Rice distillers' byproduct improved growth performance and reduced enteric methane from "Yellow" cattle fed a fattening diet based on cassava root and foliage (*Manihot esculenta* Cranz)

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Abstract

The aim of the study was to evaluate the effect of rice distillers' byproduct (RDB) on growth and enteric methane emissions from local "Yellow" cattle fed a basal diet of cassava root fermented with yeast, urea, di-ammonium phosphate (DAP), cassava foliage and rice straw. Sixteen local "Yellow" cattle with initial weight of 67.5Â±3.5 kg were housed in four pens each with four animals. The treatments were rice distillers' byproduct at a predicted level of 4% of diet DM (RDB) or none (No RDB). Two pens received the "RDB" treatment and two pens received the "No RDB" treatment at random. The experiment lasted 120 days at the end of which concentrations of methane and carbon dioxide in eructed gas mixed with air were determined in a closed chamber in which the animals were kept for 10 minutes prior to measurement.

Recorded intake of rice distillers' byproduct was 2.75% of diet DM. This was because the fermented cassava root was offered ad libtum and actual intakes were some 30% higher than had been predicted. Growth rate and feed conversion were improved by 40 and 20% respectively, when the diet of fermented cassava root and cassava foliage was supplemented with the rice distillers' byproduct. Rice distillers' byproduct supplementation increased the concentration of propionic acid in the rumen VFA and reduced by 26% the ratio of methane:carbon dioxide in the mixed eructed gas and air in the measurement chamber.

Key words: *byproduct, forage, climate-smart, feed conversion, global warming, greenhouse gas, propionic acid*

Introduction

Livestock play an important role in Lao PDR, which extends beyond the traditional supply of meat and milk. They are used for multiple purposes as draft power, means of transportation, capital, credit, social value, hides, and provide a source of organic fertilizer for seasonal cropping. Cattle numbers in Lao PDR have increased from 1.3 to 1.7 million head from 2005 to 2013; while the buffalo population increased from 1.1 to 1.19 million head. Due to population growth and changing diets, there is strong market demand for Lao “Yellow” cattle and buffaloes from neighboring countries of the People’s Republic of China and Vietnam as well as domestically (DLF 2013, 2014).

In the future, however, economic benefits from cattle and buffalo may be offset by their contribution to global warming (Steinfeld et al 2006). The major culprit is methane produced by enteric fermentation and from decomposing manure (IPCC 2014). Enteric CH_4 emissions are often predicted from the chemical analysis of the diets (Hristov et al 2013; Moraes et al 2014); however, these methods do not seem sufficiently accurate and appropriate for all feeding situations.

Leng (1991) pointed out that the first step in developing methane mitigating strategies is to increase productivity, as methane is produced irrespective of whether the animal is at maintenance, or is expressing its genetic potential to produce milk and meat. Thus, on any diet and particularly diets based on agro-industrial byproducts, improving live weight gain and feed conversion efficiency by supplementation leads to a significant decrease in methane production per unit of production (Figure 1).

Figure 1. Relationship between live weight gain and enteric methane production per unit live weight gain (Klieve and Ouwerkerk 2007)

Feeding systems based in cassava products and by products have great potential for increasing ruminant productivity and reducing methane production. The root is composed of highly digestible carbohydrate in the form of starch with little fiber. The foliage is rich in protein which, allied with low levels of tannin (Netpana et al 2001; Bui Phan Thu Hang and Ledin 2005), enables some of the dietary protein to escape from the rumen and, following intestinal digestion, contribute to the animal’s requirements for essential amino acids directly at the sites of metabolism. The presence of cyanogenic glucosides which are converted to HCN in the rumen is a major problem but appears to be involved in a reduction in methanogenesis (Phuong et al 2015).

The study which gave impetus to the idea that cassava could be the basis of an intensive system for fattening cattle was a response to the finding of 10,000 tonnes of cassava pulp, the residue from industrial production of starch from cassava roots, that had been deposited as waste over a 4-year period in an open pit at the Indo Chine Tapioca factory in Vientiane, Lao PDR (Phanthavong et al 2014). Extraction of samples to a depth of 10m demonstrated that despite the pit being open to the elements the cassava pulp had been naturally ensiled with a pH of 3.2. *In vitro* incubation of samples showed that the ensiled pulp was only slightly inferior in feeding value to the original fresh cassava root (Phanthavong et al 2014). It was subsequently demonstrated that with appropriate supplementation (urea to provide fermentable rumen nitrogen, brewers’ grains as source of bypass protein and rice straw for fiber), growth rates of 800 g/day with DM feed conversions of 6:1 were achievable with local cattle, indicating that cassava pulp could be the basis of an intensive cattle fattening system (Phanthavong et al 2016).

