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Effect of *Lactobacillus paracasei* Inoculation at Different Level on Fermentation Quality and Chemical Composition of Ensiled Total Mixed Ration (eTMR)

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ABSTRACT: The present research aimed to evaluate effect of *Lactobacillus paracasei* at different levels of inoculation on fermentation quality and chemical composition of the ensiled total mixed ration (eTMR). The treatments were divided into 6 groups: 1) fresh total mixed ration (fresh TMR), 2) TMR without inoculation (eTMR), 3) TMR with 10^4 CFU/g of TMR of *L. Paracasei* (LP4), 4) TMR with 10^5 CFU/g of TMR of *L. Paracasei* (LP5), 5) TMR with 10^6 CFU/g of TMR of *L. Paracasei* (LP6) and 6) TMR with 10^7 CFU/g of TMR of *L. Paracasei* (LP7). The statistic was fixed by effects of ensiling process, (Fresh TMR vs. eTMR) inoculation with *L. paracasei* or without (eTMR vs. LP4, LP5, LP6 and LP7). The samples were collected at 21 days of ensiling times for analysis of fermentation quality and chemical compositions. The result shows that *L. paracasei* inoculation significantly decreased pH values and ammonia nitrogen (NH_3-N). Latic acid tended to be decreased by inoculation. High level of *L. paracasei* inoculation affected pH and NH_3-N. Ensiling process decreased ether extract (EE) and hemicellulose. In addition, *L. paracasei* inoculation tended to prevent the loss of EE. Moreover, acid detergent lignin (ADL) was reduced by *L. paracasei* inoculation. *L. paracasei* inoculation reduced acid detergent fiber (ADF) content and decreased loss of hemicellulose from the ensiling process. Despite the fact that the ensiling process appears to lower eTMR pH values, the mean concentrations of NH_3-N and lactic acid increased. Additionally, it reduces nutritive values of eTMR (EE, neutral detergent fiber (NDF) and hemicellulose) but increases ratio of nonstructural carbohydrate (NSC), ADF, and cellulose. *L. paracasei* inoculation can enhance fermentation quality by reducing pH values and NH_3-N. It can prevent loss of EE from the ensiling process and reduce ADL content. *L. paracasei* inoculation at different levels provide different results. 10^7 CFU/g *L. paracasei* inoculation resulted well fermentation quality but chemical composition optimized by 10^5 CFU/g. Consequently, selection level of lactic acid bacteria inoculation should be considered by species of lactic acid bacteria and cost of production importantly.
Keywords: total mixed ration; *Lactobacillus paracasei*; ensiled total mixed ration; fermentation

INTRODUCTION

Ensiled total mixed ration (eTMR) is storage of total mixed ration (TMR) in sealed container or plastic bag for 21 days in anaerobic condition (Wongnen et al., 2009). The crucial key of the ensiling process is lactic acid bacteria (LAB) which is gram-positive, non-produced catalase enzyme, and non-spores forming. They are divided into several genera, including *Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, Enterococcus, and Lactococcus.* Temperatures about 25 and 40°C are appropriate for their growth. The main function of LAB is to

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ferment water-soluble carbohydrates (WSC) into organic acid as their major products (ethanol, volatile fatty acid, succinic acid, and largely lactic acid) (Madrid et al., 1999), which decline pH value of silage to between 3–5 based on the strains of LAB and the type of forage (Holzapfel et al., 1992). The accumulation of lactic acid rapidly declines pH value of eTMR causing inhibition of spoilage microbial and extending shelf life of TMR (Wongnen et al., 2009). However, natural fermentation process causes loss of nutrients during ensiled process such as respiration, fermentation, and biochemist change (Ramos et al., 2016).

