{c}	17.19^{e}	0.145^{e}
2 d.	4.57^{c}	38.91^{d}	20.31^{d}	92.27^{b}	84.72^{ab}	5.74^{c}	20.70^{d}	0.198^{d}
4 d.	4.26^{b}	42.07^{e}	30.80^{c}	92.13^{b}	84.50^{ab}	5.80^{c}	31.45^{a}	0.274^{c}
8 d.	4.16^{ab}	46.88^{f}	39.92^{b}	92.18^{b}	84.74^{ab}	5.79^{c}	30.98^{a}	0.313^{a}
14 d.	4.00^{ab}	50.19^{g}	47.41^{a}	92.22^{b}	84.65^{ab}	5.79^{c}	29.48^{b}	0.299^{ab}
25 d.	3.96^{ab}	50.53^{gh}	47.68^{a}	92.43^{ab}	84.41^{ab}	6.20^{d}	28.88^{c}	0.305^{ab}
35 d.	3.90^{a}	50.70^{h}	47.90^{a}	92.19^{b}	84.19^{b}	6.19^{d}	28.86^{c}	0.288^{bc}
SEM	0.08	1.06	1.24	0.06	0.07	0.09	0.57	0.01
P-value								
S	0.001	0.001	0.001	0.740	0.135	0.001	0.001	0.001
T	0.001	0.001	0.001	0.008	0.203	0.001	0.001	0.001
S*T	0.424	0.001	0.230	1.000	1.000	0.001	0.001	0.207

C: corn silage applied without inoculants, Y: corn silage applied with *yeast (Saccharomyces cerevisiae D-47)*

T, corn silage applied with *Trichoderma harzianum*, Y+T: corn silage applied with *Saccharomyces cerevisiae D-47* plus *Trichoderma harzianum*, ND: not detected, SEM: standard error of means, S: Silage,

T: Time and S*T: interaction

$^{a,b,c \text{ and } d}$ means within each column with different superscript differ significantly.

Effect of yeast and fungal inoculants on corn silage microbial counts

Table (4) presents the effect of corn silage inoculants on microbial counts. There was a significant (P< 0.001) effect on total bacteria (log_{10} cfu/g DM), lactic acid bacteria (log_{10} cfu/g DM) and total yeasts (log_{10} cfu/g DM). Treating silages with inoculant significantly increased total bacteria (5.51, 7.69, 7.69 and 7.81 log_{10} cfu/g DM) for the control, Y, T and Y+T, respectively.

Similarly treating silages with inoculant significantly (P< 0.001) increased lactic acid bacteria (6.46, 6.89, 6.97 and 7.03 log_{10} cfu/g DM) for the control, Y, T and Y+T, respectively. Results of total yeasts (log_{10} cfu/g DM) followed the same pattern being less for

control (5.45) and increased with the inoculant treatment being 6.57, 6.44 and 6.89 for the same respective order. Differences were significant (P< 0.001). Ensiling time lead to increase linearly of total bacteria, yeast followed the same pattern being linearly increased with ensiling time; however results indicated that lactic acid bacteria decreased from zero time to 2d after which the lactic acid bacteria increased sharply to reach maximum value at 35d of ensiling. Differences were significant (P< 0.001). The results generally indicated that inoculation with either inoculant or both together led to production of good quality silage. The production of corn silage requires incorporating the entire plant, as Richard *et al*. (2007) showed, and the storage of corn silage is based on the principle of preservation in anaerobic circumstances with the development of lactic acid bacteria. The pH is naturally lowered by these bacteria to a level that is regarded unfavorable for the growth of clostridia and most mild bacteria. Sun *et al*., (2021) reported that Lactobacillus dominated the bacterial community after two day of ensiling and had a decline in abundance during the stable phase in whole-plant corn silage with low (pH < 4.0).

Table (4): Effect of corn silage inoculants at different ensiling times on corn silage microbial count

Item	Measurements		
	Total bacteria (log_{10} cfu/g DM)	**Lactic acid bacteria (log_{10} cfu/g DM)**	**Total yeasts (log_{10} cfu/g DM)**
Treatments			
C	5.51^{b}	6.46^{b}	5.45^{c}
Y	7.69^{a}	6.89^{a}	6.57^{b}
T	7.69^{a}	6.92^{a}	6.44^{b}
Y+T	7.81^{a}	7.03^{a}	6.89^{a}
Ensiling times			
0 hr.	6.88^{c}	6.90^{b}	6.47^{bc}
5 hr.	6.89^{c}	6.93^{b}	6.86^{b}
10 hr.	6.98^{bc}	6.60^{b}	6.76^{b}
20 hr.	6.94^{bc}	5.85^{c}	6.94^{b}
2 d.	7.08^{bc}	4.75^{d}	6.90^{b}
4 d.	7.17^{bc}	5.76^{c}	7.61^{a}
8 d.	7.33^{ab}	6.60^{b}	6.11^{c}
14 d.	7.13^{bc}	8.16^{a}	5.33^{d}
25 d.	7.67^{a}	8.33^{a}	5.24^{d}
35 d.	7.69^{a}	8.36^{a}	5.16^{d}
SEM	0.10	0.12	0.10
P-value			
S	0.001	0.001	0.001
T	0.001	0.001	0.001
S*T	0.996	0.226	0.002