Subsequent research showed that:

(i) fresh cassava foliage could replace all or part of the brewers’ grains as the source of bypass protein (Keopaseuth et al 2017);

(ii) “bitter” cassava foliage could be used as the sole source of bypass protein and fiber provided that “synergistic” quantities of brewers’ grains (4% of diet DM) were fed as part of the diet (Binh et al 2017);

(iii) a local byproduct (Quilao) from rice wine production could replace the brewers’ grains (Sengsouly et al 2017).

The objective of the study reported in this paper was to determine if rice distillers’ byproduct would improve growth performance of local cattle when the basal diet was cassava root fermented with yeast, urea and di-ammonium phosphate, with fresh cassava foliage as the source of bypass protein.

Materials and methods

Location and duration

The experiment was carried out on a farm in Nongboakham village, Luang Prabang province, Lao PDR.

Experimental design and animals

Sixteen local “Yellow” cattle with initial weight in the range of 64 to 71 kg (67.5Â±3.5 kg) were used. They were vaccinated against epidemic diseases and drenched against internal parasites. They were housed in four pens with two pens per treatment. The treatments were:

RDB: Rice distillers’ byproduct at 4% of predicted DM intake
No RDB: No rice distillers’ byproduct

The basal diet was: cassava root fermented with yeast, urea and DAP (Table 1) fed ad libitum; fresh cassava foliage at 1% of live weight (as DM); and rice straw at 0.25% of live weight. The experiment lasted 120 days.

Table 1. Ingredients in the fermented cassava root

	DM basis, %	**CP in DM, %**
Cassava root	93	2.32
Yeast	3	1.35
DAP	1	1.30

Urea	2	5.60
S-rich minerals	1	

Photo 1. The local "Yellow" cattle

Feeding and management

The cassava roots (Photo 2) bought from local farmers were chopped into small pieces (1-2cm) by machine. Urea, DAP, yeast and sulphur-rich minerals were added (Table 1) and the mixture stored in sealed plastic bags for fermenting over 7 days before feeding. Rice distillers' byproduct (Photo 3) was bought from farmers who make "Lao Kao" wine in Luang Prabang province. The cattle were gradually introduced to the diets over a period of 2 weeks. The fermented cassava root was offered ad libitum.

Cassava, grown on the farm, was managed as a perennial forage with daily harvesting of the 2-3-month-old re-growth (Photo 4). Rice straw was collected from nearby farmers. Rice distillers' byproduct was mixed with part of the fermented cassava root and given as the first feed in the morning; the remaining fermented cassava root, cassava foliage and rice straw were fed separately in different troughs. The cassava foliage and rice straw were offered two times daily, at 7.00 am and 4.30 pm. Water was freely available.

Photo 2. Cassava root

Photo 3. Rice distillers' byproduct

Data collection and measurements

The offer level of rice distillers' byproduct was set at 4% of the expected DM intake which was estimated to be 2.5% of live weight. Feeds offered were weighed before giving them to the cattle. Feed refusals were collected each morning prior to offering fresh feed and weighed to measure the feed intake. The live weights of the cattle were taken at the beginning, every 15 days and at the end of the experiment, using an electronic balance.

Concentrations of CO_2 and CH_4 in mixed eructed gas and air were measured on the last day of the experiment following the procedure proposed by Madsen et al (2010). Each animal was put in a plastic-covered cage (Diagram 1; Photo 5) and after a period of 5 minutes for equilibration with the surrounding air, the concentrations of methane and carbon dioxide were recorded over a 10 minute period, using a GASMET 4030 meter (Gasmet Technologies Oy, Pulttitie 8A, FI-00880 Helsinki, Finland). The CH_4 and CO_2 concentrations in background air were also recorded. The final methane to carbon dioxide ratios were calculated as:

Ratio CH_4/CO_2 = (a-b)/(c-d)

Where "a" is methane concentration in mixed eructed gas plus air, "c" is carbon dioxide concentration in mixed eructed gas plus air, "b" is methane in the air in the cattle shed and "d" the carbon dioxide in cattle shed air.

On the penultimate day of the experiment, rumen fluid samples were taken by stomach tube two hours' post feeding in the morning. The pH was measured with a digital pH meter, prior to addition of sulphuric acid for subsequent analysis of ammonia by steam distillation (AOAC 1990) and of volatile fatty acids by high pressure liquid chromatography (Water model 484 UV detector; column novapak C18; column size 3.9mm x 300mm; mobile pahse 10mM H_2PO_4 pH 2.5; Samuel et al 1997) .

Diagram 1. A schematic view of the method for measuring CH_4 and CO_2 from cattle using GASMET infra-red analyzer

Photo 5.
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Chemical analysis

Samples of feeds offered and residues were collected over a 24 h period every 15 days to determine dry matter (DM), ash and crude protein (CP) following AOAC (1990) procedures. The solubility of the protein in cassava leaves was determined by shaking 3g samples with 100 ml 1M NaCl for 3 hours, filtering through Whatman No.4 filter paper and determining nitrogen in the filtrate.