Homofermentative LAB is used most widely in previous silage production by reason that they produce high lactic acid. (Kim et al., 2021). Presently, many researchers use heterofermentative LAB as silage additives which expect acetic acid production that leads the silage has more aerobic stability. Therefore, inoculation of LAB used for starter culture for storage of feed. The LAB inoculation can expeditiously complete the fermentation process (Weinberg et al., 1993) and decrease of nutrient losses. Ávila et al. (2010) found that the effect of the inoculant is more than species of LAB when evaluated the effect of different LAB species inoculation in sugar cane silages *(L. plantarum, L. paracasei, L. brevis, andL. buchneri*). Blajman et al. (2018) found that reducing of undesirable yeast and mold growth in LAB inoculation. Likewise, it can improve aerobic stability and LAB count in corn silage. LAB inoculation at present has many species of LAB such as *L. burcneri* inoculation at 10^4 and 10^5 CFU/g in sugarcane silage, *L. plantarum* inoculation at 10^6 CFU/g in eTMR, and *P. pentosaceus* inoculation at 10^5 CFU/g in eTMR. All studies show LAB inoculation improve fermentation quality by decreased pH values of silage and affect nutritive value based on the type of feed silage (Schmidt et al., 2014; Lei et al., 2017; Jiang et al., 2020). Oliveira et al. (2017) studied a meta-analysis of LAB inoculation in feed silage and showed that the most widely used level application of inoculation in a laboratory scale is 10^6 CFU/g (60.1%) following by 10^5 CFU/g (34.1), 10^4 CFU/. g (3.5%), and 10^7 CFU/g (2.5%), respectively.

L. paracasei is LAB in heterofermentative group, high acetic and lactic acid production, grow in high temperature (45°C) and high growth rate. This species was used for starter cultures of LAB in variety of fermented food products such as fermented vegetables (Argyri et al., 2013) and ripened cheeses (Rossi et al., 2012). In addition, this strain was used in a term of probiotic (Ortigosa et al., 2006). Additionally, Sofyan et al. (2013) reported that *L. paracasei* which is isolated from King grass silage has a potential as anti-pathogenic bacteria. *L. paracasei* have been reported by EFSA (2011) that they can improve the fermentation quality by reducing the pH values and increasing the preservation of dry matter. Lee et al. (2020) reported that effect of *L. paracasei* inoculation in Italian ryegrass silage produces a better quality of silage and significantly higher CP and lower NDF, ADF contents compared among the LAB. The hypothesis of this study is *L. paracasei* inoculation at different levels can improve fermentation quality and reduce loss of nutrients from ensiling process together with optimizing the level of *L. paracasei* inoculation. Consequently, the objective of the present research is to evaluate the effect of *L. paracasei* inoculation at different levels on fermentation quality and chemical composition of ensiled total mixed ration (eTMR).

MATERIALS AND METHODS

eTMR preparation: Ingredients of TMR was shown in **Table 1**. The TMR was calculated for obtaining 16% of CP and 68% total digestible nutrients. TMR was performed by TMR mixer machine (Jaylor, Canada) for feed uniformity. The treatments were divided into 6 groups.

Treatments 1 fresh total mixed ration (fresh TMR)

Treatments 2 total mixed ration without inoculation 0.85% NaCl (eTMR)

Treatments 3 total mixed ration with 10^4 CFU/g of TMR of *L. paracasei* (LP4)

Treatments 4 total mixed ration with 10^5 CFU/g of TMR of *L. paracasei* (LP5)

Treatments 5 total mixed ration with 10^6 CFU/g of TMR of *L. paracasei* (LP6)

Treatments 6 total mixed ration with 10^7 CFU/g of TMR of *L. paracasei* (LP7)

TMR was ensiled and vacuumed for anaerobic condition in plastic bag. The samples were collected at 21 days of ensiling times and stored in a -20°C fridge for analysis of fermentation quality and chemical compositions later.

Starter culture preparation: *L. paracasei* were cultured in de man, rogosa and sharpe (MRS) broth (De man et al., 1960) and incubated at 37°C for 24 hours for procured $1x10^9$ CFU/ml of *L. paracasei*. MRS broth were centrifuged and harvested only bacterial cell. Then, Bacterial cell were mixed 0.85% of normal saline for 10^9 CFU/ml. The solution was sprayed on TMR for 10 ml per 1 kg of TMR for procured final concentrate of *L. paracasei as* 1×10^7 CFU/g of TMR, later continually dilution for 10^8, 10^7 and 10^6 CFU/ml for procured final concentrate of *L. paracasei* as 10^6, 10^5 and 10^4 ml per 1 kg of eTMR.

