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Efficiency of lactic acid bacteria as the potent degradative microorganism to digest the total mixed ration *in vitro*

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Abstract

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The objective of this research was to investigate the efficiency of lactic acid bacteria on *in vitro* digestibility of total mixed ration (TMR) containing pineapple plant silage 55%, rice straw 15%, cassava ship 5.6%, soybean meal 12.6%, palm kernel pulp l4.2%, rice bran 5.6%, premixed 0.15% and molasses 2%. Guinea grass (*Panicum maximum*) was used for isolation and identification of microorganisms from silage. Treatments were used as TMR treated with lactic acid bacteria as follows: Treatment 1 – non treated; Treatment 2 – TMR treated with *Lactobacillus pantarum*; Treatment 3 – TMR treated with *Pediococcus pentosaceus*; Treatment 4 – TMR treated with *Pediococcus acidilactici*; Treatment 5 – TMR treated with *L. pantarum* + *P. pentosaceus* + *P. acidilactici*. The treated TMR were mixed and put in polyethylene bags and stored at ambient temperature for 7 days before analysis. The *in vitro* digestibility test used pepsin cellulase *in vitro* digestibility method. The results showed that there were significant differences ($P < 0.01$) of the nutritive value of TMR by proximate analysis and fiber analysis which treated lactic acid bacteria. For the digestibility, results showed that the TMR treated with LAB using pepsin cellulase *in vitro* digestibility which applied LP, PP, PA and LPP could improve the digestibility of TMR, which significantly increased in digestibility of TMR ($P < 0.01$). The rate of increase and decrease of digestibility and fiber in TMR treated with LAB showed an increase in dry matter and a decrease in fiber. Therefore, it was suggested that TMR treated with lactic acid bacteria of TMR may possible to develop as biological animal feed for ruminant.

Keywords: lactic acid bacteria; digestibility; total mixed ration
Abbreviations: TMR – total mixed ration; LAB – lactic acid bacteria; CRD – completely randomized design; DM – dry matter; ASH – ash; CF – crude fat; EE – ether extract; CP – crude protein; CF – crude fiber; AIA – acid insoluble ash; NDF – neutral detergent fiber; ADF – acid detergent fiber; ADL – acid detergent lignin; DMRT – Duncan's Multiple Range Test; LP – *Lactobacillus pantarum*; PP – *Pediococcus pentosaceus*; PA – *Pediococcus acidilactici*; LPP – *L. pantarum* + *P. pentosaceus* + *P. acidilactici*

Introduction

Forage crops or roughage is mainly important feed for ruminants. Most of farmers provide forage from nature with low quality in various kinds of forage crops, age and geology. In general, digestibility of fiber of forage crops in tropical zone is lower than in temperate zone by 13%, which causes lower ruminant production (Smith et al., 1988). The undeveloped feeding and management are factors limiting the production. Used concentrates are separated from roughage for feeding and that is widely applied in ruminants. It is unsuitable for the high production performance. Therefore, it is important to improve the nutrition of farmers' ruminant animals. Use of total mixed ration (TMR) may reduce this problem, and it could be considered to enhance the productivity of ruminants by improving consistency of selected diet nutrient content. It has been known that TMR improve nutrient utilization due to balance intake for roughage and concentrates leading to stabilization of rumen function (Nocek et al., 1985). The main objective is to produce TMR which digested rapidly by using potent degradative microorganisms especially lactic acid bacteria (LAB) (McDonald et al., 1991). The efforts are focused on finding potent LAB to be used in TMR for animal feed. The activities of LAB in TMR contribute to break down the forage fiber (Wallace et al., 2001). The objective of this research was to study the digestibility of TMR from effective lactic acid bacteria *in vitro*.

Materials and Methods

Silage preparation

Guinea grass (*Panicum maximum*) 45 day-old was chopped to 2-3 cm by knife. The material sample were mixed with 1% $NaCl_2$, put into compressed polyethylene bags and stored at ambient temperature for 21 days. After that the sample of 1 kg was used for isolation and identification of lactic acid bacteria.

