HUE UNIVERSITY
UNIVERSITY OF AGRICULTURE AND FORESTRY

SANGKHOM INTHAPANYA

UTILIZATION OF LOCALLY AVAILABLE FEED RESOURCES FOR INCREASING PERFORMANCE AND REDUCING ENTERIC METHANE PRODUCTION OF LOCAL YELLOW CATTLE IN LAO PDR

DOCTOR OF PHILOSOPHY IN ANIMAL SCIENCES

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SUPERVISOR 2: PROFESSOR. DR. LE DUC NGOAN

HUE, 2019

Guarantee

I hereby guarantee that the scientific work in this thesis is mine. All the results described in this thesis are righteous and objective. They have been published in Journal of Livestock Research for Rural Development (LRRD) *http://www.lrrd.org*.

Hue University, 2019

Sangkhom, PhD student

Dedication

To my parents, my wife Vilanout Silaphet, my son, Nopphasinh Inthapanya and my daughter, Phimphisa Inthapanya.

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Abstracts

This study was aimed at utilizing of locally available feed resources for increasing performance and reducing enteric methane production of local yellow cattle in Lao PDR. There were five experiments presented in five research chapters of this thesis. Experiments I, III and IV were to study gas and methane production in an *in vitro* rumen fermentation. Experiment II was to study intake, digestibility and N balance in local yellow cattle and finally, experiment V was to study the growth rate and enteric methane production from local yellow cattle.

The main findings of the study were that (i) gas production and methane content of the gas were reduced when ensiled cassava root replaced the dried cassava root as a carbohydrate source, and when cassava leaf meal replaced water spinach meal as a protein source; (ii) Adding brewers' grains at 5% dry matter (DM) to the diet of ensiled cassava root supplemented with either cassava foliage or water spinach as the main protein source increased DM feed intake, the apparent DM digestibility and increased by 42% in nitrogen (N) retention of local yellow cattle; (iii) Total gas production was lower for fermented than ensiled cassava root but was increased by supplementation with brewers' grains and rice distillers' by-product, methane concentration in total gas production was lower for the fermented rather than the ensiled cassava root, while methane production per unit substrate DM fermented was less for the fermented compared to the ensiled cassava root and was reduced by supplementation with brewers' grains and rice distillers' by-product; (iv) Total gas production was highest for the fermented cassava root supplementation, and methane content of the gas was highest for the control treatment, while methane production per unit digested DM showed the same trend as the methane percentage in total gas; (v) and growth rate and feed conversion ratio (FCR) were improved by 40 and 20% respectively, when the diet of fermented cassava root and cassava foliage were supplemented with the rice distillers' by-product, and rice distillers' by-product supplementation increased the concentration of propionic acid in the rumen VFA and reduced by 26% the ratio of methane to carbon dioxide in the mixed eructed gas and air in the measurement chamber.

The results of this thesis implicated that rumen fermentation can be modified by the use of locally available feed resources such as cassava root and foliage, brewers' grains and rice distillers' by-product, thus mitigate methane production, and at the same time increase cattle performance.

Key words: *By-product, Cassava root, Cassava foliage, Brewers' grains, Rice distillers' by-product, Cattle performance, Methane*

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List of abbreviations, symbols and equivalents

ADF	Acid detergent fibre
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BG	Brewers' grains
CSF	Cassava foliage
CR	Cassava root
CF	Crude fiber
CH_4	Methane
CO_2	Carbon dioxide
CP	Crude protein
CT	Condensed tannins
CLM	Cassava leaf meal
ECR	Ensiled cassava root
DM	Dry matter
DAP	Di-ammonium phosphate
DCR	Dried cassava root
FCR	Feed conversion ratio
FM	Fish meal
HCN	Hydrogen cyanide
LW	Live weight
Mekarn	Mekong basin animal research network
N	Nitrogen
NDF	Neutral detergent fibre
NH_3	Ammonia
NaOH	Sodium hydroxide
NPN	None protein nitrogen
OM	Organic matter
pH	Power of/potential Hydrogen
Prob/P	Probability

RCBD	Randomized complete block design
RS	Rice straw
RDB	Rice distiller's by-product
R	Restricted
SE Asia	South East Asia
SEM	Standard error of the mean
Sida-SAREC	Swedish international development cooperation agency Department for research cooperation
U	Urea
UTR	Urea treated with straw
WS	Water spinach
Y	Yeast

INTRODUCTION

1. PROBLEM STATEMENT

Agriculture is one of the most important sectors of the Lao PDR with its contribution to 23.3% of gross domestic product (GDP). Livestock production contributes around 3.6% to national GDP and has a growth rate of 5.9% (MPI, 2017). The 9th National Congress of the Lao People's Revolutionary Party determined the key socio-economic development goals to 2015 with the vision to 2020 consisting of (i) ensuring political stability, national unity and national harmony, peace, security and social order; (ii) ensuring sustainable economic growth and create solid foundation for industrialization and modernization; (iii) eradicating poverty, achieving the millennium development goals, and preserving and developing national culture, and (iv) strengthening regional and international economic integration (NAFRI and IPSARD, 2013).