Fermentation quality analysis: To receive extracted eTMR, 90 ml distilled water was added to 10 g TMR and eTMR samples and stored in the refrigerator at 4°C before being filtered through 4 layers of cheesecloth. A pH meter was immediately used to test the pH of silage extract (Bal et al., 1997). For the organic acid detection, the filtrate was centrifuged at 12,000 × g for 10 minutes at 4°C, and the supernatant was filtered through a 0.22 μm membrane filter. High-performance liquid chromatography was used to examine the volatile fatty acids of eTMR, which included acetic acid, propionic acid, butyric acid, and lactic acid (adapted from Scherer et al., 2012). The samples were analyzed on a C18 column (150 × 4.6 mm The mobile phase was composed of 20% of acetonitrile and 80% of KH_2PO_4 (adjust pH to 2.6 by HCl). The flow rate was 0.5 mL/minute, and the UV detector was operated at a wavelength of 210 nm. Ammonia nitrogen was measured by method of Chaney and Marbach (1962).

Table 1 Ingredients of ensiled total mixed ration in this study (%as fed basis)

Ingredients	Amount (%)
Fresh Napier grass	**60.00**
Maize husk	**10.00**
Ground corn	**10.00**
Dried brewer's grain	**8.00**
Soybean meal	**5.00**
Rice bran	**3.60**
Molasses	**2.00**
Dicalcium phosphate (DCP)	**0.50**
Premix	**0.50**
Urea	**0.40**
Total	**100.00**

Chemical composition analysis: Fresh TMR and eTMR were dried for 48 hours in an air circulation oven at 60°C. The dried sample then were ground by grinder (CT293 Cyclotec TM, FOSS Analytical A/S, Hilleroed, Denmark) and passed a 1 mm mill screen for subsequent determination of chemical analysis including by dry matter (DM), organic matter (OM), ether extract (EE). Kjeldahl method was used for determination of crude protein (CP) (AOAC, 2000). The procedures of Van Soest et al. (1991) were used to analyze the acid detergent fiber (ADF) contents, neutral detergent fiber (NDF) contents and acid detergent lignin content (ADL). Hemicellulose was calculated as NDF–ADF, and cellulose as ADF–ADL.

Statistical analysis: All data were analyzed using analysis of variance by IBM SPSS Statistics 25. Statistical models included the fixed effects of ensiling process, (Fresh TMR vs. eTMR) inoculation with *L. paracasei* or without (eTMR vs. LP4, LP5, LP6 and LP7) and multiple comparisons among level of *L. paracasei* means were performed by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980). The statistical analysis was performed with 95% significant level.

RESULTS AND DISCUSSION

Ensiling effects

The ensiling process reduced pH values in eTMR compared with Fresh TMR (4.80 vs. 5.95) (**Table 2**) due to during the anaerobic conditions, LAB ferment water-soluble carbohydrate and release strong acid as a product (lactic acid) caused reducing pH value (Huyen et al., 2020; Muck, 2010). NH_3-N content significantly increased in eTMR when compared to Fresh TMR (28.67 vs. 5.07 mg/dl) because the activity of microbial in the ensiling process uses nutritive values of feed especially protein via deamination and decarboxylation causing an increase in NH_3-N. (Oliveira et al. 2017; Abbasi et al., 2018). The lactic acid was not detected in Fresh TMR and increased in eTMR. There was no difference in acetic acid content between fresh TMR and eTMR while propionic acid was not detected. Butyric acid

significantly increased in eTMR compared with fresh TMR (0.072 vs. 0.002 %DM). The increase in butyric acid was caused by clostridia bacteria that produced butyric acid and led to spoilage feed (Li *et al*., 2020).