C: corn silage applied without inoculants, Y: corn silage applied with *yeast (Saccharomyces cerevisiae D-47)* .T, corn silage applied with *Trichoderma harzianum*, Y+T: corn silage applied with *Saccharomyces cerevisiae D-47* plus *Trichoderma harzianum*, hr: hours . d: day, SEM, standard error of means, S: Silage, T: Time, and S*T: interaction

$^{a,b,c \text{ and } d}$ means within each column with different superscript differ significantly.

Effect of yeast and fungal inoculant on IVDMD and IVOMD of corn silage

Data in Table (5) show the effect of inoculant on *in vitro* corn silage digestibility. Inoculants increased IVDMD significantly (P< 0.001); Values were 42.59, 46.09, 44.66 and 47.64% for C, Y, T and Y+T, respectively. The best value of IVDMD was that of corn silage inoculated with both yeast + trichoderma. Values of IVOMD followed the same pattern being low for C (63.3%) and higher for Y (65.5%) and T (65.25%) and highest for Y+T (67.25%). Time of ensiling had almost no effect on *in vitro* digestibility. Values of IVDMD ranged between 44.5 and 45.9%; the respective values of IVOMD ranged between 64.8 and 65.9%. Muck *et al.* (2017) reported that silage additives are expected to directly inhibit clostridia and other detrimental microorganisms, enhance aerobic stability, improve cell wall digestibility.

Table (5): Effect of corn silage inoculants at different ensiling times on IVDMD and IVOMD of corn silage

Item	Measurements	
	IVDMD	**IVOMD**
Treatments		
C	42.59^{d}	63.30^{c}
Y	46.09^{b}	65.50^{b}
T	44.60^{c}	65.25^{b}
Y+T	47.64^{a}	67.25^{a}
Ensiling times		
0 hr	44.50^{c}	64.84^{c}
5 hr	45.10^{bc}	65.21^{bc}
10 hr	45.05^{bc}	65.18^{bc}
20 hr	45.17^{b}	64.89^{bc}
2 d	45.28^{b}	64.87^{bc}
4 d	45.20^{b}	65.95^{a}
8 d	45.43^{ab}	65.50^{ab}
14 d	45.25^{b}	65.40^{abc}
25 d	45.38^{ab}	65.50^{ab}
35 d	45.92^{a}	65.90^{a}
SEM	0.18	0.14
P-value		
S	0.001	0.001
T	0.004	0.001
S*T	0.667	0.767

C: corn silage applied without inoculants, Y: corn silage applied with *yeast (Saccharomyces cerevisiae D-47)* .T, corn silage applied with *Trichoderma harzianum*, Y+T: corn silage applied with *Saccharomyces cerevisiae D-47* plus *Trichoderma harzianum*, hr: hours . d:day, SEM, standard error of means, S: Silage, T: Time, and S*T: interaction

$^{a,b \text{ and } c}$ means within each column with different superscript differ significantly.

The majority of silage inoculants have been created in order to maximize silage nutritional value for ruminant livestock through the promotion of a beneficial fermentation. For these reasons, they have been based on homo-fermentative lactic acid bacteria, which produce lactic acid as their main end product of fermentation. As a result, they have increased the amount of true protein and available energy in silages. (Jones, 1998; Davies *et al.*, 2005; Wee *et al*., 2006; Haag *et al*., 2015 and Borreani, *et al*., 2018). Nkosi *et al.*, (2011) reported that the inoculant treatments boosted intake and apparent digestibility while also having a favorable impact on the fermentation of corn silage.

CONCLUSION

It could be concluded that the inoculation of yeast (*Saccharomyces cerevisiae D 47*) and *Trichoderma harzianum* in corn silage may improve the silage quality. The results indicate an increased CP content in corn silage parallel with increasing ensiling time. This indicates that the inoculation of yeast and T. harzianum provided a suitable environment for fermentation conditions. Although the inoculants caused less DM and nutrient loss; decrease CF, NDF and ADF, their addition increased the number of bacteria and the concentration of lactic acid. Value of pH in silage was in the appropriate range for the ensiling process leading to better digestion (*in- vitro*). Inoculated with both yeast and trichoderma recorded the best value of IVDMD and IVOMD comparing with the others treatments .The results of this study boost using microorganisms of the fourth generation such as yeast and Trichoderma, which have a probiotic effect and thus direct enhancement of animal performance. Consequently, more studies are still needed in this regard.