Livestock including beef cattle plays an important role in agriculture development in Lao PDR (MAF, 2015). Smallholder livestock owners in Laos traditionally kept their animals as a means of storing wealth, a source of income, meat consumption, draught power for transport, traditional culture, and provision of manure as fertilizer for cropping (DLF, 2015). Cattle are considered one of livestock to ensure food security, poverty alleviation, and commercial production in the government agenda. Cattle have become increasing valuable assets for smallholder farmers, particularly the poor due to an increased demand from regions such as northern, central and southern. Currently, it was reported that the cattle population has increased from 1.47 million in 2010 to 1.98 million in 2017 (MPI, 2017), of which approximately 98% were in the hand of smallholder farmers. This is despite efforts by the Laos government to develop commercial-scale farms, of which there were 180 commercial cattle farms in 2017. About 45% of the cattle are in the central region, 25% in the northern region, and 30% in the southern region, with this growth motivated by rapidly increasing requirements for livestock products by 4.1% annually, leading to expanded livestock production in Laos (MPI, 2017). However, the cattle production is still dominated by small-scale or backyard producers using traditional techniques with mostly indigenous breeds usually kept under free range situations; and ruminants graze on natural pastures, on the roadside, on fallow land, in paddy fields after the harvest, and in the forest, hence natural pastures represent the main source of ruminant feed in Laos (Napasith et al., 2018).

Economic benefits from cattle 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; Hristov et al., 2013; Moraes et al., 2014). According to Laos' MPI, the enteric methane production from cattle production was 87 thousand tones in 2017 (MPI, 2017). Moreover, reducing GHG emissions from ruminant livestock should therefore be a top priority since it could help to curb global warming (Sejian et al., 2010). Methane is produced as a by-product from feed fermentation in the rumen. Therefore, methane production can be manipulated by modifying rumen fermentation. Leng (1991) emphasized 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. Klieve and Ouwerkerk (2007) indicated that applying simple nutritional and management principles for example the improvement in utilisation of untreated straw by ruminants increased live weight grain and reduced methane production per unit of live weight grain (Diagarm 1). Thus, on any diets and particularly diets based on agro-industrial by-products, improving live weight gain and feed conversion efficiency leads to a significant decrease in methane production per unit of products such as meat or milk.

Cassava (*Manihot esculenta* Crantz) is an annual crop grown widely in the tropical and subtropical regions (Osakwe and Nwose, 2008; Lebot, 2009). It is currently the third most important crop in Laos, after rice and maize (Department of Agriculture, 2014). It is widely grown throughout the country by upland farmers but in small areas using local varieties and with very few inputs. Cassava has become a major crop in Lao PDR mainly because of the export of starch that is extracted from the cassava root (MAF, 2014; CIAT, 2015).

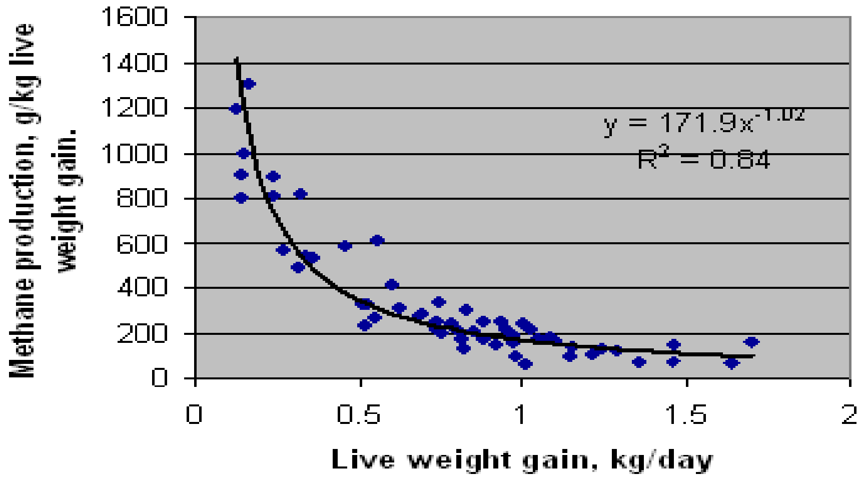

Diagram 1: Relationship between live weight gain and enteric methane production per unit live weight gain (Klieve and Ouwerkerk, 2007)

Cassava products are needed not only as a major source of income for rural households but they are also used for feed of livestock particularly cattle. The root is composed of highly digestible carbohydrate in the form of starch with little fiber (Kang et al., 2015; Polyorach et al., 2013). 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. Cassava leaves are thus considered a good source of bypass protein for ruminants (Ffoulkes and Preston, 1978; Wanapat, 2001; Keo sath et al., 2008). It has been fed successfully to improve performance of sheep (Hue et al., 2008), goats (Phengvichith and Ledin, 2007) and cattle (Wanapat et al., 2000; Thang et al., 2010) in fresh, wilted or dried form. The presence of cyanogenic glucosides in the cassava plant which are converted to hydro-cyanide (HCN) in the rumen may be a major problem but may also have positive effects as HCN appears to be involved in a reduction in methanogenesis (Phuong et al., 2015).

Water spinach (*Ipomoea aquatica*) plays an important role for farmers in rural areas; and it is easy to cultivate and has a very high yield of biomass with a short growth period (Kean Sophea and Preston, 2001). The CP content in the leaves and stems can be as high as 32 and 18% on DM basis (Ly Thi Luyen, 2003). Water spinach is widely used for human food, but at the same time this vegetable can serve as feed for all classes of livestock. It has been reported that water spinach (*Ipomoea aquatica*) supplementation of low quality diets increased the DM intake, and improved the apparent digestibility and N retention in goats (Kongmanila et al., 2007). They have been used successfully to replace part of the protein in diets based on rice by-products (Chhay Ty et al., 2005; Chittavong Malavanh et al., 2008).

