Tropentag 2022
September 14-16, 2022
Conference on International Research on Food Security, Natural Resource Management and Rural Development
organised by the Czech University of Life Sciences, Prague, Czech Republic

Effect of *Lactobacillus rhamnosus* C6 Inoculation on Fermentation Quality and Rumen Degradability of Maize Cob and Hush Silage

Saowaluck Yammuen-Art[a] Siwat Sangsritavong[b], Narongrit Jaipolsaen[b] Siriporn Umsook[c]

a Chiang Mai University, Faculty of Agriculture, Department of Animal and Aquatic Science, Thailand
b National Science and Technology Development Agency, National Center For Genetic Engineering And Biotechnology (BIOTEC), Thailand
c Veterinary, Conservation and Research Section, Animal Management Division, Chiang Mai Night Safari , Thailand

Introduction

Chiang Mai Province is the biggest area of maize production (157,000 rai) with an estimated annual crop residues production of 42,000 tons. The crop residues such as cob and husk can be use as the roughage for ruminants. Because of the huge amount of crop residues, therefore the preservation is necessary. Silage is the feed preservation by ensiling method. Lactic acid bacteria is a common species in silage and has an important role in ensiling process. (Muck, 2010) Lactic acid bacteria can change water-soluble carbohydrate to lactic acid. The accumulation of lactic acid leads to reduction of pH. The low pH condition inhibits the growth and nutrient utilization of desirable microorganism which results in reducing loss of nutrient. (McDonald *et al*., 1991) Natural fermentation of silage can cause loss of nutrient by epiphytic bacteria. The quality of silage could be improved by lactic acid bacteria inoculants, consequently lactic acid production occurs more quickly and loss of nutrients during ensilage can be reduced. (Widyastuti, 2008) *Lactobacillus rhamnosus* was able to ferment various carbohydrates substrates. *L. rhamnosus* AT195 showed a good survival performance in the silage fermentation process of both maize and sorghum and improved the fermentation quality of the forages by reducing the ammonia nitrogen content (Salime *et al*., 2007). The objective of this study were to evaluate effect of *Lactobacillus rhamnosus* C6 inoculation on fermentation quality and rumen degradability of maize cob and hush silage.

Material and Methods

Starter culture and silage preparation

L. rhamnosus C6 was isolated from rumen fluid of Thai native cattle. The isolates was grow in Nutrient agar (NA) plate at 37°C for 48 hours in anaerobic condition. Maize cob and hush silage were divided into 2 groups as control and supplemented with *L. rhamnosus* C6 with 10^6 cfu/g. The silage were ensiled for 21 days for later analysis

Fermentation quality analysis

The fermentation quality was evaluated by pH measurement and VFA analysis. To receive extracted silage 90 ml distilled water was added to 10 g silage 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 silage, 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 KH2PO4 (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).

Feed sample analyses

The silage samples were dried at 60˚C for 48 h. Dry matter (DM), crude protein (CP), ether extract (EE) and crude fiber (CF) were analyzed according to AOAC Methods (AOAC, 2000). The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed by detergent methods (Van Soest *et al.* 1991).

***In vitro* gas production and estimated parameters of maize cob and husk**

Ruminal degradability was determined using *in vitro* gas production technique. Ruminal fluid was obtained from 4 rumen fistulated Thai native cattle. The silage samples were passed through a 1 mm colander until they were ground. Each sample of 230 ± 5 mg was accurately weighed out in 3 replicates and placed into 100 mL glass syringes that were fitted with plungers. They were then incubated in a shaking water bath at 39°C. Three empty syringes containing only the incubation medium were incubated as blanks in order to correct for any gas production resulting from the activity of the rumen fluid. Gas production was recorded after incubation at 2, 4, 8, 10, 12, 24, 48, 72 and 96 hr. To estimate the fermentation kinetic parameters, data on cumulative gas production were established using the exponential model that had been proposed by Ørskov and McDonald (1979).

Results and Discussion

The pH value of maize cob and hush silage inoculated with *L. rhamnosus* C6 were lower than control group. Lactic acid concentration was higher in inoculated groups. LAB inoculation triggered reducing of pH, values led to inhabitation of bacterial ligninolytic enzymes, including the three main lignin-degrading enzymes: lignin peroxidase, manganese peroxidase, and laccase, functioning at pH values around 8 (Rahman *et al*., 2013), causing ADL to be higher in *L. rhamnosus* C6 inoculation than control.

Table 1 Fermentation quality of fermented maize cob and husk with (LAB.) or without (CON.) *L. rhamnosus* C6 at 21 day of ensiling time**.**

Item	Treatment		SEM	*P-value*
	Control	*L. rhamnosus C6*		
pH	4.48	4.30	0.47	0.01
Acetic (%DM)	5.65	7.99	0.68	<0.001
Propionic (%DM)	0.14	0.13	0.01	0.67
Butyric (%DM)	1.19	0.64	0.16	<0.001
Lactic (%DM)	0.12	0.28	0.04	0.04
Total acid (%DM)	8.97	11.48	0.73	<0.001

SEM= standard error of the mean, DM= Dry matter**.**

The chemical composition of maize cob and hush silage after 21 days of ensiling periods is shown in Table 2. Maize cob and hush silage inoculated with *L. rhamnosus* C6 showed significantly lower DM but significantly higher OM contents than control group. Maize cob and hush silage inoculated with *L. rhamnosus* C6 showed significantly lower NDF, cellulose and hemicellulose contents than control group. The fiber decreased in maize cob and hush silage

inoculated with *L. rhamnosus* C6, caused by LAB inoculation, which rapidly lowered the pH values of eTMR, prolonging the period of acid hydrolysis (fiber degradation reaction) and causing a decrease in ADF (Zhao *et al*., 2019).

