Effect of Several Hetero- and Homofermentative Lactic Acid Bacteria Inoculants on the Chemical and Proximate Composition in Corn Silage

Nasyatul, M.H.[1*], Faisal, A.B.[2], Julie-Marzlinda, M.R.[2], Sasyafezleen, M.Z.[2], Faiz, A.H.[2], Syafri-Famin, A.A.[2], Marini, A.M.[3] and Noraini, S.[1]

[1]Malaysian Agricultural Research and Development Institute, Serdang, Selangor
[2]Malaysian Agricultural Research and Development Institute, Kluang, Johore
[3]Malaysian Agricultural Research and Development Institute, Kemaman, Terengganu

*Corresponding author: nasyatul@mardi.gov.my

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Abstract

This study aimed to determine the effect of homofermentative and heterofermentative lactic acid bacteria on the chemical and nutritional components of corn silage. The variety of corn used in this study was GWG 4546 at the harvesting age of 110 days. It was ensiled with 10^5 cfu of LAB for each gram of fresh material. The strains of LAB used to ensile were *P. acidilactici* (homofermentative LAB), *L.plantarum* (hetero-homofermentative LAB) and *L. fermentum* (heterofermentative LAB). The fresh stover was packed in 5L of HDPE plastic jar and kept at room temperature for 28 days. The silages were harvested every week for chemical and proximate analysis. The experimental design was completely randomized design, with 5 treatments x 4 fermentative periods x 3 replicates. From the study, inoculation of homofermentative *P. acidilactici* produces more lactic acid at an early stage of ensiling, whereas heterofermentative *L. plantarum* produce more lactic acid at later stages. Furthermore, the crude protein content and metabolizable energy of corn stover silage inoculated with homofermetative LAB were found to be constant throughout the 28 days of fermentation and the values were significantly higher than silages in other treatments. However, there were no significant differences in fibre content, ash content, pH values, dry matter, and fat content among all groups. Therefore, it can be concluded that the addition of homofermentative *P. acidilactici* resulted in better preservation with improved energy and protein values in 110-days of grain corn stover after 28 days of ensilation.

Keywords: Corn stover silage, *Pediococcus acidilactici*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, homofermentative, heterofermentative, *Zea mays* variety GWG 4546

Introduction

The feed cost always becomes major expense in livestock production where it represents about 60-70% of the total cost, approximately about 4 to 7 million tons per year. The main ingredients used in animal feed are corn (50 to 55%) and soybean (25-30%) and Malaysia needs to import about 3.71 million tons of grain corn from other countries such as Argentina, Brazil, USA, Pakistan and France at a value of about RM 3 billion (UN Comtrade, 2019). Therefore, after proper planning in 2016, Malaysia starts to plant grain corn on 99.3 Ha and able to produce about 129.8 metric tons in 2018 and the yield was increased to 134.1 metric tons in 2019.

However, due to the pandemic Covid-19 that emerged in early 2020, the production of grain corn in Malaysia dropped to 91.5 metric tons and the imported feed price especially grain corn dramatically increased by 30 to 50%. Since this was happen, the grain corn crop has been given higher priority by the Malaysian government to open more land and planted more grain corn to support the demand and reduce the dependence on imported corn (DOA, 2020).

The corn stover which is obtained as the residue from corn production contains a good fibre source for ruminants. However, it has a low moisture content, low crude protein content and high structural carbohydrate contents, which resulted in poor fermentation and lead to the low nutritive value of silage. Generally, moist silage is based on natural lactic acid fermentation. The epiphytic lactic acid bacteria (LAB) transform the water-soluble carbohydrates into organic acid in the ensiling process. As a result, the pH is reduced and the forage is preserved (Keskin *et al*, 2005). However, LAB, especially lactobacilli, is present in forage in very low numbers. When LAB fails to produce sufficient lactic acid during fermentation to reduce the pH and inhibit the growth of clostridia, the resulting silage will be of poor quality (Sebastian *et al*, 1996; Sharp *et al*, 1994). Therefore, it is necessary to use some bacterial inoculants to control microbes in silage fermentation. Many studies have shown the advantage of using LAB as silage inoculants (Denek *et al.*, 2001; Contreras-Govea *et al.*, 2006; Huanzhe Si *et al.*, 2018). It is intended to ensure rapid fermentation that results in faster accumulation of lactic acid, lower pH values at earlier stages of ensiling, and inhibition of the growth of some pathogenic bacteria (Bolsen *et al.*, 1998). Most commercially available inoculants contain homofermentative LABs, which are fast and efficient producers of lactic acid and thus improve silage fermentation. Heterofermentative LAB is also sometimes included in inoculants for silage because they produce volatile fatty acids (VFA), which inhibit the yeasts and moulds that are activated on aerobic exposure of the silage (McAllister *et al.*, 1998). In this experiment, selected lactobacilli and *Pediococcus* strains isolated from rumen cattle were used as silage additives based on their fermentation characteristics; homofermentative and heterofermentative. *P. acidilactici* is homofermentative bacteria, *L. fermentum* is heterofermentative bacteria, *L. plantarum* has both fermentation characteristics (it can be homo and/or hetero). This study aimed to determine the effect of homofermentative and heterofermentative lactic acid bacteria on the chemical and nutritional components of corn silage.