Alternative local feed sources for ruminants are available from food processing factories including maize drying, jatropha extraction, brewers' grains, cassava processing, potatoes, rubber, coffee and sugar (MPI, 2017). Crop residues and agro-industrial by-products used in ruminant diets are rice straw, cassava pulp and wet brewers' grains as roughage, energy and protein sources. The most appropriate ways to improve feed resources for ruminants are through efficient utilization of crop residues, root of plants, and tree/shrub foliage (Leng, 1997). However, to optimize performance correct feeding methods need to be applied ensuring that rumen function is efficient and secondly ensuring efficient assimilation of nutrients by providing a source of bypass nutrients (Preston and Leng, 2009).

Brewers' grains are the major by-product of the brewing industry, representing around 85% of the total by-products generated (Mussato et al., 2006). It is a lignocellulosic material available in large quantities throughout the year. It is considered to be a good source of bypass protein (Promkot and Wanapat, 2003). Rice distillers' by-product is another potential source of high quality protein in rural areas of Asian countries particularly Laos and Vietnam. Rice distillers' by-product is the residue after distilling the alcohol derived by yeast fermentation of sticky rice (Taysayavong and Preston, 2010). The farmers in Vietnam also use rice distillers' by-product known as "hem". It is traditionally used it as a mixture with other feeds such as rice bran and broken rice in diets for pigs (Oosterwijk et al., 2003; Luu Huu Manh et al., 2000). The protein content of rice distillers' by-product ranges from 17 to 33% in dry matter with a well-balanced array of amino acids (Luu Huu Manh et al., 2003). The positive effects of using these by-products in cattle diets has been reported by Sengsouly et al., (2016), Phanthvong et al., (2016) and Keopaseuth and Preston, (2017). Another potential benefit of these by-products is their effect in reducing rumen methane production in goats (Vor Sina et al., 2016). Probably brewers' grain and rice distillers' by-products are acting as a "prebiotic/probiotic" that can manipulate modified activities in the rumen. Therefore, there is potential to mitigate greenhouse gas (GHG) emissions and at the same time to improve cattle performance, by utilizing locally available feed resources.

Overall, the problem statement of this research is that most cattle in Lao PDR are raised in traditional low input free grazing system, where they allowed to freely for feed all the year round based on the natural grassland or after the main crops have been harvested. The feed is limited in both quantity and quality, which severely limits productivity and increase methane production from local cattle production. By contrast, there are potential options for improving local cattle production at the same time reducing methane production by using local feed resources.

2. THE OBJECTIVES

The overall aim of this dissertation was to utilize locally available feed resources for increasing performance and reducing enteric methane production of local yellow cattle in Lao PDR.

The specific objectives were:

- To study effects of carbohydrate sources from ensiled or dried cassava roots supplemented with sources of protein from cassava leaf meal; water spinach meal and cassava leaf meal plus water spinach meal in an *in vitro* rumen fermentation on gas and methane production.
- To study effects of with or without brewers' grains and supplemented with sources of protein: cassava foliage and water spinach on feed intake,

digestibility and nitrogen (N) balance in local yellow cattle fed ensiled cassava root, urea and straw as a basal diet.

- To study effects on gas and methane production of ensiled cassava root compared with fermented cassava root and brewers' grains or rice distillers' by-product or nor supplement in an *in vitro* rumen fermentation.
- To determine effects on methane production of supplementing a basal diet of ensiled cassava root, urea and cassava leaf meal with rice distillers' by-product, fermented cassava root, and yeast (*Saccharomyces cerevisiae*) in an *in vitro* rumen fermentation.
- To evaluate the effect of rice distillers' by-product on growth performance 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.

3. THE HYPOTHESES

- The use of ensiled cassava root will reduce the methane production in an *in vitro* rumen fermentation compared to dried cassava root
- Methane production will be reduced when cassava leaves is used instead of the water spinach alone or combination of cassava leaves with water spinach is used as a protein source in an *in vitro* rumen fermentation
- The supplement of brewers' grains to the diet will improve feed intake, digestibility and N retention of local yellow cattle than without brewers' grains
- Cassava roots fermented with yeast, urea and DAP will reduce methane production than ensiled cassava root in an *in vitro* rumen fermentation
- Adding small quantity of protein source from rice distillers' by-product will reduce methane production than brewers' grains in an *in vitro* rumen fermentation
- Methane production will be reduced when a diet is supplemented with rice distillers' by-product, fermented cassava root or yeast (*Saccharomyces*

cerevisiae) in an *in vitro* rumen fermentation use the ensiled cassava root as a basal substrate

- Adding the rice distillers' by-product to a diet of fermented cassava root and fresh cassava foliage will improve the growth rate, feed conversion and reduce enteric methane production in local yellow cattle.

4. SIGNIFICANCE/INNOVATION OF THE DISSERTATION

This thesis is the output from five experiments; of which three experiments focus on gas and methane production in an *in vitro* rumen fermentation; one on feed intake; digestibility and N balance in local yellow cattle and the other one on growth performance and enteric methane production in local yellow cattle. This is the first series of studies and the first scientific information in Laos on utilizing locally available feed resources to manipulate rumen fermentation and thus to mitigate methane emissions and at the same time to improve cattle performance.

