

6-1-2017

Effects of proportion of cassava and lactic acid-treated cassava in rations on rumen pH and plasma lipopolysaccharide-binding protein in beef cattle

Rittichai Pilachai

Weerachai Thongdee

Yauvapol Chumpol

Suvaluk Seesupa

Jan Thomas Schonewille

Follow this and additional works at: <https://digital.car.chula.ac.th/tjvm>



Part of the [Veterinary Medicine Commons](#)

Recommended Citation

Pilachai, Rittichai; Thongdee, Weerachai; Chumpol, Yauvapol; Seesupa, Suvaluk; and Schonewille, Jan Thomas (2017) "Effects of proportion of cassava and lactic acid-treated cassava in rations on rumen pH and plasma lipopolysaccharide-binding protein in beef cattle," *The Thai Journal of Veterinary Medicine*: Vol. 47: Iss. 2, Article 4.

Available at: <https://digital.car.chula.ac.th/tjvm/vol47/iss2/4>

This Article is brought to you for free and open access by the Chulalongkorn Journal Online (CUJO) at Chula Digital Collections. It has been accepted for inclusion in The Thai Journal of Veterinary Medicine by an authorized editor of Chula Digital Collections. For more information, please contact ChulaDC@car.chula.ac.th.

Effects of proportion of cassava and lactic acid-treated cassava in rations on rumen pH and plasma lipopolysaccharide-binding protein in beef cattle

Rittichai Pilachai^{1*} Weerachai Thongdee¹ Yauvapol Chumpol¹

Suvaluk Seesupa² Jan Thomas Schonewille^{3,4}

Abstract

The objectives of this study were to investigate the effect of the proportion of cassava and lactic acid (LA)-treated cassava in rations on rumen fermentation parameters and plasma lipopolysaccharide-binding protein (LBP) in beef cattle. Four, rumen cannulated, beef cattle were randomly assigned to four experimental rations in a study with a 2 × 2 factorial arrangement in a 4 × 4 Latin square with a 16-day adaptation period, followed by a 5-day experimental period. During the 5 days of experimental period, the cattle were subjected to 4 experimental treatments, i.e. rations that contained either a high proportion or a low proportion (50% versus 35% dry matter) of cassava either untreated or LA treated. Results showed that clinical signs of subacute laminitis were influenced (P = 0.001) by the proportion of cassava, but not by the treatment of cassava. The cow fed the high proportion of untreated cassava tended to reduce (P = 0.058) the mean postprandial rumen pH, increased (P = 0.032) the area under the curve (AUC) of rumen pH less than 5.80 and tended (P = 0.087) to increase the time that rumen pH was lower than 5.80. However, plasma LBP concentrations did not respond consistently to the experimental rations. The outcome of the current study supports the idea that the treatment of cassava with LA reduces the risk of rumen acidosis when cattle are fed rations with a high proportion of cassava.

Keywords: lactic acid-treated cassava, rumen pH, lipopolysaccharide-binding protein, beef cattle

¹Faculty of Technology, Udon Thani Rajabhat University, Udon Thani 41000, Thailand

²Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

³Faculty of Veterinary Medicine, Utrecht University, 3508 TD Utrecht, the Netherlands

⁴Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima 30000, Thailand

*Correspondence: pilachai_rittichai@yahoo.com

Introduction

Cassava and rice straw are important ingredients to formulate rations for beef cattle in tropical areas (Wanapat, 2003; Pilachai, 2013). High incorporation of cassava increases energy density of the ration and thus growth performance. On the other hand, it is well known that the feeding of high amounts of rapidly fermentable starch such as cassava in combination with a low rice straw content increases the risk of rumen acidosis (Pilachai et al., 2012, 2014) and therefore bovine laminitis (Nocek, 1997). Recently, Rittichai et al. (2014) have demonstrated that the treatment of cassava meal with 1% lactic acid (LA) decreased the degradation rate of dry matter (DM) and starch under *in vitro* conditions. This observation is in line with those reported by Iqbal et al. (2009) and Khol-Parisini et al. (2015), who demonstrated that barley grain treated with LA reduced the fermentation rate of barley starch and prevented rumen acidosis (Iqbal et al., 2009) in dairy cows. Therefore, it can be speculated that the treatment of cassava with LA might prevent rumen acidosis.

Currently, there is a general consensus that the condition of rumen acidosis is related to laminitis (Nocek, 1997; Pilachai, 2013). The mechanism underlying the relationship between rumen acidosis and laminitis is still a matter of debate, but lipopolysaccharides (LPS) may play an important role. Under the condition of rumen acidosis, LPS are released in the rumen (Emmanuel et al., 2008; Ametaj et al., 2009; Khafipour et al., 2009) and may enter systemic circulation. The entrance of LPS into systemic circulation may trigger the synthesis LPS-binding protein (LBP, Li et al., 2012), which is considered to be a potentially important biomarker related to bovine laminitis (Danscher et al., 2011). The objective of the current study was to investigate the effect of the proportion of cassava and LA-treated cassava in rations on rumen pH. It was hypothesized that the use of LA-treated cassava versus untreated cassava in rations with a high proportion of cassava could prevent rumen acidosis. Moreover, plasma LBP was measured and it was expected that plasma LBP concentrations were lowered after the feeding of rations with a high proportion of LA-treated cassava instead of untreated cassava.