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تأثير استخدام سلالات معينة من الخميرة والفطر كلقاحات للسيلاج على التركيب الكيماوى وخصائص التخمر و الهضم المعملي لسيلاج الذرة

ماجد مروان محمد على[١]، جمال أحمد براغيت[١]، بركات محمد أحمد[١]، عمرو محمد المصري[٢]، مي عبدالحميد سالم[١]، أسامة ابوالعز نايل[١]

(١) قسم الانتاج الحيوانى – كلية الزراعة – جامعة المنوفية

(٢) قسم النبات اللزراعى - كلية الزراعة – جامعة المنوفية

الملخص العربى

أجريت الدراسة الحالية بمعمل التغذية بقسم الإنتاج الحيواني بكلية الزراعة جامعة المنوفية لدراسة تأثير سلالات معينة من الفطر (*Trichoderma harzianum*) والخميرة (*Saccharomyces cerevisiae D-47*) على التركيب الكيميائي للسيلاج وخصائص التخمر والهضم المعملي. وتم إجراء التجربة باختبار أربعة معاملات، وهي كالتالي: مجموعة المقارنة : (C) سيلاج الذرة بدون إضافات، المعاملة :Y سيلاج الذرة المعامل بالخميرة-Saccharomyces cerevisiae D 47،المعاملة :T سيلاج الذرة المعامل بفطر Trichoderma harzianum و المعاملة Y+T: سيلاج الذرة المعامل بمخلوط من الفطر والخميرة . تم نقل الذرة الكاملة المفرومة وتحضينها مع الغلق المحكم في أكياس بلاستيكية (١,٥ إلى ٢ كجم) وتم استخدام جهاز تفريغ الهواء لإزالة الهواء من الأكياس بعد التعبئة. تم تخزين الأكياس في درجة حرارة الغرفة لأزمنة متتالية (وقت صفر ، ٥ ساعات ، ١٠ ساعات ، ٢٠ ساعة ، ٢ ، ٤ ، ٨ ، ١٤ ، ٢٥ و ٣٥ يومًا.) وتم تقدير قيم كل من حموضة السيلاج pH - تركيز الأمونيا N -و تقدير الأحماض العضوية بالسيلاج (حمض اللاكتيك ، حمض الخليك ، حمض الفورميك، حمض الستريك وحمض السكسينيك). كما تم تقدير العدد الكلي للبكتيريا وبكتيريا حمض اللاكتيك والخمائر الكلية فى عينات السيلاج . تم استخدام تقنية المرحلتين (Tilley and Terry 1963) لتحديد معاملات الهضم المعملية لكل من المادة الجافة والعضوية.

وكانت أهم النتائج:

١- لم يكن للمعاملات تأثير معنوي على المادة الجافة بينما أدى زيادة مدة الحفظ إلى انخفاض معنوي في محتوى DM من ٣٢,٧٨٪ إلى ٣١,٩١٪ عند ٣٥ يوم - اتخذت المادة العضوية اتجاه مماثل مع التقدم في زمن الحفظ حيث انخفض محتوى المادة العضوية من٩٣,٢٣٪ إلى ٩٤,١٢٪ عند ال ٣٥ يوما التالية.

٢- كان للمعاملات تأثيرا معنويا على المحتوى البروتيني حيث كان محتوى البروتين الخام ٨,١١ و ٩,٣٢ و ٩,١٥ و٩,٠٨٪ لكل من معاملات المقارنة و Y و T وكلاهما معا Y + T على التوالي. أدى زيادة زمن الحفظ إلى زيادة المحتوى البروتيني بشكل ملحوظ في اتجاه خطي إيجابي مع ازمنة الحفظ المتتالية كما أشارت نتائج السيلاج المعامل إلى جودة بيئة الحفظ مما يعني أن البيئة الناتجة كانت جيدة وأن جودة السيلاج كانت أفضل.

٣- انخفضت نسبه الألياف الخام بشكل معنوي من ٢١,٦٢ في المعاملة المقارنة إلى ١٩,٤١ و ١٩,٥٢ و ١٩,٣٧٪ في المعاملات Yو T و.Y + T ومع مرور زمن الحفظ انخفض تركيز الألياف الخام بينما لم يلاحظ أي ارتباط بين نوع اللقاح والزمن.