Isolation of microorganism

The samples were collected from the silage in three parts of each silo – upper, middle and lower parts. The samples consisted of 100 g from each part which were mixed; 10 g randomly collected sub-samples were diluted with distilled water then mixed with 90 ml of 0.1% peptone. The sample was serially diluted up to 10^{-6}, then 0.1 ml was pipetted and spread plated on de Man, Rogosa and Sharpe broth (MRS) mixed with calcium carbonate 1% (de Man et al., 1960) and incubated at 30 ± 4°C for 48 h, the single colonies observed and isolated into pure culture.

Morphological identification of microorganism

The morphology of isolated LAB was noted based on the methods of Kandler and Weiss (1986) and Stiles and Holzapfel (1997). The following tests are used: catalase production test (South Bend Medical Foundation, 2010) and gas production tested (Hayward, 1957), a growth test with different temperature regimes (Cai et al., 1998; Yang et al., 2010), salt tolerance test at different concentrations of $NaCl_2$ (Cai et al., 1998; Yang et al., 2010), growth test for different pH levels (Kandler and Weiss, 1986; Yang et al., 2010). Morphological identification was done by following the methods of the Bergey Manual of Determinative Bacteriology (Holt et al., 1994).

Total Mixed Ration (TMR) preparation

The total mixed ration feed used in this study were pineapple plants silage and rice straw as the source of roughage mixed with concentrate. TMR is made by recipe for goat with ingredients as follows.

Ingredients	kg/100 kg
Pineapple plant silage	55
Rice straw	15
Cassava ship	5.6
Soybean meal	12.6
Palm kernel pulp	4.2
Rice bran	5.6
Premixed	0.15
Molasses	2

The premix consists (per kilogram of dry matter): vitamin A – 10,000 IU; vitamin D3 – 2,000 IU; vitamin E – 20 IU; Cu – 10 mg; Mn – 80 mg; Zn – 40 mg; Fe – 50 mg; I – 0.8 mg; Se – 0.3mg; Co – 0.3 mg.

The experiment was carried out using completely randomized design (CRD) with four replications. The total mixed rations (TMR) were treated with lactic acid bacteria in each treatment as follows: Treatment 1 – non treated; Treatment 2 – TMR treated with *Lactobacillus pantarum*; Treatment 3 – TMR treated with *Pediococcus pentosaceu*; Treatment 4 – TMR treated with *Pediococcus acidilactici*; Treatment 5 – TMR treated with *L. pantarum* + *P. pentosaceus* + *P. acidilactici*. The TMR were mixed and put in polyethylene bags and stored at ambient temperature for 7 days before analysis.

Proximate composition analysis of silage

For each treatment, 1000 g of fresh material were randomly collected to determine nutrient composition. The samples were oven dried at 60°C for 48 h prior to proximate analysis. Dry matter (DM), ash (ASH), crude fat (CF), ether extract (EE), crude protein (CP) and crude fiber (CF) were determined according to the methods of AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the method of Van Soest and Robertson (1979).

All analyses were conducted using the Fibertec System M6 (FOSS, USA). The experiments were designed as completely randomized design with 4 replications. The experiment was repeated two times. Data were computed using analysis of variance and treatment means were compared using Duncan's Multiple Range Test (DMRT) at P = 0.05 and P = 0.01.

***In vitro* digestibility test**

Pepsin cellulase *in vitro* digestibility method was used following McLeod and Minson (1978). The enzymatic digestion was determined by adding 0.5 g of feed sample to test tube, size 2.5 cm, long 10 cm with screw cap. A 500 ml of acid – pepsin (0.2% pepsin with 0.1 N HCL) was prepared the day before treatment and incubated at 39°C then added and tubes were shaken and incubated at 39°C for 48 h. After incubation, the feed samples were centrifuged for 10 min. Thereafter, the tubes were filtered by filter stick for suction water, the residues washed with distilled water and transferred to the respective test tube. A 50 ml cellulase acetate buffer solution (ONOZUKA 3S) was prepared the day before and incubated at 39°C, tubes were shaken for 10 minutes and incubated at 39°C for 48 h, then the tubes were filtered through pre-weighed sintered glass crucibles and washed with distilled water. Thereafter, the crucibles with residues were dried at 105°C for 12 h and weighed to obtain analysis of dry matter. The crucibles with residues were burnt at 500°C for 5 h in furnace cooled to room temperature and weighed to obtain to analysis for dry matter digestibility.