Materials and Methods

Animals and experimental design: The current experiment was approved by the committee of Ethics of Animal Experimentation of Udon Thani Rajabhat University, Udon Thani, Thailand. Prior to the experiment, it was decided to immediately terminate the experiment if any cow refused to eat and concurrently presented itself with severe diarrhea and clinical signs of metabolic acidosis (e.g. tachycardia and tachypnea). At the end of each 21-day study period, all cattle received an intra-ruminal dose of sodium bicarbonate (100 g/cattle) to increase the buffer capacity of the rumen contents.

Four, rumen cannulated, Native × Brahman cross-bred, beef cattle with a mean body weight of 412.0 ± 58.9 kg were used. The animals were housed on a concrete floor in individual pens (3 × 3 m²) with

natural ventilation at the Ruminant Research Unit of Udon Thani Rajabhat University, Udon Thani, Thailand.

The experimental design consisted of a 2 × 2 factorial arrangement in a 4 × 4 Latin square for a period of 21 days which consisted of a 16-day adaptation period, followed by a 5-day experimental period. The animals were randomly assigned (within the restrictions of the Latin square design) to each sequence of feeding of four experimental rations and had free access to water.

Experimental rations: Prior to the preparation of the total mixed ration (TMR), cassava chips were finely ground. Then, the ground cassava chips were either treated with 1% of lactic acid solution (DL-lactic, 85%, wt/wt) or water (untreated cassava) in a ratio of 1 to 1 wt/vol for 48 h at an ambient temperature of ~25°C. The particle length of rice straw was reduced to approximately 4 cm by means of a mechanical cutter. During the first 16 days of each run-in/washout period, the cattle were offered a TMR that consisted of (% of DM) rice straw, 50; cassava chip, 15; soybean meal, 10; rice bran, 23; urea, 0.5; sulfur, 0.5; and minerals, 1.

During the 5 days of each experimental period, the cattle were offered unrestricted amounts of an experimental TMR that contained either a high proportion or a low proportion (50% versus 35%) of cassava either untreated or LA-treated (Table 1). The rations were offered daily in two equal portions at 08:00 and 17:00 hours. Feed refusals were collected to calculate the actual feed intake.

Collection of samples: Throughout the experimental period, the rations were sampled daily and pooled, ground, and stored in sealed plastic bags at an ambient temperature (25°C) until analysis. On days 3 and 5 of each experimental period, pH of rumen contents was recorded 15 min before feeding and 9 times hourly after feeding. The pH was measured by means of a pH electrode (Mettler-Toledo GmbH 8603, Schwerzenbach, Switzerland) which was inserted into the ventral sac of the rumen through the cannula.

On day 5 of each experimental period, rumen fluid samples (~100 mL) were taken from the ventral sac of the rumen 15 min before feeding and 3, 6 and 9 h after the morning feeding. Immediately after the collection, the rumen fluid was filtered through four layers of cheesecloth and a ~55-mL filtered subsample was preserved by adding 5 mL of 1 M H₂SO₄ and stored at -20°C until analysis of volatile fatty acids (VFA) and lactate.

On days 3 and 5 of each experimental period, blood (20 mL) was taken 15 min before feeding and 9 h after feeding from the jugular vein by means of a syringe. The blood was subsequently transferred into an evacuated EDTA tube (2.5-mL), a sodium fluoride tube and a tube containing polypropylene (Zenimed Co., Ltd., Bangkok, Thailand). Thereafter, the tubes were immediately placed on ice and centrifuged at 2,500 × g for 15 min within 30 min after the collection. Directly after centrifugation, plasma and serum were collected and stored in 1.5-mL tubes at -20°C until analysis.

Throughout the experiment, at 12:00 hour on days 3 and 5 of each experimental period, all cattle were clinically monitored, including auscultation of heart rate and rumen contraction, measurement of rectal temperature, and observation of respiration frequency. Consistency of feces were inspected visually and graded as 1 = dry, firm; 2 = normal; 3 = pasty, soft; 4 = diarrhea, thin; or 5 = watery (modified from Hughes, 2001). Furthermore, all cows were clinically examined daily for signs related to subacute

laminitis which involved observation on claw inflammation and foot pain according to the definition of subacute laminitis modified by Greenough et al. (2007). Briefly, the coronary band was observed and evaluated for swelling as 0 = no swelling and 1 = swelling and pink in color. Weight shifting was defined as lateral transfer of weight by the animal from one leg to another in a monotonous manner as 0 = no, 1 = slight and 2 = marked weight shifting.

Table 1 Ingredient and analyzed composition of the experimental rations

	Low cassava		High cassava	
	Untreated cassava	LA-treated cassava	Untreated cassava	LA-treated cassava
Ingredient composition (% of DM)				
Rice straw	30.0	30.0	15.0	15.0
Cassava	35.0	-	50.0	-
LA treated cassava	-	35.0	-	50.0
Soybean meal	10.0	10.0	10.0	10.0
Rice bran	23.0	23.0	23.0	23.0
Urea	0.5	0.5	0.5	0.5
Sulfur	0.5	0.5	0.5	0.5
Mineral premix*	1.0	1.0	1.0	1.0
Analyzed composition (% of DM)				
Dry matter	66.2	66.3	59.5	59.4
Crude ash	8.2	8.1	7.7	7.6
Crude protein	11.3	11.5	11.3	11.6
NDF	29.4	28.4	22.6	20.2
ADF	10.9	10.4	9.2	8.5
Ether extract	2.6	2.7	2.4	2.3
NFC**	48.5	49.3	56.0	58.3

LA, Lactic acid; *Vitamin-mineral premix consisted of (g per kg): Mg, 10; Zn, 6; Mn, 8; Fe, 10; Cu, 1.6; (mg per kg) I, 100; Co, 20; Se, 60; (IU/kg) vitamin A, 2,000,000; vitamin D, 400,000; vitamin E, 3000 (IS Dairy mix®, Thailand); **NFC, Non fiber carbohydrates calculated as follows: (100 - crude ash - crude protein - NDF - ether extract).