Table 3 shows the chemical composition of eTMR. There was no difference in DM, OM, CP, ADL between fresh TMR and eTMR. The ensiling process reduced EE in eTMR compared to fresh TMR (2.49 vs. 3.94 %DM). Fatty acids are oxidized by some aerobic bacteria and LAB in the ensiling process via biohydrogenation. Additionally, plant enzymes can reduce fatty acids by cleaved into aldehydes and ketones (Wu et al., 2021; Bueno et al., 2020; Han and Zhou, 2013). NDF and Hemicellulose decreased in eTMR, but nonstructural carbohydrate increased in eTMR compared with fresh TMR (58.03 vs. 67.02, 29.81 vs. 41.30 and 21.79 vs. 11.99 %DM, respectively). Hemicellulose breakdown into the energic substrate for microbe has occurred during the ensiling process which releases non-structural carbohydrate as pentose (glucose, galactose, and mannose) and hexose (xylose and arabinose). Moreover, hemicellulose is easily hydrolyzed by acid (Dewar et al., 1963; Bueno et al., 2020; Houfani et al., 2020; Patel and Parsania, 2018). ADF and cellulose were higher in eTMR than fresh TMR (28.22 vs. 25.73 and 25.18 vs. 22.37, respectively). Respiratory of the plant during oxygen occurred causes loss of nutrients and energy resulting in a high ratio of ADF (Pitt, 1990).

Inoculation effects

pH values of *L. paracasei* inoculation on eTMR were significantly lower than eTMR without inoculation (4.31 vs. 4.80). This study shows eTMR without inoculation still had pH values of 4.8, which represents the uncompleted ensiling process and may cause loss of nutrients. However, Inoculation of LAB decreased pH values of eTMR. Huyen et al. (2020) reported that pH values under 4.5 reduced DM losses by inhibiting undesirable microbial growth. The amount of NH_3-N in LAB inoculated eTMR is lower than eTMR without inoculation (25.30 vs. 28.67 mg/dl) because LAB inoculation can reduce NH_3-N by inhibiting the growth of clostridia bacteria and some enterobacteria that producing NH_3-N by proteolysis (Ávila and Carvalho, 2020; Heron et al, 1989). The lactic acid tends to reduce (P=0.053) in LAB inoculation compared with uninoculated eTMR (2.01 vs. 2.94 %DM). This result is contrary to many previous studies that reported LAB inoculation increased the proportion of lactic acid in feedstuff silage (Oliveira et al., 2017). It may be due to LAB inoculation suddenly reduces pH value resulting to inhibit microbial activity that occurred during the ensiling process including LAB themselves (National Research Council, 1992). Whereas acetic acid and butyric acid were not significant between treatments.

Although there was no difference in DM, OM, CP, NSC, NDF, ADF, hemicellulose and cellulose between treatments. Ether extract tends to increase (P=0.058) in LAB inoculation compared with eTMR without inoculation (3.02 vs. 2.49 %DM). Protein loss during the ensiling process is divided into 2 phases 1) Proteolysis to an amino acid by plant enzyme or aerobic bacteria 2) Utilization of amino acid and produced NH_3-N. LAB inoculation reduces pH and inhibits the growth of aerobic bacteria (clostridia and enterococcus). Besides, it also inhibits LAB that uses amino acids and releases NH_3-N too (Kondo et al. 2016; Oliveira et al. 2017; Kim et al. 2021). The rapid reduction of pH values caused by LAB inoculation can inhibit the growth of aerobic bacteria. In addition, it can inactivate activities of lipoxygenase enzyme that function at pH around 6.5-8 (Ellis et al., 2016; Zhao et al., 2021; Han and Zhou, 2013; Bueno et al. 2020). ADL content of inoculated eTMR is less than uninoculated eTMR (2.03 vs. 3.04) since LAB can

produce lignin peroxidase that degrades the main phenolic and non-phenolic compounds in lignin (Kachouri et al., 2016; Kietkwanboot, 2013).

Level effects

The pH values among treatments (LP4, LP5, LP6, and LP7) were significantly different (**Table 2**). The pH value of LP7 was the lowest (3.98) while LP4, LP5, LP6 were not significantly different. The current result in accordance with Kung Jr and Ranjit (2001) reported that LAB inoculation in 10^5 and 10^6 CFU/g was not significantly different. The amount of NH_3-N was significantly different among treatments. The pH value of LP7 was the lowest (22.09 mg/dl) followed by LP4, LP6 and LP5, respectively (22.61, 27.32 and 29.17 mg/dl respectively). Lactic acid, acetic acid and butyric acid concentration were not affected by the level of *L. paracasei* inoculation. Even though the 10^7 CFU/g LAB inoculation resulting in well fermentation quality compared with others in terms of low pH and NH_3-N. It has a limit in cost of production, and it is difficult to use on a farm-scale (Oliveira et al., 2017).

