International Journal of Agricultural Technology 2021Vol. 17(5):1847-1856
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

The effects of *Lactobacillus plantarum* inoculation of spontaneously fermented cassava pulp (SFCP) on the quality, feed intake and growth of Lao-native beef steers

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Napasirth, P., Wachirapakorn, C. and Napasirth, V. (2021). The effects of *Lactobacillus plantarum* inoculation of spontaneously fermented cassava pulp (SFCP) on the quality, feed intake, and growth of Lao-native beef steers. International Journal of Agricultural Technology 17(5):1847-1856.

Abstract The inoculated with *Lactobacillus plantarum* at concentration of 1.0 x 10^5 CFU/g FM showed the diet's protein contents of 14.39% DM, while the uninoculated was 13.30% DM. The pH values of the uninoculated spontaneously fermented cassava pulp (SFCP) were higher than the inoculated cassava pulp (P<0.01), while the pH of the fermented total mixed ration (FTMR) was not affected by inoculant addition (P>0.05). Total feed intakes were not significantly different between the treatments except during 60 days of the experiment, which showed that the feed intake of uninoculated FTMR was higher than the inoculated FTMR (P<0.01). Overall, ruminal pH, NH_3-N, and BUN were not affected by diet. However, steers were fed FTMR inoculated with *L. plantarum*, which significant affected on ADG and FCR compared to the control during 60 days after feeding period. The study indicated that SFCP inoculation with *L. plantarum* can improve the quality of the SFCP in the laboratory, whereas inoculated FTMR with *L. plantarum* was not decreased pH value. Finally, steers fed FTMR with inoculation had shown more significant ADG and FCR at 60 days after feeding.

Keywords: Beef cattle, Fermented Total Mixed Ration (FTMR), spontaneously fermented cassava pulp (SFCP)

Introduction

Ruminant production in Laos has gradually increased over nearly half a century (Napasirth and Napasirth, 2018). For instance, total beef production has grown more than fivefold since 1961 because bovine meat is the second-most-frequently consumed meat in Laos, second to pork. By 2013, Laos' total bovine

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meat supply reached 49,371 tonnes, and annual average bovine meat consumption per capita hit 7.29 kilograms, compared to 2.76 kilograms in 1961 (FAOSTAT, 2020). Laos food demand is increasing rapidly, both on-demand from the growing population and per capita consumption. At present, the total population in Laos is 7 million people (Lao Statistics Bureau, 2020). They need more than 50,000 tonnes of bovine meat (FAOSTAT, 2020). There had been considerable investment in large-scale commercial livestock production in recent years to ensure a sustainable supply of bovine meat based on locally available feed resources. Cassava pulp is the manufacturing waste produced during starch extraction and represents 10–15% of the fresh cassava roots' weight (Sriroth *et al*., 2000). This is equivalent to about 227,000–341,000 tonnes of cassava pulp discharge each year. This amount of cassava pulp is usually neglected in the open pit. Our previous research has shown that the upper surface of cassava pulp neglected in the open pit had rotted due to aerobic conditions. However, any cassava pulp under the first meter of the open pit's surface was acceptably spontaneously fermented cassava pulp (SFCP) with pH values in the range of 3.2–3.5 based on the epiphytic lactic acid bacteria that are present on cassava pulp and convert water-soluble carbohydrates into lactic acid in the ensiling process (Vongsamphanh *et al*., 2014).

As a consequence, the pH is reduced, and the feed is preserved under anaerobic conditions (Napasirth *et al*., 2015). However, when the SFCP is exposed to air, aerobic spoilage will commence. Therefore, the SFCP from the open pit must be mixed with the feed ingredients as a fermented total mixed ration and re-fermented immediately to prevent perishment. Commonly, urea has been widely used as a non-protein nitrogen (NPN) source in ruminant diets. However, urea in the ensiling process has impacted the fermentation pH, and it acts by preventing rapid pH declines. Lactic acid bacteria (LAB) can be supplemented with silage fermentation additives to guarantee rapid and vigorous fermentation (Cao *et al*., 2010 and Napasirth *et al*., 2015). Therefore, this research aimed to demonstrate the use of SFCP from the cassava factory as the main staple in FTMR with or without lactic acid bacteria on feed intake, rumen fermentation, and growth performance in Lao-native beef steers.

