



HOKKAIDO UNIVERSITY

Title	Effect of pineapple stem starch feeding on rumen microbial fermentation, blood lipid profile, and growth performance of fattening cattle
Author(s)	Khongpradit, Anchalee; Boonsaen, Phoompong; Homwong, Nitipong et al.
Citation	Animal science journal, 91(1), e13459 https://doi.org/10.1111/asj.13459
Issue Date	2020-01
Doc URL	https://hdl.handle.net/2115/82824
Rights	This is the peer reviewed version of the following article: Effect of pineapple stem starch feeding on rumen microbial fermentation, blood lipid profile, and growth performance of fattening cattle, which has been published in final form at 10.1111/asj.13459. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.
Type	journal article
File Information	Khongpradit et al._final_text.pdf, 本文



**Effect of pineapple stem starch feeding on rumen microbial
fermentation, blood lipid profile and growth performance of fattening
cattle**

**Anchalee KHONGPRADIT,¹ Phoompong BOONSAEN,¹ Nitipong HOMWONG,¹
Yutaka SUZUKI,² Satoshi KOIKE,² Suriya SAWANON¹ and Yasuo KOBAYASHI²**

*¹ Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen,
Kasetsart University, Nakhon Pathom 73140, Thailand*

*² Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589,
Japan*

Correspondence: Yasuo Kobayashi, Graduate School of Agriculture, Hokkaido
University, Kita, Sapporo 060-8589, Japan

E-mail: kyas@anim.agr.hokudai.ac.jp

ABSTRACT

Pineapple stem starch (PS) was evaluated for its suitability as a new starch source in concentrate for fattening cattle, based on the growth performance, blood profile and rumen parameters of 36 steers in a 206-day feeding study. PS was formulated as a 40% concentrate and fed with forage in comparison with ground corn (GC) and ground cassava (CA) formulated at the same level. PS feeding improved weight gain and feed conversion ratio without affecting feed intake. PS did not obviously influence blood lipid profiles throughout the experiment. Ruminal concentration of total short chain fatty acids (SCFA) increased with PS without affecting SCFA composition throughout the feeding study. Rumen amylolytic group, especially *Ruminococcus bromii*, was dominant in the rumen microbial community, and showed increased abundance by PS feeding throughout the experiment. These results clearly indicate the potential of PS as a useful starch source for fattening cattle in terms of rumen fermentation and growth performance.

Key words: *fattening cattle, growth, pineapple stem, rumen bacteria, starch*

1. INTRODUCTION

Corn and cassava are readily available and are the most popular starch sources in Thailand and other Southeast Asian countries. However, the cost of these starch sources has risen due to increasing interest in their use for animal feed, especially in ruminant livestock. Indeed, corn and cassava as ruminant feed ingredients have become highly competitive (Wanapat *et al.* 2012). On the other hand, byproducts of agricultural and pharmaceutical industries have replaced some corn and cassava recently. One such byproduct is pineapple stem starch (PS), the residue from the extraction of bromelain enzyme, which is mainly intended for pharmaceutical use. PS is high in starch content, in particular amylose, compared with corn and cassava (Nakthong *et al.* 2017). The availability of PS in terms of amount and price may allow this material to be applied to animal feed as an alternative cost effective energy source to corn and cassava. However, no detailed evaluation in relation to demand and impact on the feed market has been made for potential application of PS to ruminant livestock, in particular fattening cattle, the main target of such new starch sources.

Ruminal hydrolysis of a starchy diet is not simply a process by which α -1, 4 and 1, 6 linkages are cleaved by amylase enzymes, as other factors besides starch breakdown exist. In fact, among the many starch sources available as a concentrate ingredient, considerable variations exist in chemical and physical characteristics including proportions of amylose and amylopectin, starch-associated lipids and proteins, and size of starch granules (Svihus *et al.* 2005;

Stevnebo *et al.* 2009), all of which affect the rate and extent of starch hydrolysis and fermentation in the rumen. Therefore, all of these factors need to be considered, especially when a new starch source is evaluated.