There were no effects of *L. paracasei* inoculation at different levels on DM, OM, CP, EE, NSC, NDF and ADL. ADF in any treatments had a significantly highest in LP4 following by LP6, LP7 and LP5. A high-level inoculation can decrease ADF in accordance with cellulose that tends to remain in LP4. Perhaps, rapid reduction of pH value may affect to longer acid hydrolysis period. There was a difference in hemicellulose. LP5, LP6 and LP7 have significantly higher hemicellulose than LP4 because high-level inoculation inhibits degradation of hemicellulose by microbe and inactivates enzyme resulting in high remain hemicellulose.

CONCLUSIONS

Ensiling process cause reduced pH values of eTMR, while increased NH_3-N and lactic acid. Additionally, it reduces nutritive values of eTMR (EE, NDF, and hemicellulose) but increases ratio of NSC, ADF, and cellulose. *L. paracasei* inoculation can enhance fermentation quality by reducing pH and NH_3-N. It can prevent loss of EE from the ensiling process and reduce ADL content. *L. paracasei* inoculation at different levels provide different results. *L. paracasei* 10^7 CFU/g result well fermentation quality but chemical composition optimized by 10^5 CFU/g. Consequently, selection level of LAB culture should be considered by species of LAB and cost of production importantly.

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Table 2 Fermentation quality of fresh TMR, eTMR and eTMR inoculation with *L. paracasei* at different level

Item	Treatments						SEM	P-value		
	Fresh TMR	eTMR[1]	LP4	LP5	LP6	LP7		Ensiling	Inoculation	Level
pH	5.95	4.80	4.35^{A}	4.58^{A}	4.33^{A}	3.98^{B}	0.134	**	**	*
Ammonia nitrogen (mg/dl)	5.07	28.67	22.61^{B}	29.17^{A}	27.32^{A}	22.09^{B}	1.718	**	**	*
Lactic acid (%DM)	ND	2.94	2.10	2.08	1.78	2.06	0.275	-	NS	NS
Volatile fatty acid (%DM)										
Acetic acid	0.745	0.840	0.845	0.860	0.865	0.890	0.015	NS	NS	NS
Propionic acid	ND	ND	ND	ND	ND	ND	-	-	-	-
Butyric acid	0.002	0.072	0.047	0.078	0.056	0.054	0.008	*	NS	NS

$^{A–B}$ show superscript significantly differences between the level of *L. paracasei* ($p<0.05$).

[1]eTMR: ensiled total mixed ration.

LP4: eTMR + *L. paracasei* 10^4 CFU/g inoculation, LP5: eTMR + *L. paracasei* 10^5 CFU/g inoculation, LP6: eTMR + *L. paracasei* 10^6 CFU/g inoculation,

LP7: eTMR + *L. paracasei* 10^7 CFU/g inoculation.

SEM = Standard error of the mean.

Ensiling: TMR vs eTMR, Inoculation: eTMR vs LP4, LP5, LP6 and LP7, Level: Compared among LP4, LP5, LP6 and LP7

ND = non-detected; NS = not significant.

*= $P<0.05$; **= $P<0.01$

Table 3 Chemical compositions of fresh TMR, eTMR and eTMR inoculation with *L. paracasei* at different level