Chemical analysis: The DM content of the concentrates and rice straw was determined by drying at 135°C for 3 h (AOAC, 1990; ID 967.03). The ash content was analyzed by combustion at 550°C for 16 h and nitrogen content was determined by the macro Kjeldahl method (International Dairy Federation, 1986) with the factor of 6.25 used to convert nitrogen into crude protein (CP). Ether extract was determined according to the procedure of the AOAC (1990; ID 954.02). Neutral- and acid detergent fiber (NDF and ADF) contents were determined according to the method described by Van Soest et al. (1991).

After thawing, rumen VFA and lactate concentrations were determined with the use of HPLC (Model 1200; UV-Detector (210 nm); flow rate 0.5 mL/mL) as described by Samuel et al. (1997). Plasma LBP concentrations were determined using ELISA kits (HK503, Hycult® Biotechnology, Uden, the Netherlands). Samples were analyzed in duplicate and absorbance values were read at 450 nm using a microplate absorbance reader (BIO-RAD® Laboratories, Inc, USA) as described by Khafipour et al. (2009). Plasma glucose was measured according to the hexokinase method (BS-400, Mindray Co., Ltd., Shenzhen, China). Concentration of lactate in the serum was measured by means of a colorimetric assay (Cobas® C502, Roche Ltd., Switzerland).

Statistical analysis: All statistical analyses were performed using SPSS version 20 for Windows. Prior

to statistical analysis, the plasma LBP values were logarithmically converted. Furthermore, average DM intake (five consecutive days), and averages of postprandial values of total and individual VFA and lactate were calculated using the values measured at 3, 6 and 9 h after feeding. Average postprandial rumen pH, and both the time and area under the curve (AUC) of rumen pH less than 5.80 were calculated using the 9 values measured at 1 h intervals. Then, all data were subjected to analysis of variance (ANOVA). The total variation was divided into cow, experimental period, proportion of rice straw, cassava treatment and interaction between the proportion of rice straw and cassava treatment. When the influence of dietary treatments reached statistical significance, Tukey's test was used to distinguish the dietary treatments that had different effects on the variable involvement. In addition, concentrations of glucose, lactate and LBP in blood were pooled across the two sampling times and Student's paired t-test was subsequently used to compare the mean differences between the sampling days. Effects of dietary treatments on the consistency of feces and signs of subacute laminitis were determined using Fisher's exact test. Throughout, data are presented as mean ± standard deviation. Statistical significance was present at $P \leq 0.05$ and trends were noted at $0.05 < P \leq 0.10$.

Results

Clinical observations and feed intake: Throughout the experiment, rectal temperature, heart rate, respiration rate and rumen contractions were found to be within the normal ranges. Mean rectal temperature, heart rate, respiration rate and rumen contractions were found to be $38.59 \pm 0.21^\circ\text{C}$, 68.12 ± 3.21 time/min, 23.52 ± 1.41 time/min, and 2.60 ± 0.21 time/2 min, respectively. During the 5 days of each experimental period, 9.4% of the cows had a feces score of 4, 84.4% of the cows had a feces score of 3 and 6.2% of the cows scored a feces consistency of 2. Feces scores of 1 and 5 were not observed. The feces consistency did not differ ($P = 0.399$) among the four dietary treatments.

Clinical signs of subacute laminitis were influenced ($P = 0.001$) by the proportion of cassava, but not by the treatment of cassava. The high proportion of cassava rations induced displayed marked reactions of weight shifting (score 2) and swelling over the coronary band (score 1) in all cases. In contrast, when the animals were fed the low cassava rations, only one out of the eight cases scored 2 on weight-shifting and 1 on the swelling over the coronary band.

Feed intake, rumen pH, volatile fatty acids and lactate:

The mean intake of DM as well as rumen pH before feeding were not affected by the dietary treatments, similar to the sampling days and across treatments the mean rumen pH before feeding was 6.62 ± 0.21 . Likewise, the mean postprandial rumen pH and both the time and area under the curve (AUC) of rumen pH less than 5.80 at day 3 of the experimental period were not affected ($P \geq 0.245$) by the proportion of cassava, treatment of cassava and their interaction (Table 2). On day 5 of the experimental period, the proportion of cassava did not significantly affect the mean postprandial rumen pH, but tended ($P = 0.066$) to increase the time that rumen pH was lower than 5.80 and significantly ($P = 0.019$) increased the AUC of rumen pH less than 5.80. The treatment of cassava with LA significantly affected ($P \leq 0.036$) the mean postprandial rumen pH, the AUC of rumen pH less

than 5.80 and the time that rumen pH was lower than 5.80. The high proportion of untreated cassava tended to reduce ($P = 0.058$) the mean postprandial rumen pH, increased ($P = 0.032$) the AUC of rumen pH less than 5.80 and tended ($P = 0.087$) to increase the time that rumen pH was lower than 5.80.