Materials and methods

Forage sample and silage preparation

Grain corn stover (*Zea mays* variety GWG 4546) was used after harvesting at the age of 110 days after. The corn stover was chopped to an approximate length of 2-3 cm. The chopped forage was inoculated with three isolated strains of homofermentative LAB (*Pediococcus acidilactici*), hetero-homofermentative LAB (*Lactiplantibacillus plantarum*), heterofermentative LAB (*Limosilactobacillus fermentum)*, and LAB consortium (a combination of *P. acidilactici*, *L. plantarum*, *L. fermentum* at ratio 1:1:1 (v/v)). All strains were isolated from the rumen of fistulated Kedah-Kelantan cattle (male cattle at the age of 5 years old) through fistula, identified by phenotype and 16S rRNA, then lyophilized in 10% of skimmed milk and stored at -80°C for further used. The stover was subsequently mixed homogeneously, packed, and compressed manually into approximately 2.5 kg, then ensiled in a 5 L laboratory plastic jar and sealed airtight with a screw top. Five

treatments were prepared; (1) no additives (control), (2) homofermentative LAB, (3) hetero-homofermentative LAB, (4) heterofermentative LAB, (5) LAB consortium. All inoculants were applied as additives at 1.0 x 10^5 CFU/g of fresh materials, and control treatment was added with equal water (non-chlorinated). A total of 60 jar were prepared (4 fermentation periods X 5 treatments X 3 replicates) and kept at room temperature. Triplicate jars for each treatment were opened on sampled on days 7, 14, 21, and 28.

Chemical analyses

The dry matter contents of pre-ensiling forages and silages were determined at 65°C in a forced-air oven for 72 hours. The pH of fresh forage and silage was measured using a pH meter (Metler-Toledo 5 series, Switzerland). Lactic acid contents of silage were analysed using a D-/L-Lactic Acid assay Kit (Megazyme, USA) following the instruction manual. Dried forage and silage samples were ground through a 1 mm mesh screen. The crude protein (CP) and ether extract (EE) were analysed following AOAC methods (AOAC, 1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were analysed using a fibre analyser (FT122 Fibertec, FOSS, Sweden) following the method by Van *et al*, 1991.

Statistical analysis

Data were analysed statistically using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) software programme. Differences in chemical and nutrient composition in silage between treatment groups were determined using procedure GLM and the variance between groups were analysed by one-way ANOVA using Duncan's Multiple Range Test (Steel and Torrie, 1980). The values were expressed as mean± SD and p-value of less than 0.05 were considered as significant.

Results and Discussion

Chemical composition of plant materials

The chemical compositions of fresh corn stover before ensiling are shown in Table 1. The initial pH of the fresh stover was 5.59 and has high dry matter content (54.88%). It also has good metabolizable energy (ME) value, but is low in crude protein content (5.34%). A high NDF value (78.13%) shows the maturity of the plant as it was harvested at the age of 110 days. The ME is the energy available for use by the cattle for maintenance of body systems, activity, milk production, pregnancy and weight gain. The higher the energy values in Megajoules, the better the quality of the feed (Moran, 2005).