Table 2. Chemical composition (DM basic %) of maize cob and husk inoculated *L. rhamnosus* C6 at 21 day

Chemical composition	**Treatment**		**SEM**	***P-value***
	Control	***L. rhamnosus C6***		
Dry matter (DM)	43.59	42.73	0.24	<0.001
Organic matter (OM)	96.33	96.76	0.12	<0.001
Crude protein (CP)	2.97	2.95	0.01	0.39
Ether extract (EE)	1.75	1.82	0.05	0.35
Crude fiber (CF)	85.18	90.02	3.88	0.39
Neutral detergent fiber (NDF)	80.87	78.62	0.68	<0.001
Acid detergent fiber (ADF)	44.41	44.86	0.25	0.22
Acid detergent lignin (ADL)	8.22	7.88	0.14	0.07
Hemicellulose	36.45	33.75	0.79	<0.001
Cellulose	72.64	70.74	0.57	<0.001

Gas production at 48 and 72 hr after incubation was significantly greater in inoculated groups. The inoculated groups revealed the gas production rate (c) and the potential extent of gas production (|a|+b) were significantly higher than in inoculated groups.

Table 3. *In vitro* gas production and estimated parameters of maize cob and husk inoculated *L. rhamnosus* C6

Item	**Treatment**		**SEM**	***P-value***
	Control	***L. rhamnosus* C6**		
***In vitro* gas production (ml/0.2g DM)**				
2 hours	1.63	1.96	0.34	0.03
4 hours	2.33	3.29	0.47	0.53
8 hours	5.62	7.53	0.70	0.05
10 hours	9.59	11.05	0.49	0.53
12 hours	13.57	14.89	0.57	0.16
16 hours	20.82	22.65	0.65	0.16
24 hours	31.00	32.41	0.63	0.49
48 hours	34.94	42.87	3.18	0.002
72 hours	43.35	50.71	3.00	0.02
96 hours	48.50	55.10	2.84	0.06
Kinetic of gas production				
a(ml)	-3.28	-5.41	0.56	0.04
b (ml/0.2g DM)	64.0	104.34	9.93	0.10
c (% hr.)	.04	.07	0.01	0.01
IaI+b	67.31	109.76	10.46	0.03
Estimated parameters				
OMD (%)	43.07	44.27	0.54	0.48
ME (Mj/Kg DM)	5.80	6.02	0.09	0.48
SCFA (mol)	0.68	0.71	0.01	0.54
NE_L (Mj/Kg DM)	3.03	3.04	0.01	0.21

Conclusions

It could be concluded that maize cob and hush silage inoculated with *L. rhamnosus* C6 had better quality fermentation than natural fermentation as *L. rhamnosus* C6 stimulated the fermentation process and accelerated lactic acid production, resulting in a sharp drop in pH and faster ruminal degradability after 48 hr of incubation

References

A.O.A.C. 2000. Official Methods of Analysis ofthe Association of Official Analytical

Bal, M. A., Coors, J. G., & Shaver, R. D. 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(10), 2497–2503.

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

McDonald, P., A. R. Henderson, and S. J. E. Heron. 1991. The biochemistry of silage. 2nd ed. Chalcombe Publications, Marlow, Bucks, UK.

Muck, R. E. 2010. Silage microbiology and its control through additives. Rev. Bras. Zootec. 39:183–191.

Ørskov, E., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci.92(2),499-503.

Rahman, N. H. A., Abd Aziz, S., & Hassan, M. A. (2013). Production of ligninolytic enzymes by newly isolated bacteria from palm oil plantation soils. Bioresources, 8(4), 6136–6150.

Salimei,E. V. Capilongo, A. Simoni, P. G. Peiretti, C. Maglieri, C. A. Romano, L. Mannina, R. Coppola, and E. Sorrentino. *Lactobacillus rhamnosus* as Additive for Maize and Sorghum Ensiling.Journal of Agricultural and Food Chemistry 2007 55 (23), 9600-9607

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

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

Widyastuti, Y. 2008. Fermentasi silase dan manfaat probiotik silase bagi ruminansia. Med. Pet. 31:225-232.

Zhao, J., Dong, Z., Li, J., Chen, L., Bai, Y., Jia, Y., & Shao, T. (2019). Effects of lactic acid bacteria and molasses on fermentation dynamics, structural and nonstructural carbohydrate composition and in vitro ruminal fermentation of rice straw silage. Asian-Australasian Journal of Animal Sciences, 32(6), 783.