The proportion of cassava, but not the treatment of cassava, significantly ($P = 0.050$) affected the concentration of total VFA in rumen fluid and the values were found to be 11% greater when the high proportion of cassava was fed (Table 3). The proportions of acetic acid and propionic acid of total VFA and their ratio were not affected by the content of cassava in the ration or the cassava treatment. The proportion of butyrate of total VFA was found to be 1.27 times greater ($P = 0.033$) when the LA-treated cassava was fed. The concentrations of total VFA and molar proportions of VFA were not affected by the interaction between the proportion of cassava and cassava treatment. The concentration of lactate in rumen fluid was not affected by the dietary treatments.

Blood glucose, lactate and LBP concentrations: The plasma values of glucose, lactate and LBP (Table 4) were not affected by the sampling days ($P \geq 0.369$) and they were, therefore, pooled across the two sampling times. The plasma glucose concentrations were similar among the dietary treatments ($P = 0.099$). The plasma lactate concentration was significantly ($P = 0.024$) affected by the proportion of cassava, but not by the treatment of cassava ($P = 0.108$). The plasma lactate concentrations were significantly ($P = 0.010$) affected by the interaction between the proportion of cassava and cassava treatment; the highest plasma lactate values were found after the feeding of TMR containing the high proportion of untreated cassava. Upon ANOVA, the plasma LBP values were found to be significantly ($P = 0.025$) affected by the dietary proportion of cassava, but Tukey's test did not identify specifically the dietary treatments that produced different values ($P = 0.111$).

Table 2 Rumen pH after feeding of the experimental rations

	Low cassava		High cassava		Pooled SEM	P-value		
	Untreated cassava	LA-treated cassava	Untreated cassava	LA-treated cassava		C	T	C × T
Dry matter intake (kg/d)	9.07	9.59	8.08	8.69	1.144	0.122	0.358	0.958
Rumen pH								
Day 3								
Average	5.99	6.08	5.81	6.09	0.224	0.725	0.396	0.739
Time rumen pH <5.8 (min/d)	151.20	126.90	404.10	103.50	147.468	0.466	0.313	0.385
Area pH <5.8 (min × pH/d)	13.40	9.25	30.37	6.56	10.864	0.535	0.245	0.400
Day 5								
Average	6.09 ^{ab}	6.14 ^{ab}	5.57 ^b	6.19 ^a	0.121	0.108	0.036	0.058
Time rumen pH <5.8 (min/d)	131.10 ^{ab}	59.70 ^b	493.35 ^a	76.50 ^b	84.393	0.066	0.028	0.087
Area pH <5.8 (min × pH/d)	8.92 ^b	4.00 ^b	41.48 ^a	6.26 ^b	5.452	0.019	0.010	0.032

^{a, b} Means in the same row with different superscript letters are significantly different; *Time rumen pH <5.8, min/0-9 h after feeding; LA, Lactic acid; C, Effect of proportion of cassava; T, Effect of cassava treatment; C × T, Interaction effect between proportion and treatment of cassava.

Table 3 Selected indices of rumen fermentation after feeding of the experimental rations

	Low cassava		High cassava		Pooled SEM	P-value		
	Untreated cassava	LA-treated cassava	Untreated cassava	LA-treated cassava		C	T	C × T
Total VFA (mM)	78.49 ^b	81.64 ^b	93.89 ^a	84.05 ^a	6.543	0.050	0.410	0.136
Individual VFA (mol/100 mol)								
Acetate	60.59	59.95	68.17	63.58	2.870	0.098	0.397	0.516
Propionate	28.61	28.36	23.01	23.33	3.161	0.143	0.997	0.931
Butyrate	10.81	11.69	8.82	13.09	0.942	0.764	0.033	0.121
Acetate to Propionate ratio	2.21	2.12	3.63	2.63	0.629	0.176	0.416	0.518
Lactate (mM)	2.07	1.77	1.92	2.20	0.367	0.151	0.512	0.437

^{a, b} Means in the same row with different superscript letters are significantly different; LA, Lactic acid; C, Effect of proportion of cassava; T, Effect of cassava treatment; C × T, Interaction effect between proportion and treatment of cassava.

Table 4 Plasma concentrations of glucose, lactate and lipopolysaccharide-binding protein (LPB) after feeding of the experimental rations

	Low cassava		High cassava		Pooled SEM	P-value		
	Untreated cassava	LA-treated cassava	Untreated cassava	LA-treated cassava		C	T	C × T
Glucose (mM)	66.50	64.00	54.38	62.88	3.403	0.099	0.412	0.157
Lactate (mM)	0.83	1.14	1.20	1.10	0.056	0.024	0.108	0.010
LPB (µg/mL)	14.52	12.33	18.88	17.52	Log value*	0.025	0.373	0.944

*SEM value derived from log transformed LBP values was found to be 0.047; LA, Lactic acid; C, Effect of proportion of cassava; T, Effect of cassava treatment; C × T, Interaction effect between proportion and treatment of cassava.

Discussion

The outcome of the current study supports the idea that the treatment of cassava with LA reduces the risk of rumen acidosis when cattle are fed rations with a high proportion of cassava. Currently, there is no general agreement on the definition of rumen acidosis in terms of pH (Gozho et al., 2005; Krause and Oetzel, 2005; Plaizier et al., 2008). In the current paper rumen acidosis is arbitrarily defined as a rumen pH below 5.80 for at least 3 consecutive hours (Dohme et al., 2008; Zebeli et al., 2008). Although the various indicators of rumen acidosis at day 3 were numerically in line with those at day 5, the differences among the dietary treatments did not reach statistical significance at day 3 of the experimental period. This lack of statistical significance at day 3 is most likely to be explained by the greater variation of the selected indicators of rumen pH (Table 2). The greater variation in rumen pH at day 3, compared to day 5, is not easy to explain but it may be speculated that rumen fermentation adapted to a lesser extent at day 3 after the diet change. During the run-in/washout periods, the ration contained 50% rice straw (DM basis) while during the experimental periods the ration contained 15 to 30% rice straw (DM basis).