Cassava roots fermented with urea, di-ammonium phosphate and yeast (specifically *Saccharomyces cerevisiae*) was used as an energy source. The cassava foliage was used as a source of bypass protein. The presence of cyanogenic glucosides in the root and foliage, which are converted to hydro-cyanide (HCN) in the rumen was involved in a reduction in methanogenesis. Brewers' grains and rice distiller by products were a source of bypass protein and acted as a "prebiotic" providing habitat enabling the evolution of rumen microbial communities capable of detoxifying the HCN when the cassava foliage was consumed by the cattle.

Total gas and methane production *in vitro* incubation was lower for the fermented cassava root, and then for the ensiled cassava root than for the dried root. In addition, total gas and methane production was reduced when cassava leaf meal replaced water spinach meal and when supplementing with brewers' grains and rice distillers' by-product. Moreover, rice distillers' by-product supplementation in substrate increased the concentration of propionic acid in the rumen and reduced by 26% the ratio of methane to carbon dioxide in the eructed rumen gas.

Adding 5% of brewers' grains to a diet of ensiled cassava root, urea and rice straw supplemented with either cassava foliage or water spinach as a main protein source increased the apparent DM digestibility and N retention in local yellow cattle. Growth rate and feed conversion ratio in local yellow cattle were improved by 40 and 20%, respectively when a diet of fermented cassava root and cassava foliage was supplemented with 2.75% (in DM) of rice distillers' by-product.

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CHAPTER 1:
LITERATURE REVIEW

I. CATTLE PRODUCTION IN LAO PDR

Agriculture is one of the most important sectors of the Lao PDR with its contribution to 23.3% of gross domestic product (GDP). Livestock production contributes around 3.6% to national GDP and has a growth rate of 5.9% (MPI, 2017). Cattle play an important role in Lao PDR, which extends beyond the traditional supply of meat (DLF, 2014). 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 (DLF, 2015). Cattle are considered as one of livestock production to ensure food security, poverty alleviation, and commercial production in the government agenda (DLF, 2015). Cattle have become increasing valuable assets for smallholder farmers, particularly the poor due to increased demand from regions (MPI, 2017). They provide up to 50% of smallholder household annual cash income (Nampanya et al., 2013). Improving livestock productivity is an important national goal that can provide sustainable development of the economy and potentially, reduce rural poverty and food insecurity (Windsor, 2011; Nampanya et al., 2010; Khounsy and Conlan, 2008).

1.1 CATTLE POPULATION AND BEEF CONSUMPTION

1.1.1 Cattle population

Cattle have become increasing valuable assets for smallholder farmers, particularly the poor due to increased demand from regions. According to MPI (2017) cattle population has grown by an average 5% per year since 2010 (1.47 million in 2010 to 1.98 million heads in 2017), with smallholder farmers holding approximately 98% of the total cattle (MPI, 2017). This is owned despite efforts by the Laos government to develop commercial-scale farms, of which there were 180 commercial cattle farms with total of cattle 26,600 heads in 2017. About 45% of the cattle in the central region, 25% in the northern region, and 30% in the southern region, with this growth are motivated by rapidly increasing requirements for livestock products by 4.1% annually, leading to expanded livestock production in Laos (MPI, 2017) (Figures 1 and 2).

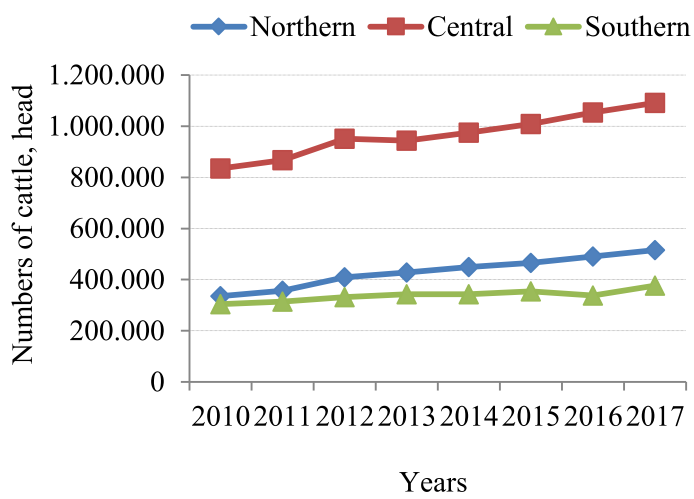

Figure 1: Cattle distribution in regions in Lao PDR

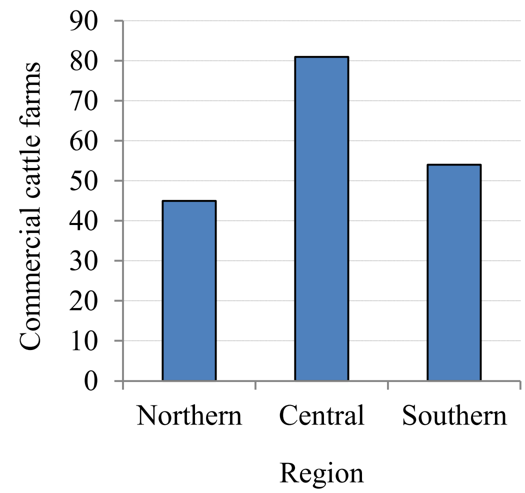

Figure 2: Commercial cattle farms in 2017

1.1.2 Cattle beef consumption

The beef production has tended to increase, with consumption at 4.1 kg/capita/year in 2017 (FAOSTAT, 2017). The growth in cattle production is mainly to meet the increasing domestic demand for breef. Recently, there has been a trend towards higher quality food and food diversity, mainly because of the growing economy and the open market systems (DLF, 2017). Mostly, beef consumption in Laos is almost 100% of the total meat production of the country, whereas high quality beef needs to be imported for restaurants and supermarkets in Vientiane, the Laos capital. The average price of cattle meat has increased more than double from 4.09 US$ per kilogram in 2009 to 8.05 US$ per kilogram, with a gross national income per capital of $ 2,150 in 2017 (World Bank, 2018).