Materials and methods

Sample collection

Collecting undisturbed cassava pulp samples from the open pit for this experiment was conducted using the PVC pipe (8 m long and 10 cm in diameter). The pipe was pressed vertically from the cassava pulp's surface up to the bottom of the pit. Then, the cassava pulp 1 m from the top of the pipe was removed to eliminate the infected pulp. Undisturbed cassava pulp samples were taken at 2, 3, 4, 5, 6, and 7 m below the surface and mixed. A total of 70 samples were collected at different sites around the open pit. The pH of fermented cassava pulp was immediately measured using a pH meter. Three kilograms of each sample were taken and placed in vacuum storage bags to maintain anaerobic conditions and avoid sample damage for analyzing the chemical composition of raw materials.

Treatment preparation and experimental diets

SFCP fermented with or without the commercial *Lactobacillus plantarum* strain was prepared using small-scale fermentation (Cai *et al*., 1999) to analyze the chemical composition of raw materials, the main changes that occur during the fermentation process, and in vitro gas production. In the feeding trial, the SFCP from the cassava factory was used as the main staple in the FTMR (Table 1); the dietary treatments were SFCP without additive (Uninoculated) and SFCP with *L. plantarum* inoculum (1.0 x 10^5 CFU/g FM) (Inoculated). Before ensiling, Napier grass was chopped into sections of approximately 2–4 cm in length. After proper mixing, the FTMR was packed thoroughly into circular concrete tanks (0.8m diameter and 1.2m high). The tops of the tanks were covered with a plastic sheet. After that, old car tires were used as weight material. They were carefully pressed to remove air and then stored outdoors at ambient temperature (30–40 ℃) for at least 30 days for evaluation in beef steers.

Chemical analysis

At each fermentation tank opening time, the samples were mixed, and 1 kg of each sample was taken for chemical analyses and the determination of silage fermentation characteristics. The samples were dried in a forced-air oven at 50 ℃ for 96 hours and then ground to pass through a 1-mm screen. Dry matter (DM), crude protein (CP), and crude ash were analyzed according to the methods of AOAC (1990). Cell wall components were analyzed using the methods of Goering and Van Soest **(**1970). The fermentation products of the FTMR from cold water extracts were analyzed. Ten grams of cassava pulp silage and FTMR were homogenized with 90 ml of sterilized distilled water (Cai *et al*., 1999)**.** The FTMR pH was measured with a glass electrode pH meter **(**MP230; Mettler Toledo, Greifensee, Switzerland**)**.

Animals

Eight 1-year-old Lao-native beef steers averaging 92 ± 2.20 kg at the beginning of the experiment were divided into two equal groups of four steers to evaluate the treatment effects on growth performance in the feeding trial. They were placed in individual cages of 1.2m × 2.5m with a concrete floor Clean tap water and trace-mineralized salt blocks were freely available to the animals at all times. Before the experiment, all the animals were vaccinated against foot-and-mouth disease. All the animals were given an injection of a vitamin supplement (AD_3E) at the beginning of the operation.

Sample and data collection

Throughout the experiment, the dietary treatments were weighed daily and provided to individuals equally twice a day between 9 a.m. and 5 p.m. after the feed residue had been removed. Every week, samples of all dietary ingredients and FTMR were collected and mixed individually for analyzing. Cattle were weighed monthly to monitor growth before feeding in the morning**.** On the feeding experiment**'**s final day, the blood samples were collected via the jugular vein before the morning feeding and four hours after feeding**.** The blood samples were transferred to a tube containing heparin to obtain the plasma and centrifuged for 20 minutes at 3,500 rpm at 4 ℃**.** The samples were kept at **-**20 ℃ until further analysis**.** One hundred milliliters of ruminal fluid were collected at the same time as the blood by using a stomach tube with a suction pump**.** Approximately 50 ml of ruminal fluid was discarded before the sample collection to avoid saliva contamination**.** The pH was immediately measured using a pH meter **(**MP230; Mettler Toledo, Greifensee, Switzerland**)**.