The current finding that the LA-treated cassava was instrumental in the prevention of rumen acidosis is consistent with data reported by Rittichai et al. (2014) and Khol-Parisini et al. (2015), who showed that the treatment of cassava meal with 1% lactic acid decreased the ruminal degradation rates of dry matter and starch. Moreover, Iqbal et al. (2009, 2012) reported that LA treatment of barley resulted in a reduced fermentation rate of barley starch and thereby prevented rumen acidosis in dairy cows. The

underlying mechanism by which lactic acid reduced the degradation rate of starch in ruminant has not been fully understood yet, but LA has the potential to modify starch granules (Liljeberg et al., 1995) and the microscopic structure of starch (Deckardt et al., 2014). It has been shown by Liljeberg et al. (1995) and Östman et al. (2002) that at least in human, this change in starch or starch granules provides a barrier for enzymatic degradation of starch. Unfortunately, it is currently unknown whether this explanation can be extrapolated to the rumen. Nevertheless, *in situ* ruminal degradation rate of barley is reduced after LA treatment (Deckardt et al., 2016).

The use of LA-treated cassava instead of untreated cassava in rations with a high proportion of cassava did not lower the plasma LBP concentrations; therefore, the present study rejected the hypothesis concerning plasma LBP. However, apart from the aberrant low value found at day 3 (Fig. 1), the values were found to range from 13.58 to 19.40 µg/mL. These values are in the upper range when compared to values previously reported in other literature (Table 5). It can, therefore, be speculated that the animals responded to the LPS entrance into systemic circulation. The port of entry into systemic circulation, however, might be different among the experimental rations. One option is that plasma LBP might respond to LPS that was released during rumen acidosis (Emmanuel et al., 2008; Ametaj et al., 2009; Khafipour et al., 2009). Alternatively, a high intake of starch which is not degraded by rumen microbiota (i.e. by-pass starch) can lead to an excessive supply of starch to the hindgut, leading to high rates of fermentation and high LPS concentrations at that location (Reynolds, 2006; Li et al., 2012). It can, therefore, be speculated that plasma LBP

might also respond to LPS that originated from the hindgut instead of the rumen.

It was suggested by Danscher et al. (2011) that LBP could be considered as a biomarker related to bovine laminitis. However, in the current study a clear relationship between plasma LBP and the occurrence of clinical signs related to subacute laminitis was not observed. It is well known that plasma LPS induces production of other acute phase proteins such as heptoglobin and serum amyloid A, and pro-

inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor-necrosis factor α (Sweet and Hume, 1996; Plaizier et al., 2012). It was suggested by Nocek (1997) that these cytokines might play a role in the etiology of laminitis in dairy cattle. Perhaps, the relationship between fore mentioned cytokines and clinical signs related to subacute laminitis is clearer. Clearly, further studies are warranted to investigate the relationship between the pro-inflammatory cytokines and the etiology of laminitis.

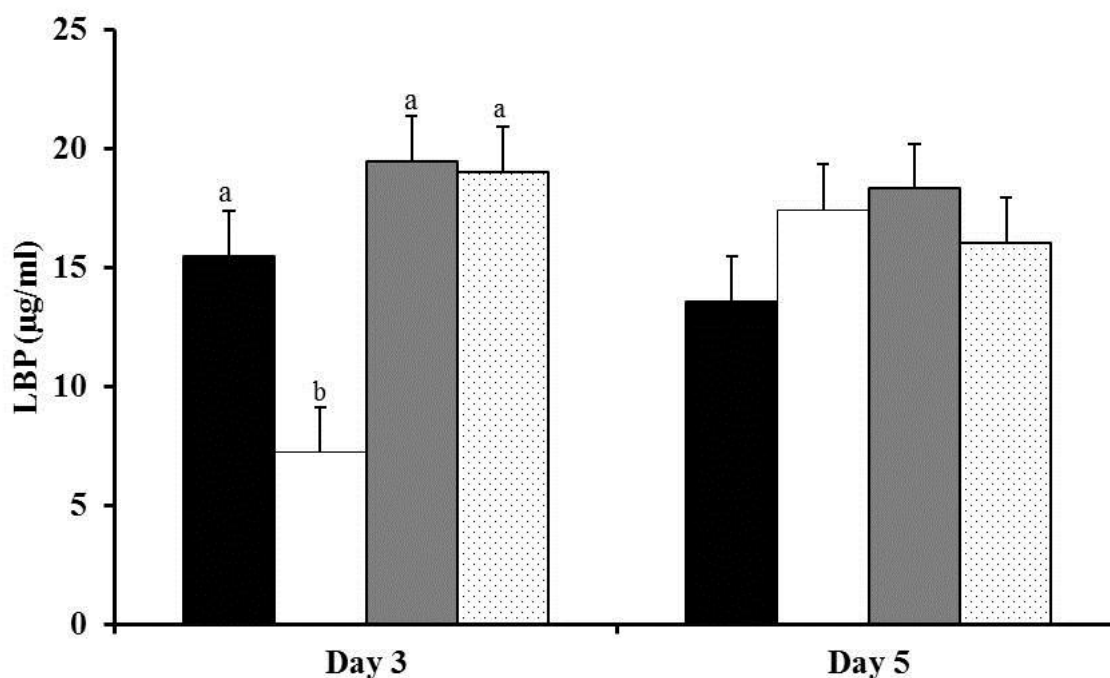


Figure 1 Mean plasma lipopolysaccharide-binding protein (LBP) concentrations in cattle fed low proportion of untreated cassava (black bar), low proportion of lactic acid (LA)-treated cassava (white bar), high proportion of untreated cassava (gray bar), and high proportion of lactic acid (LA)-treated cassava (dotted bar). Error bars indicate the standard error of the means. ^{a, b} indicate a borderline significant difference ($P = 0.054$) between the dietary treatments at day 3 of the experimental period.