Table 1. The chemical and nutritional content of fresh corn stover harvested at day 110 before ensiling

Items	Fresh corn stover[1]
pH	5.59 ± 0.41
Dry matter (%)	54.88 ± 0.70
Gross energy (Kcal/g)	3.81 ± 0.02
Metabolizable energy, (MJ/kg)	11.53 ± 0.47
Nitrogen-free extract (%)	50.91 ± 0.20
Total digestible nitrogen, TDN (%)	75.44 ± 2.88
Crude protein (%)	5.34 ± 0.54
Ether extract (%)	0.53 ± 0.43
Ash (%)	5.15 ± 0.21
Neutral detergent fibre, (%)	78.13 ± 2.71
Acid detergent fibre (%)	44.88 ± 1.23
Crude fibre (%)	38.12 ± 0.39
Hemicellulose (%)	33.26 ± 3.08
Cellulose (%)	36.25 ± 2.63
Lignin (%)	8.62 ± 1.98

[1]*Zea mays* variety GWG 4546

Fermentation characteristics of grain corn stover silage

The changes in the chemical and nutritional composition of corn stover silages treated with different inoculants were shown in Table 2 (at 7 days of ensiling), Table 3 (at 14 days of ensiling), Table 4 (at 21 days of ensiling), and Table 5 (at 28 days of ensiling). The results show that he treatment, the ensiling period and their interaction were significantly influenced the value of pH, DM, and lactic acid content ($p<0.05$) of the silages. Overall, the values of pH in all treated silages were decrease after 14 days of fermentation to approximately pH 4 as compared to the fresh material (pH 5.63). Hetero-homo fermentative LAB (*L. plantarum*) silage records the fastest pH reduction as early as 7 days of fermentation. However, after 28 days of fermentation, there are no significant differences in pH values ($p>0.05$) in all treatment groups. This may happen because the moisture content in the silage in this study was balanced at 70%. This step is important because moisture level (or dry matter of crop) will affect the chemical changes during the fermentation process. The amount of dry matter has an impact on the number of bacteria, the rate of fermentation, and the quantity of carbohydrates required for full fermentation. As DM content rises, fermentation is impeded. Silages that are drier have a tendency to stabilize at a higher pH with less fermentation acids (Jaster, 1995). A rapid pH decrease is necessary for crops with less than 55% DM to maximize quality and reduce proteolysis (Muck, 1988). Forages ensiled at the dry matter of more than 30% would have a greater risk for clostridial growth (Bates *et al.*, 1989). While, forage that is ensiled too dry (with more than 50% DM) often has a restricted fermentation and would have a limited amount of fermentation acids and is more likely to undergo secondary heating when exposed to air and associated dry matter lost (Kung and Shaver, 2001).

Homofermentative LABs are more efficient in lactic acid production than heterofermentative LABs and they can ferment a wide variety of substrates and quickly produce large amounts of lactic acid (McDonald *et al.*, 1991). In well-preserved silages, acidification was initiated by homofermentative strains, and the heterofermentative strains whose tolerance to acetic acid will grow and dominate the silage up to 85% after 4 days of fermentation (Blajman *et al.*, 2020). From this study, we found that the lactic acid content in corn stover silages was influenced by the strains of the LAB inoculants. Lactic acid was produced rapidly during the first 7 days in silage treated with homofermentative LAB (*P. acidilactici*) as compared to other LAB strains and the value was high until 14 days of fermentation. However, the production of lactic acid by *L. plantarum* was significantly higher in later days (day 21 and day 28); 18.49 mM and 15.71 mM, respectively. Similar results were found with Seale (1986), who suggested that heterofermentative acid-tolerant Lactobacilli sp. may dominate the final stages of ensiling and in this study; lactic acid also was produced in high amounts in the silage without the addition of LAB. The presence of epiphytic LAB on the plant is able to convert carbon source to lactic acid under anaerobic conditions but because the numbers is low, the acid production will happen in the later stage (after 21 days of fermentation). However, the finding was contradictory to the study reported by Sifeeldein *et al.* (2018), where the values of lactic acid silage inoculated with *L. plantarum* was higher than *P. acidilactici* as early as 7 days of fermentation. This may be due to the plant material used in their study (Napier grass), as the effect of lactic acid bacteria are depending on several factors including the raw material and the types of inoculants (Wang *et al.*, 2017).