One of the most important measures in the evaluation of starch sources is the response of rumen microbes and associated fermentation products. The amylolytic activity of rumen bacteria increases with starch availability (Noziere & Michalet-Doreau 1997). The rumen amylolytic group is mainly composed of *Ruminococcaceae* (Xia *et al.* 2015) including *Ruminococcus bromii*, *Prevotellaceae* represented by *Prevotella ruminicola*, and the genus *Butyrivibrio* (Wallace *et al.* 1997). Of these, *R. bromii* is of interest for its superior hydrolysis and fermentation of starch (Mukhopadhyaya *et al.* 2018) as well as the involvement of amylases (Jung *et al.* 2019). This species is considered to be an ecological indicator of efficient and safe starch utilization in the rumen of fattening cattle on a high grain diet (Koike *et al.* unpublished results). Similarly, *R. bromii* is known to be a dominant bacterial species in the rumen of cattle fed a barley-based diet (Klieve *et al.* 2007). Thus, it is of interest to determine ruminal responses to the new candidate starch source PS under a practical feeding condition of fattening cattle in areas where PS is available.

The present study aimed to evaluate the applicability of PS as a feed ingredient for fattening cattle through tracking growth performance, blood lipid profile and rumen microbial responses to a dietary formulation of PS.

2. MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Animal Usage and Ethics Committee of Kasetsart University, Thailand (ACKU62-AGK-007).

2.1 Animals, experimental design and feeding

The experiment was conducted at the Ruminant Research Unit, Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom, Thailand. Thirty-six Holstein steers with an average body weight of 453 ± 35.3 kg at 22 months of age were managed in open-air pens (11.25 m² for two steers per pen) with rubber mats on concrete floors. The steers were divided into three groups of twelve steers each, according to body weight. Steers were allotted to one of three dietary treatments including a concentrate of corn, cassava or pineapple stem starch. Steers were fed the concentrate *ad libitum* with Napier grass silage (3 kg/head/day in DM) from day 1 to 74. From day 75 to 206, steers were fed the same concentrate with Napier grass silage (2 kg/head/day in DM) and rice straw (0.9 kg/head/day in DM) as roughage. The experimental diet (concentrate and forage) was offered twice a day at 07:00 and 16:00 h

The three different starch sources (Table 1) in concentrate were: i) ground corn (GC), ii) ground cassava (CA), and iii) pineapple stem starch (PS), and these were formulated as a 40% concentrate so that the starch content was equalized (Table 2). Ground corn and ground cassava were purchased from Cowboyfriend Co., Ltd., Nakhon Pathom, Thailand, while pineapple stem starch was obtained from Hong Mao Biochemicals Co., Ltd., Rayong, Thailand. Napier grass silage was prepared at the farm of Kasetsart University. The chopped length of the silage was

approximately 2–3 cm. Rice straw was purchased from a dealer in Suphanburi province, Thailand.

Dry matter intake (DMI) was recorded daily by weighing the offered and refused amounts. Body weight was measured using a walk-through scale before morning feeding once monthly during the experiment. Average daily gain (ADG) and feed conversion ratio (FCR) were calculated to evaluate growth performance.

2.2 Feed sampling and analyses

Starch sources and feeds were sampled every two weeks. Silage was assayed for DM (AOAC 2016) in order to adjust the amount fed. All samples were frozen (–20 °C) and mixed to obtain a representative sample for the analysis of crude protein, ether extract, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude ash (AOAC 2016) and starch (ISO 2000).

The starch granule size distribution of the three starch sources was analyzed using an image analyzer (KS400 v2; Carl Zeiss, Cambridge, UK) connected to a Carl Zeiss Axiophot 2 microscope. Before analysis, starch samples were mixed with an 80% sucrose solution following the method described by Baldwin (1994).

2.3 Blood sampling and analyses

Blood samples (5 mL) were obtained from steers at 4 h after morning feeding on days 74, 147 and 206 of the experiment by puncture of the jugular vein of each steer. Blood was collected in a heparinized glass tube. Plasma was separated by centrifugation for 15 min ($3,000 \times g$) at 4 °C. The plasma was used for analysis of low-density lipoprotein (LDL) with a MULTIGENT direct LDL reagent kit (Abbott,

Abbott Park, IL, USA. High-density lipoprotein (HDL) was also measured with an Ultra HDL reagent kit (Abbott). Triglycerides and cholesterol were analyzed using a triglyceride reagent kit and a cholesterol reagent kit, respectively (Abbott). Very low-density lipoprotein (VLDL) was calculated according to the equation: $VLDL = \text{triglycerides}/5$ (Friedewald *et al.* 1972).

