A low concentration (4% in diet dry matter) of brewersÂ’ grains improves the growth rate and reduces thiocyanate excretion of cattle fed cassava pulp-urea and Â“bitterÂ” cassava foliage

Phuong Le Thuy Binh, T R Preston[1], Khang Nhuyen Duong and R A Leng[2]

Nong Lam University, Viet Nam
binhphuonglt@gmail.com
[1] **Centro para la InvestigaciÃ³n en Sistemas Sostenibles de ProducciÃ³n Agropecuaria (CIPAV), Carrera 25 No 6-62 Cali, Colombia**
[2] **University of New England, Armidale, NSW, Australia**

Abstract

Twelve Lasind cattle were the experimental animals in a completely randomized block design with 3 treatments and 4 replicates. The basal diet was ensiled cassava pulp plus 0.7% urea (in fresh basis of pulp) fed ad libitum together with a mineral mixture containing 7.5% sulphur, 40% dicalcium phosphate and 52.5% sodium chloride. The treatments during the first 56 days (Period 1) were: BG-RS: Brewers’ grains at 1% of live weight and rice straw 0.9% of live weight (on DM basis); CFB: Fresh foliage from a bitter variety (KM 94) of cassava at 1% of live weight (DM basis), replacing the brewers’ grains and rice straw in the BG-RS control treatment; CFS: Fresh foliage from a sweet variety (Gon) of cassava at 1% of live weight (DM basis). For the 2nd period of 56 days the treatments BG-RS and CFS remained the same but treatment CFB was modified by adding a supplement of brewers’ grains at 4% of the diet DM.

In Period 1 the cattle fed bitter cassava foliage had lower DM intake than those fed sweet cassava foliage and gained only 61 g/day compared with 383g/day for those fed sweet cassava foliage. In period 2 when brewers’ grains at 4% of the diet were added to this treatment, the DM intake increased by 47% and the live weight gain (380g/day) did not differ from the 410g/day for cattle fed sweet cassava foliage. In both periods the DM intake and growth rate were better for cattle fed brewers’ grains and rice straw, rather than sweet cassava foliage, as the source of bypass protein and fiber. Urine excretion of thiocyanate was highest in cattle fed cassava pulp-urea supplemented only with bitter cassava foliage, was reduced to half by addition of 4% brewers grains to the bitter cassava foliage diet, was present at a low level in the urine of cattle fed sweet cassava foliage, and was not detected in the urine of cattle fed cassava pulp-urea, brewers’ grains and rice straw. There was a tendency for the acetate: propionate ratio in rumen fluid to be lower when cassava pulp-urea was supplemented with brewers’ grains and rice straw compared with cassava foliage. The ratio of methane relative to carbon dioxide in mixed eructed gas-air decreased in the order of treatments in which the cassava pulp-urea diet was supplemented with brewers’ grains, sweet cassava and bitter cassava, respectively.

Key words: *bypass protein, HCN, methane, sweet cassava foliage, variety*

Introduction

Cassava pulp, the byproduct from industrial extraction of starch from cassava roots, contains 70% starch but is very low in crude protein (less than 2% in DM) (Sriroth et al 2000).When used as a production feed for ruminants, supplementation with deficient nutrients is mandatory to provide ammonia for micro-organisms, bypass protein to complement that produced in the rumen fermentation, and coarse fiber to ensure rumen motility and to avoid sub-acute acidosis (Preston and Leng 1987). Urea is the cheapest source of crude protein to provide rumen ammonia for microbial growth and, in the tropical context, following the research of Ffoulkes and Preston (1978), cassava foliage appeared to be the appropriate source of both bypass protein and fibre (Promkot and Wanapat 2003).