Table 2. Changes in chemical and nutritional content of corn stover[1] silages (n=3) on 7 days of ensiling

Parameters	Treatments				
	Control	*Pediococcus acidilactici*	*Lactiplantibacillus plantarum*	*Limosilactobacillus fermentum*	LAB consortium[2]
pH	5.00 ± 0.09^{b}	4.99 ± 0.15^{b}	4.19 ± 0.14^{a}	4.26 ± 0.01^{a}	4.30 ± 0.15^{a}
DM (%)	29.93 ± 2.26^{b}	33.18 ± 0.94^{ab}	33.69 ± 1.80^{ab}	34.70 ± 3.00^{a}	33.94 ± 3.26^{ab}
Lactic acid (mM)	13.48 ± 0.88^{b}	17.28 ± 0.22^{a}	12.39 ± 1.09^{b}	14.22 ± 1.21^{b}	16.40 ± 1.11^{a}
GE (Kcal/g)	3.90 ± 0.03^{a}	3.87 ± 0.03^{ab}	3.89 ± 0.05^{a}	3.90 ± 0.03^{a}	3.82 ± 0.04^{b}
ME (Mj/kg)	11.53 ± 0.37^{a}	11.13 ± 0.42^{a}	11.20 ± 0.08^{a}	11.46 ± 0.47^{a}	10.39 ± 0.44^{b}
NFE	55.02 ± 0.69^{a}	50.51 ± 1.99^{b}	52.71 ± 0.59^{ab}	52.37 ± 0.86^{b}	52.31 ± 1.89^{b}
TDN	75.44 ± 2.23^{a}	73.05 ± 2.55^{a}	73.42 ± 0.49^{a}	75.00 ± 2.84^{a}	68.55 ± 2.64^{b}
CP	5.01 ± 0.31^{a}	4.85 ± 0.39^{a}	4.84 ± 0.06^{a}	5.17 ± 0.50^{a}	4.00 ± 0.43^{b}
EE	0.48 ± 0.22	0.51 ± 0.06	0.69 ± 0.17	0.73 ± 0.06	0.58 ± 0.16
Ash	4.68 ± 0.17^{a}	4.96 ± 0.06^{a}	5.42 ± 0.29^{b}	5.64 ± 0.23^{b}	5.42 ± 0.17^{b}
NDF	79.53 ± 0.52^{ab}	78.39 ± 0.84^{a}	81.97 ± 1.46^{b}	79.49 ± 1.08^{ab}	80.13 ± 2.94^{ab}
ADF	45.50 ± 3.80	46.69 ± 2.30	46.18 ± 1.59	42.74 ± 2.03	46.25 ± 3.16
CF	34.84 ± 1.26^{a}	39.21 ± 2.22^{b}	36.40 ± 1.08^{ab}	36.16 ± 1.43^{ab}	37.73 ± 1.74^{ab}
Hemicellulose	34.03 ± 3.31^{ab}	31.70 ± 2.23^{a}	35.79 ± 3.03^{ab}	36.74 ± 1.14^{b}	33.88 ± 2.24^{ab}
Cellulose	35.98 ± 4.01	38.47 ± 4.90	37.49 ± 1.71	34.01 ± 1.55	35.73 ± 4.76
Lignin	9.52 ± 2.00	8.21 ± 2.63	8.69 ± 1.19	8.73 ± 0.82	10.52 ± 2.46

^{abc} means with different superscripts in same column were significantly different at P<0.05; [1]*Zea mays* variety GWG 4546; [2] inoculants mixture of *P.acidilactici, L. plantarum, L. fermentum* (at ratio 1:1:1 (v/v)

Table 3. Changes in chemical and nutritional content of corn stover[1] silages (n=3) on 14 days of ensiling