2.4 Rumen sampling and analyses

Rumen fluid (approximately 50 mL) was collected by using a manual vacuum pump connected to a stomach tube at 4 h after the morning feeding on days 74, 147 and 206 of the experiment. The collected samples were immediately strained through two layers of cheesecloth. Ruminal pH was immediately measured (pH meter probe, Oakton WD 35634-30, Vernon Hills, IL, USA). These samples were frozen to determine ammonia nitrogen ($\text{NH}_3\text{-N}$), short chain fatty acids (SCFA), lactate ($-20\text{ }^{\circ}\text{C}$) and microbes ($-80\text{ }^{\circ}\text{C}$). $\text{NH}_3\text{-N}$ was spectrophotometrically analyzed using a phenol-hypochlorite assay (Weatherburn 1967). Lactate was determined using a commercial kit (Megazyme Inc., Wicklow, Ireland). SCFA was analyzed using a gas chromatograph (Shimadzu, Kyoto, Japan) equipped with an ULBON HR-20M fused silica capillary column (0.53 mm i.d. $\times$ 30 m length, 3.0 μm film; Shinwa, Kyoto, Japan). The procedures were as described in Watabe *et al.* (2018).

2.5 Microbial analysis

Extraction of rumen DNA for microbial analysis was conducted using the repeated bead-beating plus column method (Yu & Morrison 2004). Briefly, 250 μL

of ruminal fluid was mixed with glass beads and 1 mL of lysis buffer, and then physically processed to disrupt the microbial cells. The DNA was then precipitated with ethanol. The quality and quantity of DNA were assessed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Thereafter, DNA was stored at -80°C .

DNA was used for real-time PCR (qPCR) to quantify rumen representatives including total bacteria, *Butyrivibrio* group, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, *Ruminococcus albus*, total bifidobacteria, genus *Prevotella*, *Ruminococcus bromii*, *Prevotella ruminicola*, *Selenomonas ruminantium*, *Ruminobacter amylophilus*, *Succinivibrio dextrinosolvens*, *Prevotella bryantii*, *Streptococcus bovis*, genus *Treponema*, *Treponema bryantii*, *Megasphaera elsdenii*, *Anaerovibrio lipolytica*, total methanogens and total protozoa.

Detailed information regarding qPCR such as primers, standards, PCR conditions, and calculations followed that of Ohene-Adjei *et al.* (2008) (for methanogen) and Koike *et al.* (2007) (for other microbes). For assaying *R. bromii*, the newly developed primers targeting the highly amylolytic group (namely, *R. bromii* C1 group) (forward, 5'-TGAGTGAAGTAGAGGCAGGC-3'; reverse, 5'-CCTATTAGAGTGCTCTTGCG-3'), were used under an optimized condition (annealing temperature, 59°C ; extension time, 2min) (Koike *et al.* unpublished results). DNA was diluted to a final concentration of $10\text{ }\mu\text{g}/\mu\text{L}$ with $1\times\text{ TE}$ (Tris-EDTA) buffer, and was then added to the reaction mixture ($10\text{ }\mu\text{L}$) consisting of $1\text{ }\mu\text{L}$ DNA, $0.1\text{ }\mu\text{L}$ each of forward / reverse primer, 5.0 of KAPA Taq PCR kit (Kapa Biosystems, Charlestown, MA, USA) and $3.8\text{ }\mu\text{L}$ of PCR-grade H_2O .

2.6 Statistical analysis

The experiment was carried out in a completely randomized design arrangement to analyze the effect of the dietary starch source. All data were analyzed using R (3.6.2 Version) software (R Core Team 2020) by a one-way analysis of variance. Tukey's honestly significant difference test was then conducted for multiple comparisons post hoc. The statistical model was as follows:

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} is the dependent variable in the treatment i for replication j , μ is the overall mean, T_i is the treatment effect i and ε_{ij} is the random residual error in the treatment i for replication j . Statistical significance was considered at $P < 0.05$ and a trend was defined as $P < 0.10$.