The use of cassava pulp as the basis of an intensive system of cattle fattening (Phanthavong et al 2016a) was thus predicated on the model developed by Ffoulkes and Preston (1978), with cassava pulp-urea replacing molasses-urea. However, at the time and place of initiating the experiment (mid-point of the dry season in Lao PDR) cassava foliage was not available and the decision was made to replace it by a combination of wet brewers' grains (a known source of bypass protein; Promkot and Wanapat 2003) and rice straw. The results were impressive with growth rates of local "Yellow" cattle exceeding 700 g/day (Phanthavong et al 2016a). However, when cassava foliage became available in the subsequent rainy season, the attempt to replace the brewers' grains-rice straw with fresh cassava foliage was a complete failure with close to zero growth rates (Phanthavong et al 2016b). Fortunately, the observation that the cattle on the cassava foliage treatment showed a 'craving' for the brewers' grains being fed to their "control" neighbors - led to the positive outcome in which 50% replacement of the cassava foliage by brewers' grains raised the growth rate to close to that on the 'control' diet of brewers' grains-rice straw (Phanthavong et al 2016b). Allied to this response was the understanding that: (i) the cassava foliage used by Phanthavong et al (2016b) was from a bitter variety (it was sourced from the fields of farmers supplying cassava roots to the starch factory); and (ii) the cassava foliage used successfully by Ffoulkes and Preston (1978) was from a "sweet" variety traditionally cultivated for root production destined for human consumption.

Varieties of cassava can be broadly classified into "sweet" and "bitter" ecotypes according to the level of cyanogenic glycosides that produce hydrocyanic acid (HCN) by plant enzymes when cassava is chopped and also when it is chewed by the animal. HCN is also produced in the rumen by microbial enzymes acting on the glycosides.

The positive growth response to the partial substitution of the bitter cassava foliage by brewers' grains (Phanthavong et al 2016b) led to the inference that the brewers' grains were a source, not just of bypass protein, but also of some factor [eg: a probiotic (see Fuller 1997); or a support to specific biofilms (Leng 2014)] favoring tolerance to, or capacity to biodegrade, the HCN or its precursors in the "bitter' cassava foliage. The logical next step was the idea that, theoretically, the benefits of brewers' grains should be manifested by much smaller proportions in the diet. This idea was tested in an experiment with growing cattle fed a diet of ensiled cassava root and cassava foliage (Inthapanya et al 2016), in which the brewers' grains was fed at the low level of 5% of the diet DM. The result was an increase of 16% in DM intake and of 48% in N retention attributable to the 5% of brewers' grains in the diet.

Hypothesis

Against this background, it was hypothesized that addition of 4% of fresh brewers' grains to a basal diet of cassava pulp-urea and cassava foliage would improve the growth rate of cattle and aid in the detoxification of the HCN associated with the feeding of "bitter" cassava foliage.

Materials and methods

Location and duration

The experiment was conducted in the Center of Research and Technology Transfer, Nong Lam University, Ho Chi Minh city, Vietnam from September 2015 to January 2016.

Treatments and experimental design

Twelve Lasind cattle (product derived from crossing of "Yellow" breed females with Red Sindhi males) were the experimental animals in a completely randomized block design with 3 treatments and 4 replicates. The basal diet was ensiled cassava pulp plus 0.7% urea (in fresh basis of pulp) fed ad libitum together with a mineral mixture containing 7.5% sulphur, 40% dicalcium phosphate and 52.5% sodium chloride.

The treatments during the first 56 days (Period 1) were:

BG-RS: Fresh brewers' grains at 1% of live weight and rice straw 0.9% of live weight (both on DM basis) **CFB:** Fresh foliage from a bitter variety (KM 94) of cassava at 1% of live weight (DM basis), replacing the brewers' grains and rice straw in the BG-RS control treatment **CFS:** Fresh foliage from a sweet variety (Gon) of cassava at at 1% of live weight (DM basis)

For the 2nd period of 56 days the treatments BG-RS and CFS remained the same but treatment CFB was modified by adding a supplement of brewers' grains offered at 4% of the diet DM.

Animals and housing

The 12 Lasind cattle with initial live weight from 116 to 160 kg were housed in individual pens, with bamboo slatted floors and roof of Nipa Palm leaves. The cattle were vaccinated against foot and mouth disease and de-wormed with Ivermectin before starting the experiment.

Feeding and management

Cassava pulp (pH 3.2) was purchased from the Wusen cassava starch factory, Binh Phuong province. It was stored in closed polyethylene bags prior to feeding. Cassava plots of the two varieties 'KM 94' and 'Gon' were established in the University farm. The foliage was harvested at successive periods of 4-5 months regrowth. All the feeds were offered three times a day, at 8.00 am, 12.00 am and 5.00 pm.