Table 5 Ration characteristics and concentration of lipopolysaccharide-binding protein (LBP) in plasma of dairy cattle

Reference	Ration	LBP ($\mu\text{g/mL}$)
Emmanuel et al. (2008)	100% basal ration (85% roughage, 15% concentrate)	5.71
	85% basal ration, 15% of barley based concentrate	4.67
	70% basal ration, 30% of barley based concentrate	6.49
	55% basal ration, 45% of barley based concentrate	10.06
Ametaj et al. (2009)	55% silage, 45% barley	15.00
	9% silage, 91% barley	23.00
Iqbal et al. (2009)	73% basal ration, 27% of rolled barley grain	3.53
	73% basal ration, 27% of LA-treated rolled barley grain	3.37
Li et al. (2012)	70% roughage, 30% grain based concentrate	8.90
	30% roughage, 70% grain based concentrate	9.50
	36% roughage, 64% alfalfa based pellets	12.10
Current study	65% basal ration, 35% of untreated cassava	14.52
	65% basal ration, 35% of LA-treated cassava	12.33
	50% basal ration, 50% of untreated cassava	18.88
	50% basal ration, 50% of LA-treated cassava	17.52

LA, lactic acid

Ingestion of high rice straw rations resulted in lower concentrations of rumen VFA and therefore a greater pH ($r = -0.528$, $P = 0.035$). This finding can be explained by the low content of fermentable organic matter of rice straw (Pilachai et al., 2012). Consequently, a greater proportion of rice straw provides lower amounts of substrate to the rumen microbes to be converted to rumen VFA. The substitution of cassava for rice straw did not affect the proportion of propionate of total VFA. This observation was somewhat unexpected because it is widely accepted that the ingestion of rations rich in starch promotes the synthesis of rumen propionate (Owens et al., 1997). The lack of effect of starch on the proportion of propionate of total VFA may be explained by the low level of DM intake in the current study. This explanation is in line with the observations of Robinson et al. (1986), Schonewille et al. (2000) and Pilachai et al. (2012). Robinson et al. (1989) and Schonewille et al. (2000) could not demonstrate a clear effect of readily fermentable carbohydrates on the proportion of propionate after the intake of a ration containing 20% starch at a level of 5.5-6.5 kg DM/day. Likewise, rations containing up to ~40% cassava did not affect the proportion of propionate at a level of 11.10 kg DM/day (Pilachai et al., 2012). Interestingly, the LA treatment of cassava was associated with a greater proportion of butyrate. The higher proportion of butyrate in the rumen is not easy to explain, but it may be speculated that LA-treated cassava induces a prolonged lag time to degrade starch, thereby causing a shift in the VFA profile. This reasoning is in line with the results of Iqbal et al. (2012), who also reported a greater percentage of butyrate of rumen VFA when cows were fed LA-treated barley.

In the current study, rumen lactate concentrations (Table 3) were found to be somewhat greater than those reported by Iqbal et al. (2009), but are consistent with previous findings reported by Khafipour et al. (2009), who observed ruminal lactate concentrations ranging from 1.65-2.29 mM. However, rumen lactate concentrations < 5 mM are considered within the physiological range (Nagaraja and Lechtenberg, 2007).

From a practical viewpoint, the most important result found in the current study is the increase in rumen pH when LA-treated cassava was fed to the cattle. Moreover, the ingestion of high cassava rations was clearly associated with clinical signs of laminitis. The relationship between LBP and laminitis, however, was not straightforward in the current study.

Acknowledgements

The study was financially supported by Udon Thani Rajabhat University, Thailand.

References

Ametaj BN, Emmanuel DGV, Zebeli Q and Dunn SM 2009. Feeding high proportions of barley grain in a total mixed ration perturbs diurnal patterns of plasma metabolites in lactating dairy cows. *J Dairy Sci.* 92(3): 1084-1091.