Item	Treatments						SEM	P-value		
	Fresh TMR	eTMR	LP4	LP5	LP6	LP7		Ensiling	Inoculation	Level
DM (%as fed)	38.80	37.94	37.15	37.76	43.75	42.32	1.075	NS	NS	NS
Chemical compositions (%)										
OM	98.62	97.79	97.80	97.99	98.21	98.32	0.086	NS	NS	NS
CP	15.67	15.47	16.20	16.15	15.85	16.50	0.108	NS	NS	NS
EE	3.94	2.49	2.88	3.52	2.91	2.71	0.140	**	NS	NS
NSC	11.99	21.79	21.26	20.57	20.33	21.33	0.652	**	NS	NS
NDF	67.02	58.03	57.47	57.74	60.13	57.77	0.680	**	NS	NS
ADF	25.73	28.22	30.15^{A}	25.98^{C}	28.61^{AB}	26.71^{BC}	0.378	*	NS	*
ADL	3.22	3.04	2.80	1.51	2.44	1.37	0.215	NS	*	NS
Hemicellulose	41.30	29.81	27.32^{B}	31.76^{A}	31.52^{A}	31.06^{A}	0.812	**	NS	*
Cellulose	22.37	25.18	27.35	24.47	26.17	25.34	0.371	*	NS	NS

$^{A–C}$ show superscript significantly differences between the level of *L. paracasei* ($p<0.05$).

[1]eTMR: ensiled total mixed ration

LP4: eTMR + *L. paracasei* 10^4 CFU/g inoculation, LP5: eTMR + *L. paracasei* 10^5 CFU/g inoculation, LP6: eTMR + *L. paracasei* 10^6 CFU/g inoculation,

LP7: eTMR + *L. paracasei* 10^7 CFU/g inoculation.

SEM = Standard error of the mean.

Ensiling: TMR vs eTMR, Inoculation: eTMR vs LP4, LP5, LP6 and LP7, Level: Compared among LP4, LP5, LP6 and LP7

NS = not significant

*= P<0.05; **= P <0.01

REFFERENCES

Abbasi, M., Y. Rouzbehan, J. Rezaei, and S. E. Jacobsen. 2018. The effect of lactic acid bacteria inoculation, molasses, or wilting on the fermentation quality and nutritive value of amaranth (*Amaranthus hypochondriaus*) silage. Journal of Animal Science. 96(9): 3983-3992.

AOAC. 2000. Official Methods of Analysis of AOAC. 17th Edition. Gaithersburg, MD, USA.

Argyri, A. A., G. Zoumpopoulou, K. A. G. Karatzas, E. Tsakalidou, G. J. E. Nychas, E. Z. Panagou, and C. C. Tassou. 2013. Selection of potential probiotic lactic acid bacteria from fermented olives by *in vitro* tests. Food Microbiology. 33(2): 282-291.

Ávila, C. L. D. S., A. R. Valeriano, J. C. Pinto, H. C. P. Figueiredo, A. V. D. Rezende, and R. F. Schwan. 2010. Chemical and microbiological characteristics of sugar cane silages treated with microbial inoculants. Revista Brasileira de Zootecnia. 39(1): 25-32.

Ávila, C. L. S., and B. F. Carvalho. 2020. Silage fermentation—updates focusing on the performance of micro-organisms. Journal of Applied Microbiology. 128(4): 966-984.

Bal, M.A., J.G. Coors, and R.D. Shaver. 1997. Impact of the maturity of corn for use as silage in the diets of dairy cows on intake, digestion, and milk production. Journal of Dairy Science. 80: 2497-2503.

Blajman, J. E., R. B. Paez, C. G. Vinderola, M. S. Lingua, and Signorini, M. L. 2018. A meta-analysis on the effectiveness of homofermentative and heterofermentative lactic acid bacteria for corn silage. Journal of Applied Microbiology. 125(6): 1655-1669.

Bueno, A.V.I., G. Lazzari, C. C. Jobim, and J. L. P. Daniel, 2020. Ensiling total mixed ration for ruminants: A review. Agronomy. 10(6): 879.

Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clinical Chemistry. 8(2): 130-132.

De Man, J. C., D. Rogosa, and E. M. Sharpe. 1960. A medium for the cultivation of lactobacilli. J Journal of Applied Bacteriology. 23(1): 130-135.

Dewar, W.A., P. McDonald, and R. Whittenbury. 1963. The hydrolysis of grass hemicelluloses during ensilage. Journal of the Science of Food and Agriculture. 14(6): 411-417.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). 2011. Scientific Opinion on the safety and efficacy of *Lactobacillus paracasei* (DSM 16245) as a silage additive for all species. EFSA Journal. 9(9): 2363.