٤- انخفضت نسبة ألياف المذيبات المتعادلة NDF بشكل معنوي من ٤٧,٦٤ في المجموعة المقارنة إلى ٤٦,٦٣ و ٤٦,٨٦ و ٤٥,٦٨٪ في التلقيح Y و T و Y + T على التوالي كما انخفضت نسبة الألياف المتعادلة NDF مع زمن

الحفظ تدريجياً ولوحظ ارتباط كبير بين اللقاح وزمن الحفظ واتخذت الألياف الحمضية ADF نفس نمط الألياف المتعادلة.NDF

٥- أدت اللقاحات إلى انخفاض الأس الهيدروجيني PHبشكل كبير من ٥,٠٩ في المعاملة المقارنة إلى ٤,٦٤ و ٤,٦٩ و ٤,٥٤ لكل من Y و T و Y + T على التوالي وانخفضت قيم الأس الهيدروجيني PH مع زمن الحفظ تدريجياً من ٦,٠١ وقت الصفر إلى ٣,٩ عند ٣٥ يوم.

٦- انخفضت نسبة نيتروجين الأمونيا معنويا من ٣٩,٠٦ في المجموعة المقارنة مقارنة بـ ٣٧,٩٥ و ٣٨,٤٤ و ٣٧,٠ جم / كجم إجمالى النيتروجين وكانت الفروق معنوية ($P < 0.001$) .ازدادت نسبة نيتروجين الأمونيا تدريجياً بصورة معنوية بزيادة زمن الحفظ بانحدار موجب بعلاقة خطية.

٧- كان تركيز حامض اللاكتيك في سيلاج المعامله المقارنة ٣٦,٧٥ جم / كجم .DM وأدت معاملة السيلاج بالخميرة (Y)والفطر trichoderma (T) إلى زيادة معنويه في تركيز حمض اللاكتيك بواقع ٣٩,٥ و ٣٨,٩٩ جم / كجم مادة جافة على التوالي. كانت أفضل قيمة هي معاملة Y + T (٤٠,٧٧ جم / كجم مادة جافة)

٨- كان تركيز حمض الفورميك في السيلاج لكل من المعاملات: المقارنة و Yو T و Y + T كان ٩٢,٥٢ و ٩٢,٣٩ و ٩٢,٣٦ و ٩٢,٣٢ مجم / كجم على التوالي . بينما كانت القيم الخاصة بحمض الستريك ٨٤,٩٥ و ٨٤,٦٨ و ٨٤,٦٢ و ٨٤,٤٩ ملجم / كجم من المادة الجافة و ٤,٤٩ و ٥,٦٦ و ٥,٦١ و ٦,٢٦ ملجم / كجم DM لحمض السكسينيك. ارتبط زمن الحفظ سلبياً بتركيز حامض الفورميك والستريك بينما كان مرتبطاً إيجابياً بتركيز حمض السكسينيك.

٩- أشارت النتائج إلى أن تركيز حامض الخليك في سيلاج المعاملة المقارنة كان ٢٧,٢٨ جم / كجم DM. أدت معاملة السيلاج بالخميرة (Y) أو الفطر (T) أو كليهما (Y + T) إلى انخفاض في تركيز حامض الخليك بواقع ٢٦,٤٢ و ٢٦,٧٨ و ٢٦,٦٩ جم / كجم المادة الجافة على التوالي.

١٠- كان هناك تأثير معنوي للمعاملات على العد الكلى للبكتيريا وبكتيريا حمض اللاكتيك والخمائر الكلية حيث أدت معالجة السيلاج باللقاح إلى زيادة البكتريا الكلية (٥,٥١ ، ٧,٦٩ ، ٧,٦٩ ، ٧,٨١) للمجموعة المقارنة وY و T و Y + T على التوالي.

١١- أدت معالجة السيلاج باللقاح إلى زيادة معنوية في بكتيريا حمض اللاكتيك (٦,٤٦ ، ٦,٨٩ ، ٦,٩٧ ، ٧,٠٣ للمجموعة المقارنة وY و T و Y + T على التوالي. نتائج الخمائر الكلية (log10 cfu / g DM) اتبعت نفس النمط حيث كانت أقل للمعاملة المقارنة (٥,٤٥) وتزداد مع زيادة زمن الحفظ.

١٢- أدت اللقاحات الى زيادة معنوية فى قيم معاملات الهضم المعملية للمادة الجافة والعضوية . وسجلت المعاملة Y+T (سيلاج الذرة المعامل بمخلوط من الفطر والخميرة) القيم الأعلى لمعاملات هضم المادة الجافة والعضوية.