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Study of microbial inoculants effect on the quality of corn stover silage planted in different areas in Kelantan

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Study of microbial inoculants effect on the quality of corn stover silage planted in different areas in Kelantan

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Abstract. Corn stover silage serves as high-energy forage for the ruminant animal as it consists of a relatively high energy content, good digestibility and palatable. The microbial inoculants applied to silage will increase the good bacteria concentration, resulting in a higher lactic acid level, resulting in a quicker drop in the pH. The present study aimed to investigate microbial inoculants' effects on the quality of corn stover silage planted in different areas in Kelantan. The samples of corn plant collected from PPK Tanah Merah and Agropark, UMK Jeli Campus. Each sample was treated with an effective microorganism (EM) as the treatment with the ratio used was 1: 1: 1⁄2: 4: 1, for molasses, salt, urea, water and EM. In contrast, the sample with no inoculation of EM served as the control. All samples were ensiled for 21 days to obtain complete fermentation. The chemical composition of the silage determined by proximate analysis. The pH value and physical characteristics of the silage were recorded. All collected data were subjected to analysis of variance. After 21 days, moisture (66.47% and 69.70%) and crude protein (13.8% and 21.2%) content were significantly higher ($p<0.05$) for both treatment samples from Agro Techno Park (AGPK) Universiti Malaysia Kelantan and Persatuan Peladang Kawasan (PPK), Jedok, Tanah Merah compared to the control. The results showed a significantly lower pH value (3.76 –3.81) ($p<0.05$) for samples treated with EM compared to control. All samples' physical characteristics expressed a good ranging of quality silage as the texture was firm and not slimy with greenish-brown and yellowish-brown colour with a pleasant odour. There was no presence of mould observed for all samples except for control from AGPK. As a conclusion, the microbial inoculants applied to corn stover silage and fermented for 21 days had improved the moisture and crude protein content and decreased the contamination level, thus improved the quality of corn stover silage.

1. Introduction

Due to the higher price of widely used feed materials, such as ground corn, soybean meal and high quality forages, the benefit of ruminant production in Malaysia has decreased in recent years [1]. In general, as the feed costs are the largest component of most livestock animal industry overall operating expenditure, reducing feed costs will be an efficient way to increase profitability [2]. Besides, to maintain good health and better performance of the animals, sufficient supplies of high-quality feed are required for the whole period [3]. However, due to high feeding costs, the producer tends to provide a less expensive but low quality of feed for their ruminant animals [4]. The feed given should be of acceptable quality in nutritional value to fatten the ruminants and achieve better carcass quality. The utilisation of available crop residues may help ruminant producers cut the animal's cost [5]. In 2019, the area used for cultivating sweet corn plants in Malaysia was estimated to be more than 10,362 hectares, making corn an important economic crop for Malaysia [6].

From a global perspective, corn is an elementary and essential feed for farm animals in all forms. Feed products consisting of a corn plant have approximately high energy nutrients and a relatively low crude protein value with a low biological value [7]. To promote a better quality of corn stover silage, the selected microbes were identified as a stimulant. Furthermore, corn stover silage production is cost-efficient as it used the waste of agriculture product. Usually, the additives were added at ensiling, improving the ensiling rate, reducing losses, reducing aerobic deterioration, improving the silage's hygienic quality, limiting secondary fermentation, improving aerobic stability, and finally, increase the nutritional content of the silage [8]. Non-protein nitrogen (NPN) as listed in Table 1, has been added to raise the crude protein content in corn stover silage [9]. Likewise, it can be preserved for a more extended period in proper storage and feed livestock during unpleasant situations. Thus, the present study's objective was to evaluate the effect of microbial inoculation in corn stover silage compared to the untreated control on the physical characteristics, pH value and chemical composition. Our objective was to test whether the inoculation strategy affected the silage quality of corn planted in two different areas in Kelantan.

Table 1. Commonly used NPN additives for corn stover silage [8].

Urea type	Nitrogen content (%)	Urea equivalent*
Anhydrous ammonia	82	0.55
Aqueous ammonia	21	2.14
Urea, 42% N	42	1.07
Urea, 45% N	45	1.00
Urea, 46% N	46	0.98
Urea, 47% N	47	0.96

*The amount of each source given will provide non-protein nitrogen (NPN) equivalent to 1 pound of 45% N urea.

2. Materials and Methods

2.1. Experimental design and preparation of the samples

The primary raw materials that were used in this research are the entire harvest of whole-crop sweet corn (except fruits) (*Zea mays* L.) and Effective Microorganism (EM) consists of Lactic Acid Bacteria (LAB), Photosynthetic bacteria, Actinomycetes and yeast. The corn stover was collected from two different areas in Kelantan which are from a cornfield at Persatuan Peladang Kawasan (PPK) Jedok at Tanah Merah, Kelantan and Agro Techno Park (AGPK), Universiti Malaysia Kelantan (UMK), Jeli Campus with the average of 80 days of cultivation period. The corn stover silages from both areas received the same inoculation treatment. Treatments were assigned as follows: control (no inoculation of EM) and EM treatment (the ratio used for this treatment is 1: 1: 1⁄2: 4: 1, which means 1% of molasses, 1% of salt, 1⁄2 % of urea, 4% of water and 1% of EM). For both the control and treatment samples, there were three replications produced. All treatment samples were compacted into airtight plastic containers and were degassed, sealed and stored at room temperature (27 °C) for 21 days.