Ellis, J. L., I. K. Hindrichsen, G. Klop, R. D. Kinley, N. Milora, A. Bannink, and J. Dijkstra. 2016. Effects of lactic acid bacteria silage inoculation on methane emission and productivity of Holstein Friesian dairy cattle. Journal of Dairy Science. 99(9): 7159-7174.

Han, L., and H. Zhou. 2013. Effects of ensiling processes and antioxidants on fatty acid concentrations and compositions in corn silages. Journal of Animal Science and Biotechnology. 4(1): 1-7.

Heron, S. J., R. A. Edwards, and P. Phillips. 1989. Effect of pH on the activity of ryegrass *Lolium multiflorum* proteases. Journal of the Science of Food and Agriculture. 46(3): 267-277.

Holzapfel, W. H. 1992. Culture media for non-sporulating Gram-positive food spoilage bacteria. International Journal of Food Microbiology. 17(2): 113-133.

Houfani, A.A., N. Anders, A. C. Spiess, P. Baldrian, and S. Benallaoua. 2020. Insights from enzymatic degradation of cellulose and hemicellulose to fermentable sugars–a review. Biomass and Bioenergy. 134: 105481.

Hu, X., W. Hao, H. Wang, T. Ning, M. Zheng, and C. Xu. 2015. Fermentation characteristics and lactic acid bacteria succession of total mixed ration silages formulated with peach pomace. Asian-Australasian journal of Animal Sciences. 28(4): 502.

Huyen, N. T., I. Martinez, and W. Pellikaan. 2020. Using lactic acid bacteria as silage inoculants or direct-fed microbials to improve *in vitro* degradability and reduce methane emissions in dairy cows. Agronomy. 10(10): 1482.

Jiang, D., B. Li, M. Zheng, D. Niu, S. Zuo, and C. Xu. 2020. Effects of *Pediococcus pentosaceus* on fermentation, aerobic stability, and microbial communities during ensiling and aerobic spoilage of total mixed ration silage containing alfalfa (*Medicago sativa L*.). Grassland Science. 66(4): 215-224.

Kachouri, F., K. Setti, H. Ksontini, M. Mechmeche, and M. Hamdi. 2016. Improvement of antioxidant activity of olive mill wastewater phenolic compounds by *Lactobacillus plantarum* fermentation. Desalination and Water Treatment. 57(56): 27125-27137.

Kietkwanboot, A. 2013. Decolorization and Biodegradation of Phenolics in Palm Oil Mill Effluent by White Rot Fungi Immobilized on Oil Palm Residues. M.Sc. Thesis in Environmental Management. Prince of Songkla University, Songkla.

Kim, D. H., K. D. Lee, and K. C. Choi, 2021. Role of LAB in silage fermentation: Effect on nutritional quality and organic acid production—An overview. AIMS Agriculture and Food. 6(1): 216-234.

Kondo, M., K. Shimizu, A. Jayanegara, T. Mishima, H. Matsui, S. Karita, M. Goto, and T. Fujihara. 2016. Changes in nutrient composition and *in vitro* ruminal fermentation of total mixed ration silage stored at different temperatures and periods. Journal of the Science of Food and Agriculture. 96(4): 1175-1180.

Kung Jr, L., and N.K. Ranjit. 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. Journal of Dairy Science. 84(5): 1149-1155.

Lee, K., T. D. Marbun, S. Kim, J. Song, C. H. Kwon, D. Yoon, J. Kang, C. Lee, S. Cho, and E. J. Kim, 2020. Effect of lactic acid bacteria treatment on nutritive value and *in vitro* ruminal fermentation of Italian ryegrass (*Lolium multiflorum L*.) silage. Journal of The Korean Society of Grassland and Forage Science. 40(3): 182-189.

Lei, C. H. E. N., X. J. Yuan, J. F. LI, S. R. Wang, Z. H. Dong, and S. H. A. O. Tao. 2017. Effect of lactic acid bacteria and propionic acid on conservation characteristics, aerobic stability and *in vitro* gas production kinetics and digestibility of whole-crop corn based total mixed ration silage. Journal of Integrative Agriculture. 16(7): 1592-1600.