The Laos government proposes to improve beef production by increasing the live weight of the Lao-local cattle and by introducing exotic breeds. Recently, cattle farming in Laos has been established with the aim of providing high quality meat. Whilst the supply of cattle meat can fulfil domestic needs, the country relies on imports to provide breeding animals to household producers. In addition, export of livestock products from Laos is very limited and the export value of meat is low and has fluctuated over the years (MPI, 2017).

1.2 POTENTIALITIES, OPPORTUNITIES,WEAKNESSES AND CHALLENGES FOR CATTLE PRODUCTION IN LAO PDR

1.2.1 Potentialities

Cattle are considered as one of livestock production to ensure food security and income generation. The cattle management practices in the smallholder systems are dominated by small-scale or backyard producers that use traditional practices (DLF, 2014), there are 60% of cattle usually kept under free range grazing systems, which rely more on natural feed resources that available in the areas (Lao policy, 2016). Laos has 654,500 ha of grazing lands and 1.14 million ha of forest areas (MAF, 2017). The grazing land areas in the uplands may be suitable for extensive cattle production systems due to vast areas of grazing resources. In contrast, grazing land areas in the lowlands may be suitable for semi-intensive and intensive cattle production because they were also able to access to input services and by-product crops which were used for supplementary feed (MAF, 2017).

1.2.2 Opportunities

Cattle are among the most demanded agricultural trade commodities in Laos. The significant increase in demand for cattle in both domestic and international markets (particularly Vietnam, and recently China) has presented both trade opportunities for cattle smallholder farmers in Laos (DLF, 2013). The total domestic supply value approximately 75% of cattle produced are consumed domestically, with the remaining 25% (more than 100,000 heads per year trans-boundary movement) exported to neighboring countries, mainly to Vietnam and China. The free trade agreements are bringing down barriers to the flow of goods and services within the regions (DLF, 2014).

Cattle have been recently selected as one of the seven national prioritized, trade agricultural products (MAF, 2014). Agriculture, farmers and rural sector of Lao PDR plays a very important role to achieve the goals set by the 9th Party Congress (NAFRI&IPSARD, 2013). Cattle related development strategies and policies were derived from the

Agriculture Development Strategy 2025 with Vision 2030 (ADS) developed by Ministry of Agriculture and Forestry in Lao PDR. In an urgent response to the prioritized work the Department of Livestock and Fisheries (DLF) has been recently drafting the National Commercialized Livestock and Aquaculture Development Policies aiming at stimulating the growth of livestock production. There are 8 major sub-policies including: (i) Policy for promoting land use for livestock and aquaculture husbandry; (ii) Labour policy; (iii) Finance and bank policy; (iv) Energy policy; (v) Policy for promoting processing; (vi) Policy for commercialization and commodity price stabilization; (vii) Policy for livestock and aquaculture value chain entrepreneurs; and (viii) Policy for human resource development on veterinary and fisheries sub-sectors.

1.2.3 Weaknesses

Despite increasing demand and trading opportunities, cattle production in Laos is still underdeveloped (DLF, 2013), in which the problems include: (i) feed management in terms of nutritional deficiencies, especially in the long dry season (from November to April). The poor animal nutrition results in low reproductivity performance, calving rate around 41-52%, calving interval at 19-21 months, time to reach slaughter weight for male 5-8years and there are low carcass weight of 65-84kg (DLF, 2015), (ii) famers' limited knowledge on beef husbandry practice and beef farming system management, (iii) endemic diseases (FMD, HS and internal parasites) occur every year but insufficient veterinary services (calf mortality 20-30%, adult mortality 10% and currently, the vaccination coverage of small scale producers is around 30%), (iv) famer group not yet strong-not be able to negotiate or access to government policies efficiently, (v) weak cooperation between government-private sector-researchers-banks in terms of cattle development and (vi) market for live cattle is poorly organized (transfer find it difficult to source enough cattle to meet export demand, and those available sometimes do not meet quality demanded by the import countries.

1.2.4 Challenges

The Lao Government and international donors have been recognizing the importance and potentials for developing the smallholder cattle industry through the improvement of cattle productivity (MAF, 2014). Due to various major constraints in cattle production systems, the government with the assistance of donor funds has focused on building the capacity of extension services, disease surveillance and monitoring procedures, and the improvement of animal husbandry by using forage interventions. Their assistance has also been made available to support the government agencies that are responsible for disease diagnoses and vaccine production. Therefore, Lao government has strategies of the year 2025 for cattle production: (i) Transforming agency from smallholder subsistence production to commercial production systems, accompanied by the development of agri-businesses is a primary focus of the government and (ii) growth in the beef cattle subsector is a significant priority of the goal, cattle is a one of nine priority commodities list, which seeks to increase supply for domestic markets and for export to neighbouring countries such as Vietnam and China, it is so-calls “Livestock revolution”. Therefore, the vision of cattle sub-sector to the year 2030 aims at” Cattle sub-sector contributes significantly to economic growth and food and nutrition security while ensuring sustainable use of Lao’s national resources”.