Statistical analysis

The data for all response variables at the beginning and end of the experiment was tested by a group t-test by using a statistical analysis system. Differences were declared significant at $P < 0.05$.

Results

Chemical composition and fermentation characteristics

The chemical composition of the SFCP and the FTMRs after 30 days of fermentation are shown in Table 1. During fermentation, white-rot fungi were visible in some of the treatments. Although diets were formulated to contain 14% CP in the diet, CP contents of the experimental diets fed in the present experiment averaged between 13.30% and 14.39% DM for uninoculated and inoculated, respectively. When storage was prolonged up to 30 days, the pH values of the uninoculated SFCP at ambient temperature were higher than the inoculated cassava pulp (3.33 and 3.06, respectively) (P<0.01), while the inoculant was not affected on FTMR (3.84-4.04) (P>0.05).

Table 1. Ingredient and nutrient composition of the experimental diets

Feed ingredients	SFCP		FTMR	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Ingredient (% of DM)				
Chopped Napier grass			30.00	30.00
Wet brewers grains			25.00	25.00
Wet cassava pulp			42.50	45.20
Urea			1.00	1.00
Mineral mixed			0.20	0.20
Salt			0.20	0.20
Molasses			1.00	1.00
Sulfur			0.10	0.10
L. plantarum (CFU/g FM[1/])	-	1.0×10^5	-	1.0×10^5
pH	3.33[a2/]	3.06[b]	4.04	3.84
Chemical composition				
DM (% as fed)	18.35	17.53	32.79	32.53
OM (% of DM)	97.64	97.18	98.83	98.71
CP (% of DM)	2.78	2.76	13.30	14.39
NDF (% of DM)	26.07	26.77	51.23	54.45
ADF (% of DM)	17.75	18.06	80.35	70.34
EE (% of DM)	1.82	1.82	4.05	3.78

[1/]: FM, fresh matter; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; CFU, colony-forming units.
[2/]: Different superscripts in the same row indicate significant (P<0.01) (SEM = 0.42).

Feed intake

Throughout the experiment, the total feed intakes (kgDM/day, kg/%BW, and g/BW$^{0.75}$) were not significantly different between treatments (P>0.05) except during 60 days of the experiment period, which showed that the feed intake of the uninoculated FTMR was higher than the inoculated FTMR

(P<0.01). However, the nutrient intakes were not different (P>0.05) between treatments (Table 2).

Table 2. Effects of the inoculation of fermented total mixed ration (FTMR) with *L. plantarum* on feed and nutrient intake

	FTMR		SEM	*P*-value
	Uninoculated	Inoculated		
Feed intake (kgDM/day)				
30 days	3.44	3.26	0.49	0.18
60 days	3.95	3.06	0.61	<0.01
90 days	4.33	4.45	0.48	0.36
Overall	3.91	3.57	0.24	0.54
Feed intake (%BW)				
30 days	3.13	2.95	0.49	0.11
60 days	3.03	2.98	0.47	0.64
90 days	2.91	2.97	0.46	0.46
Overall	3.02	2.97	0.03	0.49
Feed intake (g/BW$0^{.75}$)				
30 days	133.90	126.49	2.85	0.13
60 days	135.37	133.01	2.60	0.62
90 days	134.20	137.25	2.15	0.41
Overall	134.49	132.25	1.50	0.54
Nutrients intake (g/BW$0^{.75}$)				
DM	43.58	42.34	0.99	0.31
OM	130.82	128.21	1.95	0.43
CP	17.58	18.65	0.72	0.06
NDF	67.82	70.67	1.41	0.16

Rumen ecology

The effects of lactic acid bacteria inoculated on ruminal pH and concentrations of ammonia-nitrogen in the rumen fluid are shown in Table 3. Overall, ruminal pH and ammonia-nitrogen (NH_3-N) were not affected by the type of diet.

Blood plasma metabolism

The effects of lactic acid bacteria inoculated of FTMR on concentrations of blood urea nitrogen (BUN) in plasma are shown in Table 3. No differences in BUN occurred in the present study.