3. RESULTS

3.1 Growth performance and feed intake

Table 3 shows the effect of dietary starch source on the growth performance and feed intake of fattening cattle. The final body weight of fattening cattle fed different starch sources did not differ. Weight gain and ADG were higher in cattle fed PS compared to those fed GC. FCR was lower (more efficient) in cattle fed PS than in those fed GC. Intake of DM, concentrate and roughage was not affected by the dietary starch source.

3.2 Blood parameters

Blood lipid profiles including cholesterol, triglycerides, HDL, LDL and VLDL of cattle are presented in Table 4. Concentrations of cholesterol, triglycerides and VLDL did not significantly differ among the treatments. HDL tended to be different at day 74, showing a higher value in PS followed by CA and then GC. LDL was affected by the starch source, showing a consistently lower level in PS compared with CA. Level of LDL in CA was higher than that for GC on days 74, 147 and 206 and higher than for PS on days 147 and 206. PS diet decreased LDL levels in a feeding duration-dependent manner.

3.3 Rumen fermentation parameters

Rumen fermentation parameters in cattle fed the different starch sources are shown in Table 5. Ruminal pH did not differ throughout the experimental period. Cattle fed PS had consistently higher levels of total SCFA than those fed GC and CA. However, molar proportions of acetate, propionate and butyrate showed no difference among the three starch sources. Concentrations of $\text{NH}_3\text{-N}$ and lactate were not significantly different among treatments.

3.4 Rumen microorganisms

Abundance of rumen microbes on days 74, 147 and 206 of the experiment is shown in Figure 1. Total bacterial abundance was not different among PS, GC and CA diets in all three sampling periods.

Butyrivibrio group and *R. bromii* as a member of amylolytic bacteria were consistently dominant throughout the experimental period, representing 11–27% and 11–42%, respectively. Particularly, *R. bromii* was detected at the highest

abundance in PS among the three treatments during the experimental period (with only day 206 not showing significant differences). In the meantime, the *Butyrivibrio* group showed the highest abundance in PS only in the sample on day 74. Although other amylolytics including *Prevotella*, bifidobacteria, *S. ruminantium* and *R. amylophilus* showed inconsistent variation with treatment and experimental period, all were minor members ($4.70\text{E}-5$ to 0.25%).

Three cellulolytic species, present at minor levels (0.0002–0.067%), did not show apparent changes with treatment and experimental period. Other microbial groups including lipolytics, intermediate-utilizers, and protozoa did not show notable changes according to treatment and experimental period. Although the relative abundance of methanogens was very high (17-25%), there was no difference between treatments and periods.

4. DISCUSSION

In discussing starch utilization in ruminants, the primary focus should be on the ruminal degradation and fermentation of starch. As shown by the higher SCFA levels in the rumen of cattle fed PS (Table 5), PS is considered to be more extensively fermented in the rumen compared to the other two starch sources. It is reasonable to suggest that the enhanced fermentation with PS could be attributable to the high activity of amylolytic rumen bacteria (Figure 1), leading to enhanced growth performance, as evidenced by ADG and FCR (Table 3), without affecting blood lipid parameters partly indicating animal health status.

Starch digestion in the rumen can be influenced by chemical and physical characteristics of the starch source, such as the amylose/amylopectin ratio, granule size, and associated lipids and proteins (Svihus *et al.* 2005). In general, starch with a high amylose/amylopectin ratio, large granule size and high content of associated components is characterized by low degradability in the rumen. This is because amylose is less degradable than amylopectin, the associated components work as a surface barrier to enzymatic action, and the large granule size limits access by rumen microbes and their enzymes. Thus, it is important to take into account these factors in discussing the digestion properties of starch sources tested in the present study.