II. UTILIZATION OF LOCAL FEED RESOURCES FOR CATTLE PRODUCTION

Natural forages are an important part of the diet of cattle, but their nutrient value, particularly in terms of protein and fiber source may be inadequate to feeding cattle particulary nutriments needed for life and production. Protein and energy are often the major focus of cattle nutritional programs.

2.1 PROTEIN AND CARBOHYDRATE/FIBER FEED FOR CATTLE

2.1.1 Dietary protein

Protein plays a crucial role in virtually all biological processes such as enzymatic catalysis, transport, storage, motion, mechanical support, immunology, as well as in the control of metabolism. Understanding the mechanism behind protein utilization in animals for maintenance and production is critical for raising animals efficiently (Deutsch and Smith, 1957). Dietary proteins that reach the small intestine of ruminants consist of two protein fractions: microbial and protein un-degradable at the rumen level. Microbial protein is produced by the action of the rumen flora, which breaks down the dietary protein to peptides, amino acids and ammonia, after which these materials are used for the synthesis of own proteins (Ružić-Muslić, 2006). Ruminants are capable of utilizing different protein sources due to their stomach physiology. Feeding management for ruminants has been one of the major concerns in livestock production.

Feeding ruminants with protein that are resistant to microbial degradation in the rumen can provide a practical way to increase dietary protein and alter the amino acid profile of the protein reaching the small intestine for digestion and absorption. However, the effects of feeding high rumen un-degradable proteins (RUP) on intestinal amino acid supply and animal performance have been inconsistent. Lack of response has been attributed to various factors including depression in ruminal microbial protein synthesis and factors related to quality of the dietary protein such as inadequate or overprotection of protein, reduced intestinal availability of amino acid or inherent amino acid limitations of the dietary protein. A considerable amount of vaiation among and within feeds in ruminal degradation and intestinal digestion of protein has been reported by Calsamiglia and Stern, (1995); Howie et al., (1996); Yoon et al., (1996). It is important that this variation is considered when determining the value of feeds as sources of protein for the ruminant animal.

2.1.2 Dietary carbohydrates

Carbohydrates are an essential component of the diet of all animals. In ruminant diets, there are two groups of carbohydrates, the first being the available carbohydrates which are virtually completely utilized not only by ruminants but all animals. The second or less readily available carbohydrates are not used to any extent by simple-stomached animals but can be used significantly by ruminants. The extent to which these carbohydrates are used depend on a number of factors such as the botanical age of the plant, the type of plant, the species of ruminant and the method of processing the plant. The available carbohydrates include the simple sugars, glucose, fructose and sucrose as well as the plant storage polysaccharides. Most of starch is widespread in terms of these and its structure and degradation in the rumen. Other storage poly-saccharides, such as the fructans and the raffinose series particularly since frutans are the major storage polysaccharide of grasses.

Carbohydrates and fats are commonly perceived as having a primary role in the diet as suppliers of energy; however, these feed fractions are not uniform in terms of their value as energy sources. The diverse fractions of carbohydrates and fats have potential to provide specific nutrients that support both microbial and animal needs in ruminants. Carbohydrates are a diverse group that can be roughly classified by digestibility in the small intestine and ferment-ability by microbes. The contribution of dietary carbohydrates to the energy or nutrient supplies of the animal is affected by location and extent of digestion, which alter types and amounts of products made available to the animal. Carbohydrates digested in the small intestine provide mono-saccharides, notably glucose from starch. Ruminally, products of fermentation are much more diverse and come in the forms of gases, organic acids, and microbial mass. Types of fermentation acids produced include acetate, propionate, butyrate, valerate, and lactate. Molar proportions of acids produced vary to some degree with carbohydrate type, with acetate predominating with pectins (Strobel and Russell, 1986) and butyrate with sucrose and lactose (DeFrain et al., 2004). Even carbon organic acids (acetate, butyrate) are lipogenic, and carbon organic acids (propionate, lactate, and valerate) are glucogenic.

2.1.3 Dietary fiber

Ruminant can use large amounts of forage with high fiber content. Fiber can be defined as carbohydrates not digested by mammalian enzymes but digestible by rumen microorganisms and lignin. Therefore, fiber includes cellulose, hemicellulose, lignin, and soluble fiber (fructans, pectans, galactans, and beta-glucans). Most fiber in plant material is found in the structural components of cell walls.

In the rumen, fiber-digesting bacteria digest structural carbohydrates, while starch-digesting bacteria digest nonstructural carbohydrates. In general, the starch digesters tolerate low pH levels, but the fiber digesters are inhibited by low pH. If the goal is to maximize forage intake and digestibility, it may be counterproductive to add grain (corn, wheat, etc.) to the diet beyond a threshold of about 0.5% of body weight daily because of reduced rumen pH effects. A supplement with low levels of starch and highly digestible fiber (soybean hulls, corn gluten feed, dried distillers grains) is more appropriate to maintain forage intake, digestibility, and rumen pH. Rumen pH can also be kept from dropping too low by buffers secreted in the animal's saliva. Salivary flow is primarily stimulated during rumination (cud chewing) by effective fiber. Cattle diets deficient in fiber can cause permanent damage to the rumen wall. The effectiveness of fiber for supporting rumen health is positively related to particle size of feeds containing the fiber and is referred to as effective fiber. A high level of fiber in the diet does not always indicate that the diet is adequate in effective fiber. If the fiber is chopped or ground too short or fine it may not be effective in promoting rumen health. A good example of this is soybean hull pellets. Soybean hulls are high in digestible fiber levels and yet have a small particle size and are relatively low in effective fiber levels. Studies show that effective fiber supplementation improves the performance of cattle fed soybean hull pellets. Therefore, soybean hull pellets should not be used as an exclusive fiber source to replace hay. Finely ground fiber will pass through the digestive system rapidly and will not meet the effective fiber requirements of cattle.