Results and Discussions

Chemical composition of total mixed ration (TMR)

The nutritive value of TMR revealed that DM was 68.28%, CP – 12.19%, CF – 15.06%, ASH – 6.54%, NDF – 32.25%, ADF – 23.74% and ADL – 5.71%, respectively. These results are closed to results obtained from Mayed (2016), who reported that TMR nutritional value was DM – 83.98%, CP -13.37%, CF – 15.49%, Ash – 5.77%, NDF – 45.96%, ADF – 22.43% and ADL – 10.60%.

Changes in chemical composition of TMR treated with lactic acid bacteria

Results of the proximate analysis of the nutritive value of TMR of treatments with *Lactobacillus pantarum* (LP), *Pediococcus pentosaceus* (PP), *Pediococcus acidilactici* (PA) and *L. pantarum* + *P. pentosaceus* + *P. acidilactici* (LPP) were showed in Table 1. The dry matter was increased from 68.28% to 79.80%, 79.79%, 79.40%, and 79.42% for LP, PP, PA and LPP, respectively. The TMR treated with LP gave significantly higher dry matter than PP, PA and LPP. The TMR treated with PA gave significantly higher protein (10.97%) than PP (10.02%), LPP (9.77%) and LP (9.65%), but it was not significantly difference in LP, PP and LPP. The reduced protein content is due to the utilization of protein in food in the diet by the microbial proteolysis process (Guo et al., 2013). The TMR treated with PP gave significantly lower fiber (13.4%) than PA (13.33%), LPP (13.64%) and LP (13.82%), but it was not significantly difference between PP with PA and LP with LPP. The TMR treated with PA gave significantly higher ash (5.79%) than LP (5.58%), PP (5.56%) and LPP (5.53%). The TMR treated with PA gave significantly lower AIA (1.29%) than PP (1.50%), LP (1.55%) and LPP (1.56%), but it was not significantly difference between LP, PP and LPP.

Results showed that fiber analysis in TMR according to Van Soest revealed that fiber digestion of the TMR treated

Table 2. Fiber analysis of TMR treated with lactic acid bacteria

Treatments	Fiber analysis (%)		
	NDF	ADF	ADL
1 Control	32.25^{B}	23.74^{A}	5.71^{A}
2. *Lactobacillus pantarum*	34.13^{A}	20.60^{D}	5.24^{AB}
3. *Pediococcus pentosaceus*	30.29^{C}	20.29^{D}	4.90^{B}
4. *Pediococcus acidilactici*	32.61^{B}	21.60^{BC}	5.08^{B}
5. *L. pantarum* + *P. pentosaceus* + *P. acidilactici*	31.69^{B}	22.59^{B}	4.88^{B}
CV	2.29	3.26	6.31

Mean followed by common letter in each column are not significantly difference (P < 0.01) by DMRT.

Table 1. Chemical composition of TMR treated with lactic acid bacteria

Treatments	Chemical composition (%)							
	DM	CP	CF	Ash	Ca	P	AIA	Digestibility
1	68.28^{A}	12.19^{A}	15.06^{A}	6.54^{A}	1.19^{A}	0.33^{A}	1.75^{A}	60.31^{C}
2	79.80^{C}	9.65^{C}	13.82^{B}	5.58^{C}	1.14^{B}	0.28^{B}	1.55^{B}	64.98^{B}
3	79.79^{C}	10.02^{C}	13.14^{C}	5.56^{C}	0.98^{D}	0.25^{C}	1.50^{B}	65.16^{B}
4	79.40^{C}	10.97^{B}	13.33^{C}	5.79^{B}	0.97^{D}	0.25^{C}	1.29^{C}	66.21^{A}
5	79.42^{B}	9.77^{C}	13.64^{B}	5.53^{C}	1.04^{C}	0.26^{BC}	1.56^{B}	66.20^{A}
CV	0.55	5.5	0.93	1.03	2.7	5.7	3.47	0.43

Mean followed by common letter in each column are not significantly difference (P < 0.01) by DMRT.