Li, R., D. Jiang, M. Zheng, P. Tian, M. Zheng, and C. Xu. 2020. Microbial community dynamics during alfalfa silage with or without clostridial fermentation. Scientific Reports. 10(1): 1-14.

Madrid, J., A. Martínez‐Teruel, F. Hernández, and M. D. Megías. 1999. A comparative study on the determination of lactic acid in silage juice by colorimetric, high‐performance liquid chromatography and enzymatic methods. Journal of the Science of Food and Agriculture. 79(12): 1722-1726.

Muck, R. E., E. M. G. Nadeau, T. A. Mcallister, F. E. Contreras-Govea, M. C. Santos, and L. Kung Jr. 2018. Silage review: recent advances and future uses of silage additives. Journal of Dairy Science. 101: 3980–4000.

National Research Council. 1992. Applications of Biotechnology in Traditional Fermented Foods. National Academies Press, Washington, D.C.

Oliveira, A.S., Z. G Weinberg, I. M. Ogunade, A. A. Cervantes, K. G. Arriola, Y. Jiang, D. Kim, X. Li, M. C. Gonçalves, D. Vyas, and A. T. Adesogan. 2017. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. Journal of Dairy Science. 100(6): 4587-4603.

Ortigosa, M., C. Arizcun, A. Irigoyen, M. Oneca, and P. Torre. 2006. Effect of lactobacillus adjunct cultures on the microbiological and physicochemical characteristics of Roncal-type ewes'-milk cheese. Food Microbiology. 23(6): 591-598.

Patel, J.P., and P.H. Parsania. 2018. Characterization, testing, and reinforcing materials of biodegradable composites. Biodegradable and Biocompatible Polymer Composites. 55-79.

Pitt, R.E. 1990. Silage and Hay Preservation (NRAES 5).

Ramos, J.P.F., E. M. Santos, A. P. M. Santos, W. H. Souza, J. S. Oliveira, and T. C. Silva. 2016. Ensiling of forage crops in semiarid regions. Advances in Silage Production and Utilization, Cap. 4: 65-84.

Rossi, F., V. Gatto, G. Sabattini, and S. Torriani. 2012. An assessment of factors characterising the microbiology of Grana Trentino cheese, a Grana-type cheese. International Journal of Dairy Technology. 65(3): 401-409.

Scherer, R., A. C. P. Rybka, C. A. Ballus, A. D. Meinhart, J. F. Teixeira, and H. T. Godoy. 2012. Validation of a HPLC method for simultaneous determination of main organic acids in fruits and juices. Food Chemistry. 135(1): 150-154.

Schmidt, P., L. G. Nussio, O. C. M. Queiroz, M. C. Santos, M. Zopollatto, S. G. D. Toledo Filho, and J. L. P. Daniel. 2014. Effects of *Lactobacillus buchneri* on the nutritive value of sugarcane silage for finishing beef bulls. Revista Brasileira de Zootecnia. 43: 8-13.

Sofyan, A., A.N. Aswari, T. Purwoko, and E. Damayanti. 2013. Screening of lactic acid bacteria from rumen liquor and king grass silage as well as their antibacterial activities. Media Peternakan. 36(3): 216-216.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd edition. McGraw-Hill, NY.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarchpolysaccharides in relation to animal nutrition. Journal of Dairy Science. 74: 3583-3597.

Weinberg, Z.G., G. Ashbell, Y. Hen, and A. Azrieli. 1993. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. Journal of Applied Bacteriology. 75(6): 512-518.

Wongnen, C., C. Wachirapakorn, C. Patipan, D. Panpong, K. Kongweha, N. Namsaen, P. Gunun, and C. Yuangklang. 2009. Effects of fermented total mixed ration and cracked cottonseed on milk yield and milk composition in dairy cows. Asian-Australasian Journal of Animal Sciences. 22(12): 1625-1632.

Zhao, S., F. Yang, Y. Wang, X. Fan, C. Feng, and Y. Wang. 2021. Dynamics of Fermentation Parameters and Bacterial Community in High-Moisture Alfalfa Silage with or without Lactic Acid Bacteria. Microorganisms. 9(6): 1225.