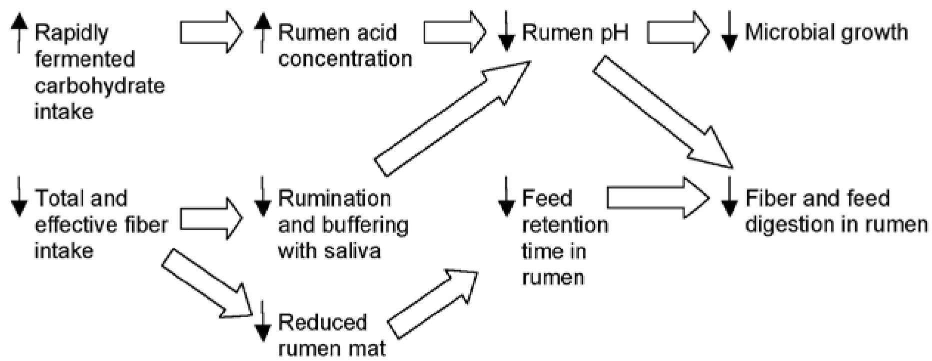

Figure 3: Rumen changes in response to decreased fiber intake

2.2 THE USE OF LOCAL FEED RESOURCES FOR CATTLE PRODUCTION IN LAO PDR

The main reason for poor performance of livestock in Laos is seasonal scarcity and qualitative fluctuations in feed. This impairs growth and reproduction of animals and results in an increased morbidity and mortality rate in animals, particularly during the dry season. However, provision of feeds with optimum quality, thus optimizing animal growth, has not been the primary objective for smallholder livestock producers and instead profit maximization has been prioritized. Many feed resources which could improve livestock production continue to be unused, undeveloped or poorly utilized. For example, locally available feeds or plant resources would minimize the production costs and therefore improve the profitability of livestock production (Wanapat, 2009).

2.2.1 Cassava by-products

2.2.1.1 Production

In Laos, cassava *(Manihot esculenta* Crantz*)* known as 'Man Ton' is one of the main food crops for smallholder farmers in remote upland areas, and it is currently the second most important crop after rice. There are 75,810 hectare of the total land for planting cassava in Laos (Soukkhamthat et al., 2016). The production of cassava increased from 51,300 tones in 2005 to 3,095,864 tones in 2016 (MPI, 2016). Recently, cassava has become an important cash crop for either domestic use or for export because it can be used for food and livestock feed as well as for industrial processing into starch, sweeteners and ethanol. According to MAF, (2014), cassava farm sizes increased from 0.1 to 0.4 hectare, while their productivity increased from 17.47 tones per hectare in 1999 to 25.08 tones per hectare in 2011 (MAF, 2015). There are 05 cassava starch factories with total planted area of 60,475 hectare, giving an average yield of fresh roots of 27 tones/hectare and annual production is of the order of 1.6 million tones (55,000 tones of cassava pulp) (Department of Agriculture, 2015; MAF, 2016). However, after starch extraction, the remaining is by-product known as cassava pulp, represents from 10-15% of the original weight of fresh roots.

2.2.1.2 Processing

Cassava (*Manihot esculenta* Crantz*)* varieties are often categorized as either as sweet or bitter, signifying the absence or presence of toxic levels of cyanogenic glucosides (HCN). However, it is known that the capacity to liberate HCN from cassava roots or foliage is reduced by processing such as sun drying or ensiling/fermentation (Wanapat, 2001).

Cassava in fresh form contains cyanide; it is highly perishable and cannot be stored for more than a few days after harvesting. Cyanide is extremely toxic to humans and animals, therefore the roots need to be processed to reduce the cyanide content to safe levels before consumption (Eggleston et al., 1992; Ihekoronye et al., 1985). Dehydrated chips are unfermented dried products of cassava. Drying is widely practised to improve the shelf life of the tuber and also helps in the process of detoxification (Irinkoyenikan et al., 2008).

Drying of cassava is widely practised to reduce post-harvest losses. The roots, after peeling and washing, are chipped into smaller sizes for faster drying. Drying is done naturally in the sun or artificially in the oven at controlled temperatures. Chips are mostly used in the production of starch and animal feeds even though it has potentials for human consumption (Irinkoyenikan et al., 2008; Oluwole et al., 2004). The other hand, sun drying or ensiling also reduce cyanide content in cassava roots or foliage which are safer for animals (Man and Wiktorsson, 2002).

Fermentation of cassava and its by-products, an alternative process has to be made between two popular fermentation techniques, namely: the liquid substrate or submerged fermentation technique and the solid substrate fermentation. Balagopalan et al., (2002) indicated the submerged fermentation technique as the one in which water is always in a free state while food nutrients in the form of carbon, nitrogen, phosphorus and others are in a suspended or dissolved state.