Parameters	Treatments				
	Control	*Pediococcus acidilactici*	*Lactiplantibacillus plantarum*	*Limosilactobacillus fermentum*	LAB consortium[2]
pH	4.09 ± 0.17	4.04 ± 0.13	4.25 ± 0.08	4.14 ± 0.20	4.37 ± 0.22
DM (%)	33.79 ± 2.20	35.31 ± 1.44	33.34 ± 2.50	33.90 ± 0.34	34.43 ± 0.61
Lactic acid (mM)	13.79 ± 0.16^{b}	15.70 ± 1.75^{a}	12.01 ± 3.43^{c}	4.53 ± 4.51^{b}	9.25 ± 1.39^{d}
GE (Kcal/g)	3.75 ± 0.06^{b}	3.74 ± 0.04^{b}	3.76 ± 0.01^{b}	3.71 ± 0.02^{b}	3.83 ± 0.05^{a}
ME (Mj/kg)	13.20 ± 0.67^{a}	13.19 ± 0.93^{a}	11.90 ± 0.65^{b}	11.82 ± 0.23^{b}	13.13 ± 0.58^{a}
NFE	54.08 ± 3.24	54.16 ± 1.85	58.04 ± 2.60	57.55 ± 1.95	55.73 ± 3.97
TDN	85.59 ± 4.07^{a}	85.52 ± 5.68^{a}	77.69 ± 3.96^{b}	77.21 ± 1.42^{b}	85.19 ± 3.54^{a}
CP	6.98 ± 0.71^{a}	6.50 ± 0.14^{a}	5.34 ± 0.61^{b}	5.29 ± 0.36^{b}	6.79 ± 0.39^{a}
EE	0.40 ± 0.12	0.57 ± 0.14	1.34 ± 0.80	0.76 ± 0.29	1.04 ± 0.61
Ash	5.82 ± 0.33^{b}	5.96 ± 0.03^{a}	6.24 ± 0.44^{ab}	6.38 ± 0.03^{ab}	7.26 ± 1.22^{a}
NDF	69.78 ± 1.93	70.61 ± 2.78	65.24 ± 3.42	65.38 ± 1.70	66.15 ± 1.12
ADF	37.66 ± 2.84^{ab}	38.64 ± 1.97^{b}	37.94 ± 1.41^{a}	35.92 ± 1.78^{a}	34.36 ± 3.42^{a}
CF	32.75 ± 3.12	32.58 ± 2.34	29.14 ± 1.83	30.08 ± 1.65	29.27 ± 3.02
Hemicellulose	32.12 ± 1.24	31.97 ± 0.88	27.30 ± 4.29	29.46 ± 3.01	31.79 ± 2.38
Cellulose	31.65 ± 2.91	30.73 ± 2.56	30.03 ± 4.61	26.47 ± 4.05	29.06 ± 4.03
Lignin	6.01 ± 0.56	7.91 ± 1.75	7.91 ± 3.43	9.44 ± 4.51	5.30 ± 1.39

^{abc} means with different superscripts in same column were significantly different at P<0.05; [1]*Zea mays* variety GWG 4546; [2] inoculants mixture of *P. acidilactici, L. plantarum, L. fermentum* (at ratio 1:1:1 (v/v)

Table 4. Changes in chemical and nutritional content of corn stover[1] silages (n=3) on 21 days of ensiling

Parameters	Treatments				
	Control	*Pediococcus acidilactici*	*Lactiplantibacillus plantarum*	*Limosilactobacillus fermentum*	LAB consortium[2]
pH	4.00 ± 0.04^{a}	3.95 ± 0.12^{a}	4.07 ± 0.06^{a}	4.20 ± 0.12^{a}	4.48 ± 0.29^{b}
DM (%)	31.64 ± 1.94	30.38 ± 2.44	33.99 ± 0.66	34.10 ± 2.46	32.74 ± 1.40
Lactic acid (mM)	17.75 ± 0.75^{ab}	16.53 ± 1.04^{b}	18.49 ± 0.23^{a}	17.86 ± 0.09^{ab}	13.30 ± 1.68^{c}
GE (Kcal/g)	3.82 ± 0.02^{b}	3.88 ± 0.03^{a}	3.85 ± 0.03^{ab}	3.87 ± 0.02^{a}	3.85 ± 0.01^{ab}
ME (Mj/kg)	11.08 ± 0.39^{b}	12.26 ± 0.71^{a}	10.99 ± 0.37^{b}	10.41 ± 0.16^{b}	10.46 ± 0.43^{b}
NFE	50.24 ± 2.34	51.64 ± 1.71	51.21 ± 0.88	49.61 ± 2.50	50.08 ± 0.85
TDN	72.71 ± 2.37^{b}	79.90 ± 4.31^{a}	72.16 ± 2.28^{b}	68.66 ± 0.97^{b}	68.96 ± 2.66^{b}
CP	5.09 ± 0.34^{b}	6.19 ± 0.55^{a}	4.90 ± 0.50^{b}	4.50 ± 0.16^{b}	4.42 ± 0.41^{b}
EE	0.66 ± 0.29	0.73 ± 0.23	0.47 ± 0.06	0.33 ± 0.18	0.31 ± 0.25
Ash	6.69 ± 0.30^{ab}	6.22 ± 0.37^{a}	6.46 ± 0.76^{ab}	7.29 ± 0.62^{b}	6.64 ± 0.33^{ab}
NDF	74.09 ± 1.12^{a}	74.01 ± 1.73^{a}	76.78 ± 0.92^{ab}	78.02 ± 2.89^{b}	79.31 ± 2.32^{b}
ADF	41.13 ± 2.54^{a}	41.26 ± 3.55^{a}	44.87 ± 0.33^{ab}	45.03 ± 1.87^{ab}	48.63 ± 2.38^{b}
CF	37.32 ± 2.65	35.22 ± 2.08	36.95 ± 0.55	38.27 ± 2.33	38.55 ± 0.91
Hemicellulose	32.96 ± 2.11^{ab}	32.76 ± 3.89^{a}	31.91 ± 1.23^{bc}	32.98 ± 1.47^{bc}	30.68 ± 2.80^{c}
Cellulose	34.49 ± 1.61	32.06 ± 0.70	37.56 ± 1.48	37.41 ± 1.30	39.21 ± 3.77^{b}
Lignin	6.65 ± 1.53	9.20 ± 2.85	7.31 ± 1.17	7.62 ± 1.08	9.43 ± 1.58