Data collection and measurements

The cattle were weighed at the beginning of the experiment and at intervals of 14 days. Feeds offered and refused were recorded daily. Samples of rumen fluid were taken by stomach tube 3 hours after the last meal at the end of the experiment for determination of concentrations of individual VFA (estimated by gas liquid chromatography following the method of Rowe et al 1979).

On the last day of the experiment: (i) samples of urine were collected 2 hours after the morning feed, frozen and stored at -20Â°C; (ii) the cattle were confined individually in a closed chamber (a bamboo structure sealed with polyethylene film) for successive measurements over 10 minute periods of the concentrations of methane and carbon dioxide in mixed air and eructed gas. The procedure was that described by Madsen et al (2010), using an infra-red gas meter (GASMET 4030; Gasmet Technologies Oy, Pulttitie 8A, FI-00880 Helsinki, Finland). For the cattle on treatment CFB-BG, after the first samplng of the eructed gas/air, and of urine, the brewers' grains were removed from the diet and for 5 days they were fed only the cassava pulp-urea and bitter cassava foliage. At the end of this period the eructed gas-air was again analyzed and samples taken of urine.

Chemical analysis

Feeds offered and refused were analysed for DM and N following the procedure of AOAC (1990). Thiocyanate in urine was measured using the procedure described in the protocol of kit D1 (http://biology-assets.anu.edu.au/hosted_sites/CCDN/five.html.

Statistical analysis

The data were analysed with the general linear model (GLM) option in the ANOVA programme of the Minitab software (Minitab 2000). Sources of variation were treatments and error.

Results

Feed intake and live weight gain

In Period 1 the cattle fed bitter cassava foliage as sole source of bypass protein had lower DM intake than those fed sweet cassava foliage and gained only 61 g/day compared with 383g/day for those fed sweet cassava foliage (Table 1a). When brewers' grains at 4% of the diet were added to this treatment in Period 2, the DM intake increased by 47% and the live weight gain (380g/day) did not differ from the 410g/day for cattle fed sweet cassava foliage (Table 1b). The trends in live weight change (Figure 1) highlight the immediate impact on growth rate when 4% of brewers' grains were included in the diet of the cattle fed bitter cassava foliage.

In both periods the DM intake and growth rate were greater for cattle fed brewersÂ' grains and rice straw, than for those fed sweet cassava foliage, as the source of bypass protein and fiber.

Table 1a. Mean values for feed intake, change in live weight and feed conversion of cattle fed cassava pulp-urea and brewers' grains plus rice straw (BG-RS), sweet cassava foliage (CFS) or bitter cassava foliage (CFB) in Period 1.

	BG-RS	CFS	CFB	SEM	*p*
Live weight, kg					
Initial	159	146	140	11.4	0.512

Final	187	169	143	13.5	0.12
LW gain, kg/d	0.487	0.383	0.061	0.0538	0.001
D**M intake, g/d**					
Cassava pulp	2401	1834	1891	67.8	0.001
Urea	16.8	12.8	13.2	-	-
Brewers grains	690				
Rice straw	1295				
Cassava foliage					
Sweet		1385			
Bitter			587		
Total DM	4386	3219	2482	81	0.001
DM conversion	9.22	8.47	-	-	-

Table 1b. Mean values for feed intake, change in live weight and feed conversion of Lasind cattle fed cassava pulp-urea and: brewers' grains plus rice straw (BG-RS), sweet cassava foliage (CFS) or bitter cassava foliage (CFB-RG) plus brewers' grains at 4% of diet DM (Period 2)

	BGRS	**CFS**	**CFB-BG**	**SEM**	***p***
Live weight, kg					

Initial	187	169	143	14	0.12
Final	222	191	163	14	0.047
LW gain kg/d	0.660	0.410	0.380	0.070	0.039
			DM intake, g/d		
Cassava pulp	3211	2737	2629	68	
Urea	22.5	19.2	18.4		
Brewers grains	1541		213		
Rice straw	1343				
			Cassava foliage		
Sweet		1267			
Bitter			810		
Total DM	6095	4004	3652	82	0.001
DM conversion	9.75	10.0	10.4	1.53	0.95

Figure 1. The negligible growth rate of Lasind cattle fed bitter cassava foliage as bypass protein source in
Period 1 was dramatically increased by adding 4% of brewers' grains to the diet in Period 2.