2.2. Preparation of corn stover silage

The whole-crop sweet corn has been chopped 10 cm height above the ground and left for four (4) to six (6) hours to wilt. Next, the crops were chopped at a range of one (1) to three (3) cm in length using chopper. The shorter in size gives better compaction during silage handling [10]. The chopped corn stover was divided into treatments with two (2) kg for each. For the treatment, samples were treated with 1% of molasses, 1% of salt, 1⁄2 % of urea, 4% of water and 1% of EM. After that, the same steps were repeated for the next samples but without EM as control (C) of this experiment. Then, samples were filled into small airtight plastic containers. Each of the containers were compressed properly to remove the remaining oxygen. The lid of containers was sealed tightly to avoid any air from entering. The containers were kept in a dry and safe place for 21 days. After 21 days, the samples were taken to the animal feed laboratory at Faculty of Agro Based Industry, UMK for further analysis.

2.3. Chemical analysis and pH determination
The collected samples were tested using a proximate analysis following the Association of Official Analytical Chemists (AOAC) International [11]. The analysis was done for the determination of dry matter (DM) content using method 934.01. Samples were dried by the oven at 70˚C for 48 hours. Next, the crude protein (CP) analysis was conducted using the Kjeldahl technique and required three steps: digestion, distillation and titration following method 2001.11. The crude fibre (CF) and ether extract (EE) analysis was carried out using a Soxtec 2055 (Method 954.02) and Fat Extraction System machine by FOSS, USA. Finally, the ash content was regulated after incinerated at 550˚C for 3 hours. The pH of the samples was defined following the method described in [10]. In 20 ml of distilled water, two grammes of sample were mixed and allowed to stand for 15 minutes and filtered with the Whatman filter paper. The LAQUAtwin Compact pH Meter by Horiba was used to measure the pH of the silage.

2.4. Physical characteristics observation
A physical analysis is an analysis of the five senses: sight, smell, hearing, taste and touch, based on the look and appearance of the sample. The physical appearance and texture, colour, presence of mould and scent of all samples were physically examined. These parameters were measured based on the guideline from [12].

2.5. Data collection and analysis
All data were subjected to analysis of variance using SPSS software (version 23). The Shapiro-Wilk test defined the normality or non-normality of distribution. A one-way ANOVA was used to analyse the collected data for chemical composition parameters and pH of corn stover silage. The differences between treatments were compared using Duncan Multiple Range Test (DMRT) at the 5% probability levels. The data are presented as the mean and standard error of the means (SEM) for all values.

3. Results and Discussion

3.1. The chemical composition and pH of corn stover silage
The comparison of chemical composition between control and EM treated samples of corn stover silage is shown in Table 2. The compositions that were analyzed include dry matter (DM), moisture, crude protein (CP), ether extract (EE) and ash content. The microbial inoculants had significantly improved the quality of corn stover silage from both AGPK and PPK compared to the control. The corn stover silage with microbial inoculant contained 30.3% and 33.5% dry matter. A study reported that dry matter content about 25 - 40 % in silage was expressed as an appropriate animal feed diet [13].

The silage that was treated with EM showed statistically significant higher ($p < 0.05$) in moisture content (69.7% and 66.5%) and crude protein (13.8% and 21.2%) compared to untreated silage. As excessive water seepage can be prevented, high-quality silage should contain 60% to 75% moisture [13, 14]. If moisture content is higher than 75 % in corn stover silage, it will result in lower nutrient content and affect the palatability [15]. The CP's content suggests that the microbial inoculant leads to an increase in the nutritional value of corn stover silage [16, 17]. There were no significant differences (p>0.05) between control and EM treated samples in ether extract (0.37% and 0.33%) and crude ash (20.3% and 4.9%). In line with current finding, Aragon [18] reported that the microbial inoculant does not affect the increase of ether extract while Aioanei and Pop [19] reported no effect on the ash content. Though the normal range for ash content in silage usually does not exceed 5% and if it is more than 10%, the sample can be classified as contaminators such as soil or manure [16, 19]. Figure 1 below shows the difference in the chemical composition of AGPK and PPK corn stover silage in each variable.

Table 2. The different in chemical composition and pH of corn stover silage from AGPK and PPK.

Composition (%)	Control	Treated with EM	*p* value
	Sample from AGPK		
Dry matter	33.38 ± 0.50^{b}	30.30 ± 0.34^{a}	**
Moisture	66.62 ± 0.50^{a}	69.70 ± 0.34	**
Crude protein	10.61 ± 0.03^{a}	13.82 ± 1.03	*
Ether extract	0.28 ± 0.02	0.37 ± 0.06	ns
Ash	24.30 ± 0.34	20.30 ± 0.34^{a}	***
pH	3.89 ± 0.03	3.76 ± 0.01^{a}	*
	Sample from PPK		
Dry matter	37.02 ± 0.43	33.53 ± 0.29	**
Moisture	62.93 ± 0.43	66.47 ± 0.29	**
Crude protein	17.51 ± 0.58	21.18 ± 0.83	*
Ether extract	0.16 ± 0.05	0.33 ± 0.08	ns
Ash	4.83 ± 0.33	4.91 ± 0.25	ns
pH	3.99 ± 0.04	3.81 ± 0.01	*

*$p<0.05$; ** $p<0.01$; *** $p<0.001$; ns - means within the same column with no letters are not significantly different.
a,b,c - means within rows with different letters are significantly different.