Stevnebo *et al.* (2009) characterized the ruminal starch digestion profile using barley cultivars with varying amylose content, and observed that barley with low amylose content showed higher degradability. However, this was not entirely related to starch per se, but was related to the higher degradability of NDF associated with the barley grain. In our study, the tested starch sources possess different NDF contents (lowest in PS < CA < GC, Table 1). This order corresponds well to that of SCFA level in the rumen of cattle fed the three different starch sources (PS > CA > GC, Table 5). In this regard, PS could be superior to the other starches with respect to rumen hydrolysis, as the low NDF content (Table 1) facilitates PS degradation and efficient fermentation. Svihus *et al.* (2005) emphasize the importance of starch structure in assessing starch degradability, i.e., the lipid complex and protein matrix associated with starch granules negatively affect starch degradation, since the digestion of such lipid and protein usually precedes starch digestion. According to the chemical properties of the three

starch sources, PS showed the lowest contents of crude protein and ether extract (Table 1), suggesting it has a simpler structure than GC and CA. In addition, PS had the smallest granule size (Table 1), which may facilitate access by rumen microbes and accelerate PS hydrolysis. Many reports have pointed out the decrease of starch digestion with the amylose/amylopectin ratio (Bednar *et al.* 2001; Saito *et al.* 2001; Abdel-Aal *et al.* 2002); however, the relationship appears to be more complex because of the different chemical and physical (structural) properties of the starch sources used in those studies. In this regard, PS used in the present study can be considered to be relatively pure and simple in chemical and structural properties compared to GC and CA, resulting in this starch source being more degradable and fermentable. Such an advantageous profile of PS could be derived from the industrial process of bromelain enzyme extraction.

Based on its fermentative nature (observed to be more degradable and fermentable), PS can be regarded as a risk factor for rumen acidosis, the rumen metabolic disorder occasionally observed under feeding of a high concentrate diet (Meyer & Bryant 2017). However, the ruminal pH was within the normal range, the ruminal lactate level remained low throughout the experiment (Table 5), and above all, no related symptoms were observed in any of the experimental cattle. Therefore, PS is considered to be a safe starch source when formulated as a 40% concentrate, as is generally done in Thailand. More detailed evaluation as to upper limit of PS supplementation is to be made in the future.

Rumen microbial results support the above scenario for PS, since amylolytic species, as represented by *R. bromii*, were observed at a higher abundance throughout the experimental period (Figure 1), possibly contributing to the higher

degradation and fermentation of PS compared to GC and CA. This could be the main reason for the higher SCFA production. Xia *et al.* (2015) showed the dominance of *Ruminococcaceae* (70–80%) in bacteria associated with barley grains, implying their contribution to starch hydrolysis. *R. bromii* is abundantly detected in the rumen of Japanese Black cattle, famous for its beef marbling under a high concentrate feeding system, suggesting the contribution of this species to rumen starch fermentation (Koike *et al.* unpublished results). The importance of *R. bromii* has been described for other ruminant livestock (Stewart & Bryant 1988; Herbeck & Bryant, 1974; Ze *et al.* 2013). These reports support our assertion that *R. bromii* plays a pivotal role in digestion of PS rich in amylose (35–36%) (Nakthong *et al.* 2017; Chen & Liu 1972).

Butyrivibrio is functionally versatile but possesses amylase activity and significantly contributes to starch hydrolysis, in particular amylopectin (Ramsay *et al.* 2006). *Butyrivibrio* of the amylolytic group increased with PS diet feeding at the initial period of the present study (Figure 1), which might partially explain the elevated hydrolysis and fermentation of the PS diet. Another major member, the genus *Prevotella*, which includes amylolytic species, did not respond to PS feeding as observed for minor amylolytic species (*S. ruminantium*, *R. amylophilus*, and others) (Figure 1).

In conclusion, pineapple stem starch feeding altered the rumen microbial community to support the growth and activity of the amylolytic *R. bromii* and *Butyrivibrio*. This promoted SCFA production in the rumen, primarily leading to improvements of body weight gain and feed conversion ratio. In this context, pineapple stem starch as a byproduct of the tropical fruit processing industry could

be applied as a feed ingredient for fattening cattle. More importantly, as this starch source did not influence feed intake and rumen pH, it is expected to be safe to use.