Figure 2. Growth curves of Sindhi cattle showing the change in live weight gain after introduction of 4% brewers' grains (as % of diet DM) to those fed bitter cassava foliage

Thiocyanate in urine

The excretion of thiocyanate in urine was highest in cattle fed cassava pulp-urea supplemented only with bitter cassava foliage, was reduced to half by addition of 4% brewers grains to the bitter cassava foliage diet, was present at a low level in the urine of cattle fed sweet cassava foliage, and was not detected in the urine of cattle fed cassava pulp-urea, and brewers' grains at 25% of diet DM plus rice straw (Table 3; Figure 3).

Table 2. Mean values for thiocyanate in the urine of cattle fed cassava pulp-urea and: BG-RS (brewers' grains plus rice straw); CFB (bitter cassava foliage in period 1), CFB-BG (bitter cassava foliage plus 4% brewers' grains in period 2) and CFS (sweet cassava foliage)

	BG-RS	**CFB**	**CFB-BG**	**CFS**	**SEM**	***p***
Thiocyanate, ppm	0	90[a]	55[b]	12[c]	6.06	<0.001

[abc] *Means without common superscript differ at p<0.05*

Figure 3. Secretion of thiocyanate in the urine of cattle fed cassava pulp-urea and BG-RS (brewers' grains plus rice straw), CFB (bitter cassava foliage), CFB-BG (bitter cassava foliage plus 4% brewers' grains), CFS: (sweet cassava foliage), or BG-RS (brewers' grains at 25% of diet DM plus rice straw 1% of diet DM)

Volatile fatty acids in rumen fluid

There was a tendency for the acetate: propionate ratio to be lower ($p<0.07$), when cassava pulp-urea was supplemented with brewers' grains and rice straw compared with cassava foliage (Table 3; Figure 4). There was no effect on VFA proportions when brewers' grains were included at 5% of the diet on the bitter cassava foliage treatment.

Table 3. Mean values for VFA proportions in rumen fluid from cattle fed cassava pulp-urea and: BG-RS: brewers' grains plus rice straw; CFB-BG: bitter cassava foliage plus 4% brewers' grains; CFS: sweet cassava foliage

Molar %

	BG-RS	CFB-BG	CFS	SEM	*p*
Acetic	50.4	64.0	66.5	3.19	0.24
Propionic	38.7	24.1	25.5	3.25	0.35
Butyric	10.9	11.9	7.97	1.62	0.28
Ac:Pr ratio	1.34	2.73	2.75	0.40	0.07

Figure 4. Mean values for VFA proportions in rumen fluid from cattle fed cassava pulp-urea and BG-RS (brewers' grains plus rice straw), CFB-BG (bitter cassava foliage plus 4% brewers' grains) and CFS (sweet cassava foliage)

Methane: carbon dioxide ratios

Levels of methane relative to carbon dioxide in mixed eructed gas-air decreased in the order in which the cassava pulp-urea diet was supplemented with brewers' grains, sweet cassava and bitter cassava, respectively (Table 4; Figure 5). This tendency was similar to the findings of Keopathseuth et al (2016) which showed a reduction in methane production when brewers' grains were replaced by cassava foliage (sweet variety) as source of bypass protein in cattle fed cassava pulp-urea as the basal diet. Several in vitro rumen studies have shown that methane production was reduced when leaves from bitter cassava replaced those from sweet cassava (Phuong et al 2012, 2015).

Table 4. Mean values of methane:carbon dioxide ratios in mixed eructed gas/air when Sindhi cattle were fed cassava pulp-urea and BG-RS (brewers' grains plus rice straw), CFS (sweet cassava foliage), CFB (bitter cassava foliage) or CFB-BG (bitter cassava foliage plus brewers' grains at 4% of diet)

	BG-RS	CFS	CFB	CFB-BG	SEM	*p*
$CH4:CO_2$ ratio	0.0364^{a}	0.0335^{ab}	0.0310^{b}	0.0308^{b}	0.00195	0.015