The pH value of samples from both AGPK and PPK treated with EM has a significantly lower value ($p < 0.05$) compared to the control with 3.76 and 3.81, respectively. When the pH is lower 4.2, the fungus and yeast would stop growing and maintain a good silage quality [7] as low pH limits the growth of undesirable bacteria during storage. Good silage consists of large lactic acid production, which results in lower pH value and prevents spoilage microorganism [20]. Wet silage contributed to high pH reading and usually contained a high level of ammonia (> 15%); this shows a poor fermentation and leads to scouring [14].

3.2. Physical characteristics of corn stover silage

The physical characteristics of the samples have been observed to know their quality in various ways. The outcome in terms of texture, colour, aroma, and mould presence of corn stover silage's physical characteristics are displayed in Table 3.

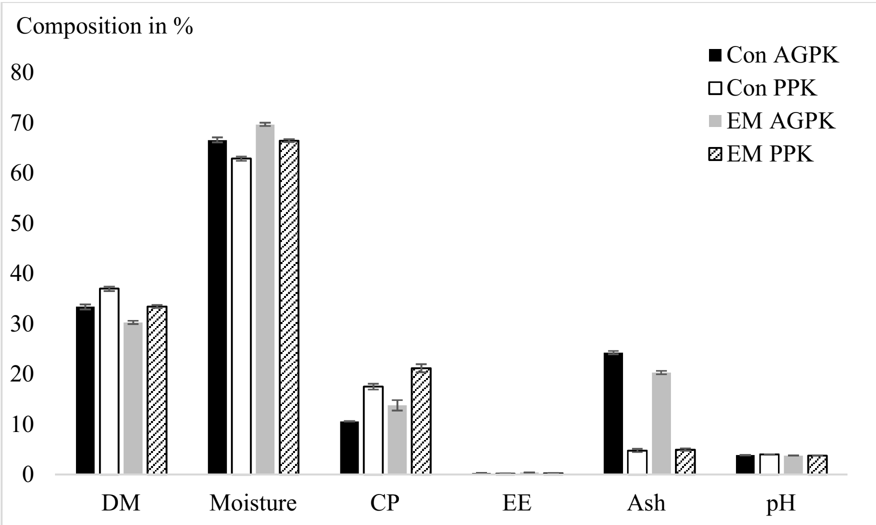

Figure 1. The comparison of chemical composition of corn stover silage from AGPK and PPK.

Table 3. The observation of physical characteristics of corn stover silage from AGPK and PPK.

Physical characteristic	Corn stover silage			
	AGPK		PPK	
	Control	EMT	Control	EMT
Texture	Firm and not slimy	Firm and not slimy	Firm and not slimy	Firm and not slimy
Colour	Greenish brown	Yellowish brown	Greenish brown	Yellowish brown
Aroma	Pleasant smell, sweet aroma and not rancid	Pleasant smell, sweet aroma and not rancid	Pleasant smell, sweet aroma and not rancid	Pleasant smell, sweet aroma and not rancid
Mould presence	white mould visible at the surface	No visible mould	No visible mould	No visible mould

The silage texture was firm and slightly moist, but not slimy for all samples from both AGPK and PPK. The water content at the beginning of silage production is critical as this affects the silage texture [19]. From both control samples, the colour of the silage identified was greenish-brown, while the EM treated samples were yellowish-brown, indicating that the silages are in good condition, depending on their ingredients [21]. The successful silage feature must have a pleasant and not rancid odour [22] as observed in all samples. The presence of mould was found only in AGPK control samples. The delay while filling the chopped crops in the container will result in excessive respiration and encourage undesirable microbes' growth, thus increasing the loss of nutrients in silage [23]. If the silo is exposed to oxygen also contributes to yeasts' growth and moulds that spoil silage [7]. Other than that, the length of chopped crops is one factor contributing to the quality of silage. If the length is too long, this will make compaction difficult, and the air will remain trapped in the silage resulting in heating and spoilage [13]. The excellent skill being used while filling the container led to rapid anaerobic condition accomplishment and minimised mould and fungi growth [24].

4. Conclusion
Inoculation of microbes in corn stover silage did not affect the physical characteristics of the silage. Inoculation of corn silage with the lactic acid bacteria (LAB), Photosynthetic bacteria, Actinomycetes and yeast increased the moisture and CP content after 21 days of the fermentation process. In contrast, it reduces the pH and DM in silages. Therefore, fermentation for 21 days with the microbial inoculants impacted the nutritional composition of corn stover silage considering the pH value, chemical composition, and physical characteristics observed during this study.

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