- AOAC 1990. Official Methods of Analysis, 15th edn. Association of Official Analytical Chemists, Arlington, Virginia.
- Dansch AM, Thoenner MB, Heegaard PMH, Ekstrom CT and Jacobsen S 2011. Acute phase protein response during acute ruminal acidosis in cattle. *Livest Sci.* 135(1): 62-69.
- Deckardt Kathrin, Ratchaneewan Khiaosa-ard, Heinrich Grausgruber and Qendrim Zebeli 2014. Evaluation of various chemical and thermal feed processing methods for their potential to enhance resistant starch content in barley grain. *Starch/Stärke.* 66(5-6): 558-565.
- Deckardt Kathrin, Barbara U Metzler-Zebeli and Qendrim Zebeli 2016. Processing barley grain with lactic acid and tannic acid ameliorates rumen microbial fermentation and degradation of dietary fibre *in vitro*. *J Sci Food Agric.* 96(1): 223-231.
- Dohme F, DeVries TJ and Beauchemin KA 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: ruminal pH. *J Dairy Sci.* 91(9): 3554-3567.
- Emmanuel DGV, Dunn SM and Ametaj BN 2008. Feeding high proportions of barley grain stimulates an inflammatory response in dairy cows. *J Dairy Sci.* 91(2): 606-614.
- Gozho GN, Plaizier JC, Krause DO, Kennedy AD and Wittenberg KM 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *J Dairy Sci.* 88(10): 1399-1403.
- Greenough PR, Bergsten C, Brizzi A and Mulling CKW 2007. Bovine Laminitis and Lameness: A Hands on Approach. 1st ed. Saunders Elsevier, Philadelphia, PA. 311 p.
- Hughes J 2001. A system for assessing cow cleanliness. *In Pract.* 23(9): 517-524.
- International Dairy Federation 1986. IDF Standard 20A. International Dairy Federation. Brussels, Belgium.
- Iqbal S, Zebeli Q, Mazzolari A, Bertoni G, Dunn SM, Yang WZ and Ametaj BN 2009. Feeding barley grain steeped in lactic acid modulates rumen fermentation patterns and increases milk fat content in dairy cows. *J Dairy Sci.* 92(12): 6023-6032.
- Iqbal S, Terrill SJ, Zebeli Q, Mazzolari A, Dunn SM, Yang WZ and Ametaj BN 2012. Treating barley grain with lactic acid and heat prevented subacute ruminal acidosis and increased milk fat content in dairy cows. *Anim Feed Sci Technol.* 172(3-4): 141-149.
- Khafipour E, Krause DO and Plaizier JC 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. *J Dairy Sci.* 92(3): 1060-1070.
- Khol-Parisini A, Humer E, Sizmaz Ö, Abdel-Raheem ShM, Gruber L, Gasteiner J and Zebeli Q 2015. Ruminal disappearance of phosphorus and starch, reticulo ruminal pH and total tract nutrient digestibility in dairy cows fed diets differing in grain processing. *Anim Feed Sci Technol.* 210: 74-85.

- Krause KM and Oetzel GR 2005. Inducing subacute ruminal acidosis in lactating dairy cows. *J Dairy Sci.* 88(10): 3633-3639.
- Li S, Khafipour E, Krause DO, Kroeker A, Rodriguez-Lecompte JC, Gozho GN and Plaizier JC 2012. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. *J Dairy Sci.* 95(1): 294-303.
- Liljeberg HGM, Lonner CH and Bjorck IME 1995. Sourdough fermentation or addition of organic acids or corresponding salts to bread improves nutritional properties of starch in healthy humans. *J Nutr.* 125(6): 1503-1511.
- Nagaraja TG and Lechtenberg HK 2007. Acidosis in feedlot cattle. *Vet Clin North Am Food Anim Pract.* 23(2): 333-350.
- Nocek JE 1997. Bovine acidosis: implications on laminitis. *J Dairy Sci.* 80(5): 1005-1028.
- Östman EM, Nilssont N, Lilberg HG, Molin M and Bjarck IME 2002. The effect of lactic acid on blood glucose and insulin responses to cereal products: mechanistic studies in healthy subjects and *in vitro*. *J Cereal Sci.* 36(3): 339-346.
- Owens FN, Secrist DS, Hill WJ and Gill DR 1997. The effect of grain source and grain processing on performance of feedlot cattle: a review. *J Anim Sci.* 75(3): 868-879.
- Pilachai R 2013. Feeding practices and potential risk factors for laminitis in dairy cows in Thailand. Ph.D. dissertation, Faculty of Veterinary Medicine, Utrecht University, the Netherlands.
- Pilachai R, Schonewille JTh, Thamrongyoswittayakul C, Aiumlamai S, Wachirapakorn C, Everts H and Hendriks WH 2012. Starch source in high concentrate rations does not affect rumen pH, histamine and lipopolysaccharide concentrations in dairy cows. *Livest Sci.* 150(1-3): 135-142.
- Pilachai R, Schonewille JTh, Thamrongyoswittayakul C, Aiumlamai S, Wachirapakorn C, Everts H and Hendriks WH 2013. Diet factors and subclinical laminitis score in milking cows of smallholder dairy farms in Thailand. *Livest Sci.* 155(2-3): 197-204.
- Pilachai R, Schonewille JTh, Thamrongyoswittayakul C, Aiumlamai S, Wachirapakorn C, Everts H, Vlaeminck B, Doekes G and Hendriks WH 2014. Hydrate sodium calcium aluminosilicate does not reduce rumen lipopolysaccharide concentrations in cows. *Anim Feed Sci Technol.* 191: 111-115.
- Plaizier JC, Khafipour E, Li S, Gozho GN and Krause DO 2012. Subacute ruminal acidosis (SARA), endotoxins and health consequences. *Anim Feed Sci Technol.* 172(1-2): 9-21.
- Reynolds CK 2006. Production and metabolic effects of site of starch digestion in dairy cattle. *Anim Feed Sci Technol.* 130(1-2): 78-94.
- Rittichai Pilachai, Walailuck Kaewwongsa, Anan Petlum, Yauvapol Chumpol and Weerachai Thongdee 2014. Rumen digestibility of lactic acid steeped cassava using nylon bag technique. *Khon Kaen Agriculture J.* 42: 567-576.
- Robinson PH, Tamminga S and Van Vuuren AM 1986. Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen fermentation in dairy cows. *Livest Prod Sci.* 15(2): 173-189.
- Samuel M, Sagathewan S, Thomas J and Mathen G 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. *Indian J Anim Sci.* 67: 805-807.
- Schonewille JTh, Van't Klooster Ath, Cone JW, Kalsbeek-Van der Valk HJ, Wouterse H and Beynen AC 2000. Neither native nor popped cornmeal in the ration of dry cows affects magnesium absorption. *Livest Prod Sci.* 63(1): 17-26.
- Sweet MJ and Hume DA 1996. Endotoxin signal transduction in macrophages. *J Leukoc Biol.* 60(1): 8-26.
- Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci.* 74(10): 3583-3597.
- Wanapat M 2003. Manipulation of cassava cultivation and utilization to improve protein to energy biomass for livestock feeding in the tropics. *Asian-Aust J Anim Sci.* 16(3): 463-472.
- Zebeli Q, Dijkstra J, Tafaj M, Steingass H, Ametaj BN and Drochner W 2008. Modeling the adequacy of dietary fiber in dairy cow based on responses of ruminal pH and milk fat production to composition of the diet. *J Dairy Sci.* 91(5): 2046-2066.