ACKNOWLEDGEMENTS

The authors thank the Agriculture Research Development Agency, Thailand, for their financial support in conducting the present study. AK was supported by the cotutelle program (initiated in 2018) for PhD students between Hokkaido University and Kasetsart University.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Abdel-Aal ESM, Hucl P, Chibbar RN, Han HL, Demeke T. 2002. Physicochemical and structural characteristics of flours and starches from waxy and nonwaxy wheats. *Cereal Chemists* **79**, 458– 464.
- AOAC. 2016. *Official methods of analysis*, 20th edn. Association of Official Analytical Chemistry, USA.
- Baldwin PM. 1994. Holes in starch granules: confocal, SEM and light microscopy studies of starch granule structure. *Starch* **46**, 341–346.
- Bednar GE, Patil AR, Murray SM, Grieshop CM, Merchen NR, Fahey GC. 2001. Starch and fiber fractions in selected food and feed ingredients affect their

- 357 small intestinal digestibility and fermentability and their large bowel
 358 fermentability *in vitro* in a canine model. *Nutrition Journals* **131**, 276–286.
- 359 Chen YM, Liu HY. 1972. Studies on stem bromelain and stem starch from
 360 pineapple plants. *Taiwania* **17**, 266–276.
- 361 Friedewald WT, Levy RI, Fredrickson DS. 1972. Estimation of the concentration
 362 of low-density lipoprotein cholesterol in plasma, without use of the
 363 preparative ultracentrifuge. *Clinical Chemistry* **18**, 499–502.
- 364 Herbeck JL, Bryant MP. 1974. Nutritional features of the intestinal anaerobe
 365 *Ruminococcus bromii*. *Applied Microbiology* **28**, 1018–1022.
- 366 ISO. 2000. Animal feeding stuffs–Determination of starch content. Polarimetric
 367 method. Standard 6493:2000. International Organisation for Standardisation,
 368 Geneva, Switzerland. Available from URL:
 369 <https://www.iso.org/standard/12866.html>
- 370 Jung JH, An YK, Son SY, Jeong SY, Seo DH, Kim MK, Park CS. 2019.
 371 Characterization of a novel extracellular α -amylase from *Ruminococcus*
 372 *bromii* ATCC 27255 with neopullulanase-like activity. *International Journal*
 373 *of Biological Macromolecules* **130**, 605–614.
- 374 Klieve AV, O’Leary MN, McMillen L, Ouwerkerk D. 2007. *Ruminococcus bromii*,
 375 identification and isolation as a dominant community member in the rumen
 376 of cattle fed a barley diet. *Journal of Applied Microbiology* **103**, 2065–2073.
- 377 Koike S, Yabuki H, Kobayashi Y. 2007. Validation and application of real-time
 378 polymerase chain reaction assays for representative rumen bacteria. *Animal*
 379 *Science Journal* **78**, 135–141.

- 380 Meyer NF, Bryant TC. 2017. Diagnosis and management rumen acidosis and
 381 bloat in feedlots. *Veterinary Clinics of North America Food Animal Practice*
 382 **33**, 481–498.
- 383 Mukhopadhyaya I, Morais S, Laverde–Gomez J, Sheridan PO, Walker AW, Kelly
 384 W, Klieve AV, Ouwerkerk D, Duncan SH, Louis P, Koropatkin N, Cockburn
 385 D, Kibler R, Cooper PJ, Sandoval C, Crost E, Juge N, Bayer EA, Flint HJ.
 386 2018. Sporulation capability and amylosome conservation among diverse
 387 human colonic and rumen isolates of the keystone starch–degrader
 388 *Ruminococcus bromii*. *Environmental Microbiology* **20**, 324–336.
- 389 Nakthong N, Wongsagosup R, Amornsakchai T. 2017. Characteristics and
 390 potential utilization of starch from pineapple stem waste. *Industrial Crops*
 391 *and Products* **105**, 74–82
- 392 Noziere P, Michalet–Doreau B. 1997. Effect of amount and availability of starch
 393 on amylolytic activity of ruminal solid-associated microorganisms. *Journal of*
 394 *the Science of Food and Agriculture* **73**, 471–476.
- 395 Ohene–Adjei S, Chaves AV, McAllister TA, Benchaar C, Teather RM, Forster RJ.
 396 2008. Evidence of increased diversity of methanogenic archaea with plant
 397 extract supplementation. *FEMS Microbial Ecology* **56**, 234–242.
- 398 R Core Team. 2020. R: A Language and Environment for Statistical Computing,
 399 Vienna, Austria; [cited 12 January 2020]. Available from URL:
 400 <http://www.R-project.org/>
- 401 Ramsay, AG, Scott KP, Martin JC, Rincon MT, Flint HJ. 2006. Cell-associated
 402 α -amylases of butyrate-producing firmicute bacteria from the human colon.
 403 *Microbiology* **152**, 3281–3290.