^{abc} *Means without common superscript differ at p<0.05*

Figure 5. Mean values for ratio of methane: carbondioxide in mixed eructed breath/air of cattle fed cassava pulp-urea and: BG-RS (25%brewers' grains plus rice straw), CFB (bitter cassava foliage), CFS (sweet cassava foliage) or CFB-BG (bitter cassava foliage plus 4% brewers' grains)

Discussion

Hydrogen cyanide is rapidly absorbed from the rumen and is detoxified by rhodanese in the liver by conversion to thiocyanate (Bordo and Bork 2002). Thiocyanate production from HCN is stimulated by the presence of sulphur essential for thiosulphate production an intermediate in the detoxification (Gleadow and Woodrow 2002). For this reason we provided additional sulphur to all animals and therefore the effect of brewers grains is not related to sulphur supplementation.

We have no precise explanation of the mechanism by which 'brewers' grains provides specific benefits to ruminants fed diets rich in foliage from bitter varieties of cassava. It is,however, clear that this is related to prevention, or moderation, of the toxic effects of the HCN released in the digestive tract of the animals consuming cassava foliage highin HCN precursors.

One explanation is that there are a number of processes that reflect the possible action of brewers' grains (BG) (and rice straw). Ensiled cassava pulp silage is physically without fiber. It is composed of starch and glucans which are fermented to volatile fatty acids by fermentative biofilms which require a surface to form on and develop (Leng 2014). So the first alternative is that BG provides surfaces on which biofilms develop with multiple syntrophic fermentative consortia (see Leng 2011) in order to solubilize solid particles but also to degrade soluble sugars in the Emden Meyerhof pathway of glycolysis to pyruvate and then to form VFA. Biofilm formation is critical as without the syntrophic metabolism the rate of fermentation is slowed or terminates with the production of lactic acid creating acute acidosis. Further metabolism of lactic acid is also supported by the biofilm. Biofilm formation with different characteristics are probably involved in the breakdown of HCN which is very toxic to fermentative organisms.t It is therfore assumed that biofilms with different characteristics will need to form in close association with, but separate from, the fermentative biofilms (Leng 2017). Physical fiber is also needed to maintain rumen health and prevent sub-acute acidosis (lactic acid production, providing surface areas and niches for the detoxifying microbes to develop. Rice straw also would provide habitat niches in therelatively unreactive lingo-cellulosic materials, but also silicon attached surfaces which would favor biofilm formation. The combination of rice straw and BG in this study (diet BG/RS) effectively facilitated complete degradation of HCN in the intestinal tract, as no thiocyanate was excreted in the urine of animals fed these supplements and cassava pulp. By contrast, thiocyanate was present in the urine of the cattle fed sweet cassava foliage, although in much smaller concentrations than was recorded when bitter cassava foliage was the only supplement (Figure 3).

The practical relevance of the results of this experiment, supporting the earlier observations of Phanthavong Vongsamphanh (2016, unpublished data), is the major increase in cattle growth rate (from 61 to 380 g/day) when cassava foliage from a bitter (high HCN) variety was supplemented with a small amount of brewers' grains. This finding has major economic advantages, specifically because by this procedure it becomes feasible to use bitter varieties of cassava as the source of bypass protein and fiber in diets based on cassava pulp or cassava roots. Bitter varieties have higher yields than the sweet varieties and may have other economic advantages in possessing greater resistance to air-borne diseases. The technology will also facilitate the use as livestock feed of the residual foliage that is left when cassava roots are grown for sale to the starch factory.

It is fairly certain that the specific advantages ascribed to brewers' grains will apply equally to the use of rice distillers' byproduct (Bay Sra, Hem, Quilao – as this byproduct is known in Cambodia, Vietnam and Lao PDR) and which would facilitate use of bitter cassava foliage as the bypass protein and fiber supplement replacing the foliage of the sweet variety used successfully by Sengsouly and Preston (2016).

Elucidating the mechanisms by which brewers' grains confer protection against HCN toxicity will require research beyond the scope of our laboratory. We suggest, however, that the critical area to pursue is the concept that brewers' grains (and rice distillers' byproduct) act as a site (substratum) for biofilm attachment of detoxifying microbes and as a source of nutrients for their "detoxifying activity". We launch these ideas as a concept that could contribute to the further development of biofilm science in the context of protecting the host animal against toxicity caused by consumption of potentially lethal elements such as cyanogenic glycosides and other potentially toxic compounds.