Statistical analysis

Live weight gains were calculated from the linear regression of live weight (Y) on days in the experiment (X). The growth data were analyzed by the general linear model option of the ANOVA program in the Minitab (2000) software, using mean values for each pen for analysis of feed intake, live weights and feed conversion. For analysis of rumen ammonia and VFA, and methane/carbon dioxide ratios in mixed eructed gas and air, values for individual animals (n=8 on each treatment) were used. In the model the sources of variation were: treatments and error. The statistical model used was:

Y_{ij} = Âµ + T_i + e_{ij}

Where: Y_{ij} is dependent variable, Âµ is overall mean, T_i is effect of treatment and e_{ij} is random error.

Results and discussion

Composition of diet ingredients

The values for DM and crude protein in the the rice distillers' byproduct (Table 2) were similar to what was reported for the same product sourced in Vietnam (Luu Huu Manh et al 2009). The values for N solubility of cassava foliage (leaves and petioles) and of the rice distillers' byproduct indicate that both products are likely to be good sources of bypass protein (see Preston and Leng 1987).

Table 2. The chemical composition of the feed ingredients

	DM %	Ash	CP	N solubility#
		% of DM		

Fermented cassava root	28.5	4.26	11.4	
Cassava foliage	28.2	9.75	19.2	30.1
Rice distillers' byproduct	7.79	5.45	23.1	37.5
Rice straw	89.3	13.0	3.57	9.42

% N soluble in M NaCl; DM: dry matter, CP: crude protein

Feed intake

The intake of all diet components was increased by supplementation with rice distillers' byproduct (Table 3; Figure 2). The average intake of rice distillers' byproduct was 2.75% of the DM consumed on the "RDB" treatment. This was less than the intended concntration of 4% of diet DM, because the recorded intakes of fermented cassava root (fed ad libitum) were some 30% greater than had been predicted when the diets were formulated.

Table 3. Mean values of feed intake by Yellow cattle fed fermented cassava root, fresh cassava foliage and straw supplemented with or without of rice distillersÂ' byproduct (RDB)

	No RDB	RDB	SEM	*p*
Fresh matter intake, g/day				
Fermented cassava root	6888	7269	66.1	0.055
Rice straw	289	302	2.58	0.069
Cassava foliage	2564	2723	25.8	0.049
RDB		1068		
DM intake, g/day				
Cassava root	1796	1979	19	0.02
Cassava foliage	652	708	5.8	0.021
Rice straw	258	270	2.3	0.069
RDB	0	83		

		3041	**26.5**	**0.012**
Total DM	**2706**			
g/kg LW	**32.5**	**34.7**		
RDB as % of diet DM		**2.75**		
CP, % in DM	12.1	12.4		

No RDB: Without rice distillers' byproduct; RDB: With rice distillers' byproduct

Figure 2. Effect of rice distillers' byproduct intake of feed ingredients (as DM)

Growth performance

The cattle growth rate was uniform (Figure 3) and was increased 40% by supplementation with rice distillers' byproduct (Table 4; Figure 4). DM feed intake (Figure 5) and DM feed conversion (Figure 6) followed the same response pattern as for growth rate with 20% improvement in DM feed conversion for supplementation with rice distillers' byproduct.

Table 4. Mean values for inital and final live weight, live weight gain, DM feed intake and DM feed conversion in Yellow cattle fed fermented cassava root supplemented or not with rice distillers' byproduct (RDB)

	No RDB	**RDB**	**SEM**	***p***
Live weight, kg				
Initial	66.8	66.5	0..56	0.78
Final	103	116	1.86	0.035
Live weight gain, g/day	299	418	10.4	0.015
DM intake, g/day	2706	3041	26.5	0.012
DM feed conversion	9.05	7.27	0.14	0.013

Figure 3. Effect of rice distillers' byproduct on growth curves of Yellow cattle fed fermented

cassava root, fresh cassava foliage and rice straw

Figure 4. Effect of rice distillers' byproduct on live weight gain of Yellow cattle fed fermented cassava root, fresh cassava foliage and rice straw

Figure 5. Effect
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Rumen parameters and ratio of methane and carbon dioxide

There was no difference between treatments in rumen pH taken 2 hours after feed ingestion, but the concentration of rumen ammonia was increased when the diet was supplemented with rice distillers' byproduct (Table 5).