^{abc} means with different superscripts in same column were significantly different at P<0.05; [1]*Zea mays* variety GWG 4546; [2] inoculants mixture of *P. acidilactici, L. plantarum, L. fermentum* (at ratio 1:1:1 (v/v))

Table 5. Changes in chemical and nutritional content of corn stover[1] silages (n=3) on 28 days of ensiling

Parameters	Treatments				
	Control	*Pediococcus acidilactici*	*Lactiplantibacillus plantarum*	*Limosilactobacillus fermentum*	LAB consortium[2]
pH	4.10 ± 0.12	3.96 ± 0.06	4.16 ± 0.20	4.07 ± 0.04	4.23 ± 0.19
DM (%)	32.43 ± 1.75	31.32 ± 3.73	32.08 ± 1.93	32.94 ± 2.81	34.67 ± 3.14
Lactic acid (mM)	15.28 ± 0.43^{ab}	15.05 ± 0.74^{ab}	15.71 ± 0.41^{a}	14.10 ± 1.22^{b}	14.73 ± 0.89^{ab}
GE (Kcal/g)	3.91 ± 0.01^{ab}	3.93 ± 0.03^{a}	3.88 ± 0.03^{ab}	3.85 ± 0.04^{b}	3.87 ± 0.03^{b}
ME (Mj/kg)	10.69 ± 0.24^{b}	12.04 ± 0.64^{a}	10.86 ± 0.49^{b}	10.69 ± 0.11^{b}	10.61 ± 0.16^{b}
NFE	49.53 ± 1.38^{c}	55.48 ± 1.71^{a}	52.53 ± 1.19^{b}	51.09 ± 0.82^{bc}	51.57 ± 0.35^{bc}
TDN	70.34 ± 1.46^{b}	78.57 ± 3.92^{a}	71.37 ± 2.96^{b}	70.36 ± 0.66^{b}	69.89 ± 0.99^{b}
CP	4.63 ± 0.35^{b}	5.63 ± 0.43^{a}	4.66 ± 0.55^{b}	4.59 ± 0.18^{b}	4.45 ± 0.16^{b}
EE	0.05 ± 0.00^{b}	0.44 ± 0.29^{ab}	0.47 ± 0.23^{ab}	0.35 ± 0.26^{ab}	0.55 ± 0.29^{a}
Ash	5.75 ± 0.12^{ab}	5.52 ± 0.29^{a}	6.26 ± 0.32^{bc}	6.34 ± 0.39^{c}	6.41 ± 0.35^{c}
NDF	79.10 ± 2.30^{b}	72.32 ± 0.65^{a}	78.18 ± 2.72^{b}	78.62 ± 1.78^{b}	78.21 ± 0.78^{b}
ADF	46.33 ± 2.87^{b}	41.29 ± 2.46^{a}	46.56 ± 1.21^{b}	47.22 ± 1.27^{b}	46.87 ± 1.21^{b}
CF	40.04 ± 0.93^{c}	32.93 ± 2.12^{a}	36.08 ± 0.94^{b}	37.64 ± 0.65^{b}	37.03 ± 1.13^{b}
Hemicellulose	32.77 ± 3.47	31.03 ± 2.79	31.62 ± 3.79	31.41 ± 1.40	31.34 ± 1.25
Cellulose	36.06 ± 4.57	32.50 ± 3.47	36.45 ± 1.49	31.69 ± 7.81	34.37 ± 2.10
Lignin	10.27 ± 1.72	8.79 ± 2.25	10.11 ± 2.10	15.52 ± 8.00	12.50 ± 2.94