In the short term, however, a number of practical options need to be pursued:

(i) Definition of the response curve to increasing levels of brewers' grains (and RDB) over the range 0 to 5% of the diet DM (ie: levels of 0, 1, 2, 3, 4 and 5% of diet DM), in each case with ensiled the cassava pulp and ensiled cassava root as the energy source and with bitter and sweet varieties of cassava foliage.

(ii) The demonstration that rice distillers' byproduct (RDB) is equally effective as brewers' grains removes the dependency on an industrial process which experience shows is not usually supportive of the needs of small and medium scale farmers. [The advantages of scale favor sales of byproducts to a single or limited number of buyers]. However, in turn, the availability of RDB is constrained by the demand for artisan production of potable ethanol.

(iii) Both brewers' grains and RDB arise as secondary products from the production of alcohol at industrial and artisan levels, using barley and rice, respectively as the feedstock. It is logical to suppose that the beneficial properties conferred on the byproducts are related to the initial step of fermentation by yeast. There are obvious advantages from producing the RDB as part of the process of manipulating the basal resource; namely, to use the cassava pulp (or cassava root) as the substrate for the fermentation by yeast, that will mimic the essential steps in the process of artisan "ethanol" (but without the ethanol).

Conclusions

- Cattle fed bitter cassava foliage as source of bypass protein and fiber in a diet of ensiled cassava pup and urea had lower DM intake than those fed sweet cassava foliage and gained only 61 g/day compared with 383g/day for those fed sweet cassava foliage.

- When brewers' grains at 4% of the diet were added to this treatment, the DM intake increased by 47% and the live weight gain (380g/day) did not differ from the 410g/day for cattle fed sweet cassava foliage.

- Urine excretion of thiocyanate was highest in cattle fed cassava pulp-urea supplemented only with bitter cassava foliage, was reduced to half by addition of 4% brewers grains to the bitter cassava foliage diet, was present at a low level in the urine of cattle fed sweet cassava foliage, and was not detected in the urine of cattle fed cassava pulp-urea, brewers' grains and rice straw.

- There was a tendency for the acetate: propionate ratio to be lower, when cassava pulp-urea was supplemented with brewers' grains and rice straw compared with cassava foliage.

- The ratio of methane relative to carbon dioxide in mixed eructed gas-air decreased in the order of treatments in which the cassava pulp-urea diet was supplemented with brewers' grains, sweet cassava and bitter cassava, respectively.

Acknowledgements

This research is part of the requirement for the PhD of the senior author in the doctoral program of Hue University of Agriculture and Forestry, Vietnam. Financial support from the Sida-financed project, MEKARN II, is gratefully acknowledged.

References

AOAC 1990 Official methods of analysis 15th ed. AOAC, Washington, D.C

Bordo D and Bork P 2002 The rhodanese/Cdc25 phosphatase super family. EMBO reports3(8), 741-746.

Ffoulkes D and Preston T R 1978 Cassava or sweet potato forage as combined sources of protein and roughage in molasses based diets: effect of supplementation with soybean meal. Tropical Animal Production. http://www.cipav.org.co/TAP/TAP33/ 331.pdf

Fuller R 1997 Probiotics 2: Applications and practical aspects. https://books.google.com.vn/books?id=qxT9jIdK23YC&pg=PA179&lpg=PA179&dq=probiotic+as+removal+of+toxic+molecules+in+fermentation&source=bl&ots=YuSTkNJwvk&sig=e4VR64W_vzQogcy5UCxzN3G0_8c&hl=vi&sa=X&ved=0ahUKEwj3keOJ1vrQAhWMmpQKHbSkAtEQ6AEISTAF#v=onepage&q=probiotic%20as%20removal%20of%20toxic%20molecules%20in%20fermentation&f=false

Gleadow R M and Woodrow I E 2002 Mini-Review: constraints on effectiveness of cyanogenic glycosides in herbivore defense. Journal of Chemical Ecology 28 (7), 1301-1313.