Table 3. The rate of increased and decreased of digestibility and fiber in TMR treated with LAB

Chemical composition	Before treatment	After treatment with lactic acid bacteria (%)							
		LP	+/–	PP	+/–	PA	+/–	LPP	+/–
Dry matter	68.28	79.8	16.87	79.79	16.86	79.4	15.76	79.42	16.31
Crude fiber	15.06	13.82	-8.23	13.14	-12.75	13.33	-11.49	13.64	-9.43
NDF	32.25	34.13	5.83	30.29	-6.08	32.61	1.12	31.69	-1.74
ADF	23.74	20.6	-13.23	20.29	-14.53	21.6	-9.01	22.59	-4.84
ADL	5.71	5.24	-8.23	4.9	-14.19	5.08	-11.03	4.88	-14.54
Digestibility	60.31	69.98	7.74	65.16	8.41	66.21	9.78	66.2	9.76

with LP gave significantly higher NDF (34.13%) than PA (32.61%), LPP (31.69%) and PP (30.20%), it was not significantly difference in NDF between PA and PP. The TMR treated with PP gave significantly lower ADF (20.29%) than LP (20.60%), PA (21.60%) and LPP (22.59%). The TMR treated with LPP gave significantly lower acid detergent lignin (ADL) (4.88%) than PP (4.90%), PA (5.08%) and LP (5.24%), it was not significantly difference in LP, PP, PA and LPP when compared with non-treated with lactic acid bacteria (Table 2).

Cell wall constituents (CWC) or NDF are components of cell wall. Mostly cellulose and hemicellulose lignin includes cutin, silica and tannin. Normally, they cannot be digested by simple stomach of animal, while ruminant can have some microorganisms in the stomach, but can be more or less dependent on the amount of lignification, cutinization as well as the amount of silification in the feed. The substances in the ADF – cellulose, lignin, cutin and acid insoluble ash (AIA) refer to ash that is insoluble in acid (Van Soest, 1963, 1964). Van Soest and Moore (1965) found that CWC digestibility was correlated with the amount or concentration of lignin present in the ADF, especially when converted into logarithm.

Results showed that the TMR treated with LAB using pepsin cellulose with *in vitro* application of LP, PP, PA and LPP, could significantly improve its digestibility. The TMR treated with PA gave significantly higher digestibility (66.21%) than LPP (66.20%), PA (65.16%) and LP (64.98%), it was not significantly difference between PA, LPP and LP, PA (Table 1). The rate of increase and decrease of digestibility and fiber in the TMR treated with LAB (Table 3) showed that the TMR treated with LP gave higher increased dry matter (+16.87%) than PP (+16.86%), LPP (+16.31%) and PA (+15.76%). The TMR treated with PP gave higher decreased in crude fiber (-12.75%) than PA (-11.49%), LPP (- 9.43%) and LP (- 8.23%). For NDF in the TMR treated with LP and PA there is an increase (+5.83% and +1.12%), but for PP and LPP the results showed that the value of NDF were decreased (-6.08% and -1.74%), respectively. The TMR treated with PP gave higher decreased ADF (-14.53%) than LP, PA and LPP (-13.23%, -9.01% and -4.84%, respectively). For ADL also the results showed a decrease when the TMR was treated with LAB. The digestibility of the TMR treated with PA gave higher increased than LPP, PP and LP (+9.78%, +9.76, +8.41% and +7.74%, respectively). *Lactobacillus* sp. could produce protease and *Pediococcus* sp. was able to produce amylase and protease. These two species can possibly be used to improve the nutritional value of the silage, TMR or roughage for ruminant production (Suphalucksana and Soytong, 2017).

Conclusions

The results show that TMR treated with lactic acid bacteria can be used as biological animal feed for ruminants. The study showed that TMR is rapidly improving the quality when using lactic acid bacteria. Feed composition analyses revealed that LP gave the highest dry matter, calcium and phosphorus. The TMR treated with PA gave significantly higher protein, ash, AIA and NDF. The TMR treated with PP gave significantly lower fiber and ADF. Results showed that the TMR treated with lactic acid bacteria with *in vitro* application of LP, PP, PA and LPP, could significantly increase its digestibility. The TMR treated with PA gave significantly higher digestibility.

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