^{abc} means with different superscripts in the same column were significantly different at P<0.05; [1]*Zea mays* variety GWG 4546; [2] inoculants mixture of *P. acidilactici, L. plantarum, L. fermentum* (at ratio 1:1:1 (v/v))

From the study, there are some changes were found in energy value and protein content in the corn stover silages during the ensiling period. A rapid reduction of protein content was observed in all treatments at 7 days of fermentation and the value was suddenly increased from day 14 until day 28 of ensiling. Early reduction of CP content may happen with regards the proteolytic activity of the forage plant and the increasing value of CP may be contributed by the formation of microbial proteins that formed from the bacterial proliferation in the silage (Hao *et al.*, 2020). Even in well-preserved silages, approximately 50% degradation of protein may take place (Cavallarin *et al.,* 2006; Guo *et al.*, 2008). However, in this study, the silage treated with *P. acidilactici* contains significantly higher (p= 0.0168) crude protein value (5.6 to 6.5% DM) throughout the fermentation period as compared to other treatments (less than 5.5% DM) and there is no significant difference (p= 0.6710) was observed in the CP value of silage treated with *L. plantarum, L. fermentum* and mixtures of all microbe strains. The inoculation of *P. acidilactici* in grain corn silage may improve the CP content as the result of microbe proliferation (more microbial proteins) during the fermentation period thus enhancing the nutritive value of the silage. The energy value of metabolizable energy (ME) in silage treated with *P. acidilactici* also was found to be significantly improved (p= 0.0050) (12 to more than 13 MJ/kg) during the 28 days of fermentation as compared to fresh stover (11.53 MJ/kg). A significant improvement (p= 0.0307) also was found in silage treated with mixture strains at the age of 14 days (13.13 Mj/kg) but it become reduced at days 21 and 28 (less than 11 Mj/kg).

Microbial inoculation usually has little or no influence on the fibre content of silages because most LAB contains little or no ability to degrade plant cell walls (McDonald *et al.*, 1991). However, in this study, a significant reduction in NDF values was observed in silage treated with *P. acidilactici* at the early 7 days of fermentation and the values decreased gradually until 28 days of fermentation. The cellulose content also was significantly reduced in the same treatment group after 14 days of fermentation which indicates a high cellulolytic activity in the silage inoculated with *P. acidilactici*. However, the NDF value was remaining high (> 70%) due to the late harvesting of the raw material. The National Research Council (NRC) in 1989 reported that forage should contain at least 25% to 30% DM of NDF in the animal's diet to meet the requirement for growth and reproduction. Therefore, corn stover at the age of 110 days was considered as a poor-quality type of forage for animal consumption.

Although the addition of *P. acidilactici* showed an improvement in the quality of the corn stover silage, the combination of *P. acidilactici* with *L. plantarum* and *L. fermentum* shows no effects on the silage quality as compared with silage in the control. This may be due to the inhibitory activity between those microbes where some studies had found that *L. plantarum* could dominate and inhibit other microbes to survive (Timmerman *et al.*, 2004). The mixture of bacteria was found to be good in terms of enhancing the activity of biochemical reactions either as probiotics or as silage inoculants. Therefore, well-designed multi strains of microbes which show synergistic and symbiotic activities towards each other are important to maximize the effects.

Conclusion

This study aimed to determine how homofermentative and heterofermentative lactic acid bacteria affected the nutritional and chemical components of corn silage. Based on the study presented, it is possible to draw the conclusion that the inoculation of

homofermentative *P. acidilactici* improved the crude protein content of the corn silage, decreased the NDF and ash, and increased the energy values of the feed, hence increasing its nutritional value. Additionally, the homofermentative *P. acidilactici*, hetero-homofermentative *L. plantarum*, and heterofermentative *L. fermentum* strains that made up the LAB consortium are not a desirable combination since they do not improve the quality of grain corn silage compared to single strain species. Future research into the best strain combinations of silage inoculants, which include both homo- and hetero-fermentative strains, may be helpful in determining the silage's maximum quality because they can speed up the fermentation process by producing lactic acid and preserve more nutrients, including proteins and energy. By providing affordable feed and lowering the quantity of trash generated by the agricultural industry, the conversion of low-quality material into high-quality output could improve farm production.

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