Inthapanya S, Preston T R and Leng R A 2016 Ensiled brewers' grains increased feed intake, digestibility and N retention in cattle fed ensiled cassava root, urea and rice straw with fresh cassava foliage or water spinach as main source of protein. *Livestock Research for Rural Development. Volume 28, Article #20.* Retrieved January 20, 2017, from http://www.lrrd.org/lrrd28/2/sang28020.htm

Keopaseuth T, Preston T R and Tham Ho Thanh 2017 Cassava (Manihot esculenta Cranz) foliage replacing brewer's grains as protein supplement for Yellow cattle fed cassava pulp-urea and rice straw; effects on growth, feed conversion and methane emissions. Livestock Research for Rural Development. Volume 29, Article #35. http://www.lrrd.org/lrrd29/2/toum29035.html

Leng R A 2011 The rumen – a fermentation vat or a series of organized structured microbial consortia: implications for the mitigation of enteric methane production by feed additives. Livestock Research for Rural Development 23, Article #258. Available at http://www.lrrd.org/lrrd23/12/leng23258.htm

Leng R A 2014 Interactions between microbial consortia in biofilms: a paradigm shift in rumen microbial ecology and enteric methane mitigation. Animal Production Science 54, 519–543

Leng R A 2017 Rumen biofilm diversity and compartmentation with diets containing deleterious compounds Animal Production Science (In Press)

Madsen J, Bjerg B S, Hvelplund T M, Weisbjerg R and Lund P 2010 Methane and carbon dioxide ratio in excreted air for quantification of the methane production from ruminants, *Livestock Science* 129, 223–227

Minitab 2000 Minitab user's guide. Data analysis and quality tools. Release 13.1 for windows. Minitab Inc., Pennsylvania, USA.

Phanthavong V, Khamla S and Preston T R 2016a Fattening cattle in Lao PDR with cassava pulp. Livestock Research for Rural Development. Volume 28, Article #10. http://www.lrrd.org/lrrd28/1/phan28010.html

Phanthavong V, Preston T R, Viengsakoun N and Pattaya N 2016b Brewers' grain and cassava foliage (Manihot esculenta Cranz) as protein sources for local "Yellow" cattle fed cassava pulp-urea as basal diet. Livestock Research for Rural Development. Volume 28, Article #196. http://www.lrrd.org/lrrd28/11/phan28196.html

Phuong L T B, Preston T R and Leng R A 2012 Effect of foliage from "sweet" and "bitter" cassava varieties on methane production in *in vitro* incubation with molasses supplemented with potassium nitrate or urea. *Livestock Research for Rural Development. Volume 24, Article #189.* Retrieved December 12, 2016, from http://www.lrrd.org/lrrd24/10/phuo24189.htm

Phuong L T B, Khang D N and Preston T R 2015 Methane production in an in vitro fermentation of cassava pulp with urea was reduced by supplementation with leaves from bitter, as opposed to sweet, varieties of cassava. Livestock Research for Rural Development. Volume 27, Article #162. http://www.lrrd.org/lrrd27/8/phuo27162.html

Preston T R and Leng R A 1987 Matching Ruminant Production Systems with Available Resources in the Tropics and Sub-Tropics.. PENAMBUL Books Ltd, Armidale, NSW, Australia. **Internet edition 2009** http://www.cipav.org.co/PandL/Preston_Leng.htm

Promkot C and Wanapat M 2003 Ruminal degradation and intestinal digestion of crude protein of tropical protein resources using nylon bag technique and three-step in vitro procedure in dairy cattle. Livestock Research for Rural Development. Volume 15, Article #81. http://www.lrrd.org/lrrd15/11/prom1511.htm

Sengsouly P and Preston T R 2016 Effect of rice-wine distillers' byproduct and biochar on growth performance and methane emissions in local "Yellow" cattle fed ensiled cassava root, urea, cassava foliage and rice straw. Livestock Research for Rural Development. Volume 28, Article #178. http://www.lrrd.org/lrrd28/10/seng28178.html

Sriroth K, Chollakup R, Chotineeranat S, Piyachomkwan K and Oates C G 2000 Processing of cassava waste for improved biomass utilization. Bioresource.Technology 71:63–69

Received 22 March 2017; Accepted 14 April 2017; Published 1 May 2017

Go to top