บทคัดย่อ

ผลของสัดส่วนของไขมันสำปะหลังและไขมันสำปะหลังแช่กรดแลคติกในสูตรอาหารต่อค่าความเป็นกรดต่างในกระเพาะหมักและระดับไลโปโพลีแซคคาไรด์บายดิงโปรตีนในพลาสมาของโคเนื้อ

ฤทธิชัย พิลาไชย^{1*} วีระชัย ทองดี¹ เยาวพล ชุมพล¹
สุวลักษณ์ ศรีสุภา² แจน โทมัส สโครนีเวลลี^{3,4}

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของสัดส่วนของไขมันสำปะหลังและไขมันสำปะหลังแช่ด้วยกรดแลคติกในสูตรอาหารต่อกระบวนการหมักย่อยในกระเพาะหมักและระดับไลโปโพลีแซคคาไรด์บายดิงโปรตีน (แอลบีพี) ในพลาสมาของโคเนื้อ ทำการศึกษาในโคเนื้อจำนวน 4 ตัวที่ผ่านการเจาะและติดตั้งท่อขนาดเล็กที่กระเพาะหมัก โคทดลองถูกสุ่มให้ได้รับอาหารทดลอง 4 ชนิด โดยจัดปัจจัยการทดลองแบบ 2 × 2 แฟคทอเรียล ในแผนการทดลองแบบ 4 × 4 ลาตินสแควร์ และแบ่งช่วงการทดลองออกเป็นระยะปรับสัตว์ 16 วัน และระยะทดลอง 5 วัน โดยในช่วงระยะเวลา 5 วันของระยะทดลอง โคถูกสุ่มให้ได้รับอาหารทดลองที่ประกอบไปด้วยไขมันสำปะหลังหรือไขมันสำปะหลังแช่กรดแลคติกในสัดส่วนที่สูงหรือต่ำในสูตรอาหาร (ร้อยละ 50 หรือ 35 ของวัตถุดิบแห้ง) การศึกษาพบว่า สัดส่วนของไขมันสำปะหลังในสูตรอาหารมีอิทธิพล ($P = 0.001$) ต่อการเกิดภาวะก๊อบก๊อบ โดยที่สัดส่วนของไขมันสำปะหลังแช่กรดแลคติกไม่มีผลต่อการสังเกตพบอาการก๊อบก๊อบ โคที่ได้รับอาหารที่มีไขมันสำปะหลังแช่น้ำในสัดส่วนที่สูงมีแนวโน้ม ($P = 0.058$) ทำให้ค่าเฉลี่ยของค่าความเป็นกรดต่างของของเหลวในกระเพาะหมักลดลงหลังการกินอาหาร พื้นที่ใต้กราฟของค่าความเป็นกรดต่างของของเหลวในกระเพาะหมักต่ำกว่า 5.80 เพิ่มขึ้น ($P = 0.032$) และระยะเวลาที่ค่าความเป็นกรดต่างของของเหลวในกระเพาะหมักลดลงต่ำกว่า 5.80 มีแนวโน้มเพิ่มขึ้น ($P = 0.087$) อย่างไรก็ตาม ค่าความเข้มข้นของแอลบีพีในพลาสมาไม่มีการตอบสนองที่สม่ำเสมอตามชนิดของอาหารทดลอง ผลการศึกษานี้แสดงให้เห็นว่า ไขมันสำปะหลังแช่กรดแลคติกสามารถช่วยลดค่าความเป็นกรดต่างของของเหลวในกระเพาะหมักของโคที่ได้รับไขมันสำปะหลังในสัดส่วนที่สูง

คำสำคัญ: ไขมันสำปะหลังแช่กรดแลคติก ค่าความเป็นกรดต่างของของเหลวในกระเพาะหมัก ไลโปโพลีแซคคาไรด์บายดิงโปรตีน โคเนื้อ

¹คณะเทคโนโลยี มหาวิทยาลัยราชภัฏอุดรธานี จังหวัดอุดรธานี ประเทศไทย 41000

²คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น ประเทศไทย 40002

³คณะสัตวแพทยศาสตร์ มหาวิทยาลัยยูเทรคต์ 3508 TD ยูเทรคต์ ประเทศเนเธอร์แลนด์

⁴คณะวิทยาศาสตร์และศิลปศาสตร์ มหาวิทยาลัยเทคโนโลยีราชมงคลอีสาน จังหวัดนครราชสีมา ประเทศไทย 30000

*ผู้รับผิดชอบบทความ E-mail: pilachai_rittichai@yahoo.com