- 404 Saito K, Ito T, Kuribayashi T, Mochida T, Nakakuki T, Shibata M, Sugawara M.
 405 2001. Effect of raw and heat–moisture treated high–amylose corn starch on
 406 fermentation by the rat cecal bacteria. *Starch* **53**, 424–430.
- 407 Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG, Russell JB. 1992. A net
 408 carbohydrate and protein system for evaluating cattle diets: II. carbohydrate
 409 and protein availability. *Journal of Animal Science* **70**, 3562–3577.
- 410 Stevnebo A, Seppälä A, Harstad OM, Huhtanen P. 2009. Ruminant starch
 411 digestion characteristics in vitro of barley cultivars with varying amylose
 412 content. *Animal Feed Science and Technology* **148**, 167–182.
- 413 Stewart CS, Bryant MP. 1988. The rumen bacteria. In: Hobson PN, Stewart CS
 414 (eds), *The rumen microbial ecosystem*, 2nd edn, pp. 10–72. Academic &
 415 Professional, an imprint of Chapman & Hall, London.
- 416 Svihus B, Uhlen AK, Harstad OM. 2005. Effect of starch granule structure,
 417 associated components and processing on nutritive value of cereal starch: A
 418 review. *Animal Feed Science and Technology* **122**, 303–320.
- 419 Wallace RJ, Onodera R, Cotta M A. 1997. Metabolism of nitrogen-containing
 420 compounds. In: Hobson PN, Stewart CS (eds), *The rumen microbial*
 421 *ecosystem*, 2nd edn, pp. 238–328. Academic & Professional, an imprint of
 422 Chapman & Hall, London.
- 423 Wanapat M, Pilajun R, Kang S, Setyaningsih K, Setyawan AR. 2012. Effect of
 424 ground corn cob replacement for cassava chip on feed intake, rumen
 425 fermentation and urinary derivatives in swamp buffaloes.
 426 *Asian–Australasian Journal of Animal Sciences* **25**, 1124–1131.

- Watabe Y, Suzuki Y, Koike S, Shimamoto S, Kobayashi. 2018. Cellulose acetate, a new candidate feed supplement for ruminant animals: *In vitro* evaluations. *Journal of Dairy Science* **101**, 10929–10938.
- Weatherburn MW. 1967. Phenol–hypochlorite reaction for determination of ammonia. *Analytical Chemistry* **39**, 971–975.
- Xia Y, Kong Y, Seviour R, Yang HE, Forster R, Vasanthan T, McAllister T. 2015. *In situ* identification and quantification of starch–hydrolyzing bacteria attached to barley and corn grain in the rumen of cows fed barley–based diets. *FEMS Microbiology Ecology* **91**. fiv077.
- Yu Z, Morrison M. 2004. Improved extraction of PCR–quality community DNA from digesta and fecal samples. *BioTechniques* **36**, 808–812.
- Ze X, Mougén FL, Duncan SH, Louis P, Flint HJ. 2013. The role of “keystone” species in the degradation of recalcitrant substrates. *Gut Microbes* **4**, 235–240.

451 **Figure caption:**

452 **FIGURE 1** Effect of different starch source on rumen bacterial abundance of
 453 fattening cattle on 74th, 147th and 206th day (at 4h post feeding). The qualified
 454 species and groups are *Ruminococcus bromii*, *Butyrivibrio* group, Genus *Prevotella*,
 455 *Prevotella ruminicola*, *Prevotella bryantii*, *Succinivibrio dextionsovlvens*,
 456 *Selenomonas ruminantium*, *Ruminobacter amylophilus*, *Streptococcus bovis*,
 457 Bifidobacteria, *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*,
 458 *Ruminococcus albus*, Genus *Treponema*, *Treponema bryantii*, *Megasphara*
 459 *elsdenii*, *Anaerovibrio lipolytica*, total methanogen and total bacteria. Means
 460 (n=12) with different letters are significantly different ($P < 0.05$). Error bar
 461 indicates SD. GC = ground corn; CA = ground cassava; PS = pineapple stem starch.