Table 5. Mean values for rumen pH, ammonia and molar VFA

	No RDB	RDB	SEM	*p*
Rumen pH	7.02	6.98	0.029	0.376
NH_3, mg/liter	246	312	5.44	<0.001
Rumen VFA, molar %				
Acetic acid	64.9	61.5	0.790	0.360
Propionic acid	26.7	29.4	0.710	0.013
Butyric acid	8.41	9.13	0.300	0.055
Ac:Pro	2.43	2.09	0.053	0.006

Supplementation with rice distillers' byproduct increased the proportion of propionic acid in the rumen VFA and decreased the ratio of acetic to propionic acid (Table 5; Figure 7). The ratio of methane to carbon dioxide in the mixed eructed gas and air was decreased 26% by supplementation with rice distillers' byproduct (Table 6; Figure 8).

Table 6. Mean values for concentrations, and ratios, of methane and carbon dioxide in mixed eructed gas and air in the closed chambers holding the cattle

	No RDB	RDB	SEM	*p*
CO_2 , ppm	3790	4334		
CH_4 , ppm	144	127		
CH_4/CO_2	0.0384	0.0285	0.0023	0.01

Figure 7. Effect of rice distillers' byproduct on acetic: propionic acid ratio in rumen fluid of Yellow cattle fed fermented cassava root, fresh cassava foliage and rice straw

Figure 8. Effect dioxide in m cass

Discussion

The positive effect of the low level (2.75% of diet DM) of rice distillers' byproduct on growth performance, and in reducing methane in eructed gas from the rumen, is similar to the responses reported by Sengsouly and Preston (2016) when rice distillers' byproduct was added at 4% to a diet differing only in the treatment of the cassava root which was ensiled without additives.

In the present experiment, the cassava root was fermented for 7 days after mixing with yeast-urea-DAP. Live weight responses to rice distillers' byproduct were similar in both experiments: from a weight gain of 300 g/d in non-supplemented animals to 440 g/day for those that were supplemented (Sengsouly and Preston 2017), compared with 299 to 418 g/day in the present experiment. The cattle breed ("Yellow") was the same; however. initial weights were less (67kg in the present experiment compared with 91kg in the experiment of Sengsouly and Preston 2016). It would seem that there is no benefit from prior fermentation of the cassava root with yeast, urea and DAP (as in the present experiment), compared with ensiling the root and adding the urea and minerals at the time of feeding (Sengsouly and Preston 2016).

The inverse relationship between the propionic acid concentration in the rumen fermentation and the proportion of methane in rumen gas has been observed in many experiments (see the mega-analysis by Syahniara et al 2016). It is in line with the understanding that hydrogen produced in fermentation is directed into propionate production and this will supply, following it being adsorbed, a source for glucose synthesis, which in ruminants appears to be an essential nutrient. This may spare the degradation of absorbed amino acids for this purpose where glucose requirements are high (late pregnancy, lactation and fast growth) (see Preston and Leng 1987). Thus the change in propionate has two benefits: it preserves a greater proportion of the metabolisable energy and provides an essential nutrient.

The mechanism by which small quantities of rice distillers' byproduct (4% of diet DM) bring about these positive effects, benefitting both animal performance, and the environment (methane is twenty times more active than carbon dioxide in its contribution to global warming) is still to be identified. Here we suggest the idea that substances in rice distillers' byproduct (perhaps ?-glucan or related compounds) support biofilm formation which in turn increases the efficiency of microbial growth (Leng

2014). Rice distillers' byproduct is rich in yeast which in turn is rich in ?-glucan, an element shown to have positive benefits on animal and human health through stimulation of the immune system. Another area that may be involved is that rumen microbes (or biofilms) degrade the cyanogenic glycosides to HCN which is then metabolized to carbon dioxide. In support of this idea are the reports that less thiocyanate was excreted in the urine in similar studies where brewers' grains were fed (in cattle - Binh et al 2017; and in goats - Vor Sina et al 2017).

The benefits of supplementing cassava diets with small quantities (2.75% of diet DM) of rice distillers' byproduct or brewers' grains are suggested to be due to:

- They supply essential amino acids as bypass protein
- The yeast cells provide ?-glucan that may support biofilm habitat for HCN metabolizing bacteria
- The improved biofilm habitat in the rumen increases the efficiency of microbial growth and the production of propionic acid for glucose synthesis in the animal.

Conclusions

- Growth rate and feed conversion in local Yellow cattle were improved by 40 and 20% respectively when a diet of fermented cassava root (with yeast, urea and DAP) and cassava foliage was supplemented with 2.75% (in DM) of rice distillers' byproduct.
- Rice distillers' byproduct supplementation increased the concentration of propionic acid in the rumen VFA and reduced by 26% the ratio of methane to carbon dioxide in the eructed rumen gas.
- Supplementation of a cassava-based cattle fattening diet with 2.75% rice distillers' byproduct provides a double benefit for "climate-smart agricultural production" (Ramirez-Restrepo et al 2017) by reducing the emission of methane: (i) per unit DM feed intake; and (ii) per unit of live weight gain (and thus of methane per unit food production).

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