Table 3. Effects of the inoculation of fermented total mixed ration (FTMR) with *L. plantarum* on rumen ecology and blood urea nitrogen

	FTMR		SEM	P-value
	Uninoculated	**Inoculated**		
Ruminal pH				
Before feeding	6.42	6.52	0.45	0.14
4 h after feeding	6.51	6.25	0.49	0.07
Ammonia nitrogen, mg/dl				
Before feeding	15.58	16.24	1.45	0.77
4 h after feeding	22.76	24.82	1.61	0.43
Blood urea nitrogen, mg/dl				
Before feeding	17.86	17.16	1.01	0.59
4 h after feeding	19.56	18.37	0.88	0.22

Growth performance

The results of the growth performance are shown in Table 4. The initial BW was similar between treatments. However, steers fed FTMR inoculated with *L. plantarum* had a more significant average daily gain (ADG) and feed conversion ratio (FCR) versus control steers during the 60 days after the feeding period.

Table 4. Effects of the inoculation of fermented total mixed ration (FTMR) with *L. plantarum* on average daily gain and feed conversion ratio

	FTMR		SEM	P-value
	Uninoculated	**Inoculated**		
Initial weight, kg	109.63	110.38	0.42	0.40
Final weigh, kg	169.22	167.86	1.21	0.61
Average daily gain, ADG (kg/day)				
30 days	0.69	0.64	0.47	0.24
60 days	0.62	0.68	0.53	0.02
90 days	0.67	0.59	0.45	0.06
Overall	0.66	0.64	0.01	0.55
Feed conversion ratio, FCR				
30 days	5.93	6.07	0.59	0.71
60 days	7.58	6.76	0.63	0.04
90 days	7.68	8.93	0.77	0.07
Overall	7.06	7.25	0.46	0.86

Discussion

Spontaneously fermented cassava pulp (SFCP) in this experiment was shown a CP lower than the one reported by Pilajun and Wanapat (2016) (2.78 and 4.8%, respectively). However, the cassava chips' composition depends on breeds, harvesting age, environmental conditions, and planting season (Saengchan *et al*., 2015). After mixing the SFCP with feed ingredients, each treatment's pH increased because of the alkalizing effect of the added urea. In general, it is expected that spoilage microorganisms would be inhibited at a lower pH than at a higher pH, which is closer to the optimum pH range of spoilage microorganisms. Napasirth *et al*. (2015) reported that silages would be well-preserved with a low pH, below 4.0 (Ryser *et al*., 1997 and Yahaya *et al*., 2002). However, this result agrees with the study of Kamphayae *et al*. (2016), which found that the pH of cassava pulp is 3.63, while ureated FTMR silages typically have a higher than usual pH (~4.0).

Average daily gain (ADG) ranged from 0.64 to 0.66 kg/day for inoculated and uninoculated fodder in the current study. However, in the period 60 days after feeding, the ADG on inoculated FTMR was more significant than that on uninoculated FTMR ($P< 0.05$). These results were higher than the report of Nampanya *et al*. (2013), which stated that fattening Lao-native beef cattle in Laos had an ADG of approximately 0.32 kg/day. The FCR of this experiment was between 7.06 and 7.25. For beef cattle, an FCR ranging from less than 5 to more than 20 that may be encountered (National Research Council, 2000). The responses in ruminal pH and BUN were similar to those in other reports. The concentration of ruminal ammonia-nitrogen level of 3.3 and 8.5 mg/dl is widely accepted as the minimum concentration at which maximum microbial growth and activity would occur in vivo (Kang-Meznarich and Broderick, 1980). In conclusion, our study indicated that the inoculation of SFCP with *L. plantarum* can improve the quality of the ensiled in the laboratory while inoculating FTMR with *L. plantarum* is not decreased the pH value. Finally, steers fed on inoculated FTMR showed a more significant ADG and a more significant FCR at 60 days after feeding.

Acknowledgements

The authors would like to express sincere thanks for research facilities support to the Thailand Research Fund (TRG5880027) for financially supported; Program in Animal Science, Faculty of Technology, Udon Thani Rajabhat University (UDRU), Thailand; Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand and Department of Livestock and Fisheries, Faculty of Agriculture (FAG), National University of Laos (NUOL), Lao PDR.

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(Received: 22 October 2020, accepted: 25 August 2021)