The main limiting factor to the use of cassava leaves as animal feed is the presence of cyanogenic glucosides, which is 10 times higher in the leaves than in the roots and have been used as a source of linamarin for standardization of methods (Haque and Bradbury, 2004). Therefore, leaves must be processed carefully to reduce cyanide (Gomez and Valdivieso, 1985), when the plant tissue is broken down by processing or during ingestion by animals. The cyanide levels in leaves are influenced by genetic, physiological, edaphic and climatic differences with the stage of maturity being perhaps the major source of variation (Ravindran, 1995). Many researches have demonstrated that the hydro-cyanide (HCN) content in leaves can be reduced by sun-drying (Bui Van Chinh and Le Viet Ly, 2001) and ensiling (Ly and Rodríguez, 2001).

2.2.1.3 Nutritive values

Cassava roots have high levels of energy (75 to 85% of soluble carbohydrate) and minimal levels of crude protein (2 to 3% CP); they have been used as a source of readily fermentable energy (Kang et al., 2015; Polyorach et al., 2013; Wanapat et al., 2013), while the top growth could be harvested at four months initially and at 2-3 months intervals subsequently. Cassava foliage is a good source of bypass protein for ruminants (Keo Sath et al., 2008; Promkot and Wanapat, 2003). It has been fed successfully to improve performance of sheep (Hue et al., 2008), goats (Do et al., 2002; Phengvichith and Ledin, 2007) and cattle (Wanapat et al., 2000; Thang et al., 2010) in fresh, wilted or dried form. Cassava leaves are known to contain variable levels of condensed tannins; It is about 3% in dry matter (DM) according to Netpana et al., (2001) and Bui Phan Thu Hang and Ledin, (2005).

The composition of cassava can be affected by factors such as growing conditions, maturity, variety, rootstock and climate; nutrient content of cassava by-product feeds is also influenced by variety as well as processing or handling. The chemical composition of various cassava fraction, including root and leaves (fresh, ensiled and dried), used in livestock feeding is presented in Table 1.

Table 1: Nutritional composition of cassava by-products used in livestock feeding programs

Items	Fresh cassava root	Cassava peel	Cassava root ships	Ensiled cassava root	Dried cassava root	Fresh cassava foliage	Sun-dried cassava foliage	Dried cassava leaves
DM, %	21.4	28.5	82.4	31.7	90	32.1	53.4	88.7
CP, %	2.28	4.2	2.81	2.7	23	22.2	26.6	22.5
CF, %	3.7*	12.7	-	3.5*	3.1*	17.7**	17.9**	56.8*
NDF, %	7.8*	48.1	-	34.3*	8**	48.7	49.9*	32.9
ADF, %	5.3*	15.2	-	5.3	5.4**	34.4	40.7*	24.4
Ash, %	2.8*	8.7	2.23	6.4	2.3*	4.48	5.7	0.64*
EE, %	0.67	1.4	-	-	2.1*	31.4	8.3*	3.29*
Soluble N, %	-	63.7*	-	10.8	25.2	31.1*	27.6	27.4
Lignin, %	1.6*	7.2*	-	-	1.7**	9.4**	8.4*	1.92
NFE, %	-	68.3*	-	-	59.2**	-	42.9**	41.8**
NFC, %	-	-	-	-	82**	-	39.9**	-
Starch, %	80.8*	80.2*	-	-	80.4**	-	-	-
ADF: acid detergent fibre;CF: crude fiber; CP: crude protein; DM: dry matter; EE: ether extract; NDF: neutral-detergent fibre; NFC: none-fiber carbohydrate; NFE: none-fiber energy								
Sources	*Phanthavong et al (2015);Heuzé & Tran (2012)**	*Dayal et al (2018); Oladunjoye et al (2014)**	*Silivong et al (2018)*	*Sengsouly et al (2016); Vanhnasin et al (2016)**	*Sengsouly et al (2016);Hang Du Thanh et al (2009)*; Heuzé &Tran (2012)***	*Silivong et al (2018); Phuong et al (2012)*; Heuzé&Tran (2012)***	*Porsavathdy et al (2017); Joomjantha et al (2008)*; Heuzé&Tran (2012)***	*Sengsouly et al (2016); Phanthavong et al (2015)*;Heuzé &Tran (2012)***

Nitrogen digestibility of cassava foliage is higher than their tubers. In contrast, the organic matter and energy digestibility was higher for tubers (dehydrated, fresh and peeled fresh) than different forms of cassava foliage (Table 2).

Table 2: Protein and energy digestibility of cassava by-products used in livestock feeding programs

Cassava fraction	Foliage dehydrate	Foliage fresh	Foliage ensiled	Peel dry	Peel fresh	Roots dehydrate	Roots fresh	Roots peel fresh
Ruminant nutritive values								
OM digest, %	68.2	63.9	62.7	56.1*	-	88.8	89.1	93.7
Energy digest, %	66.8	62.6	61.5	-	-	84.5	85	89.2
DE rumen, MJ/kg	13.2	12.4	12.3	9.5*	-	14.2	14.5	14.9
ME rumen, MJ/kg DM	10.4	9.9	9.8	8.3*	-	12.2	12.4	12.8
N digest, %	75	72.3	74	-	59.7	35.3	31.4	33.8

DE: digestible energy; ME: metabolizable energy OM: organic matter; N: nitrogen

The secondary metabolite concentrations (cyanide) indicated that the varieties of cassava cultivars were higher for cassava leaf and peels in fresh form than leaf, peels and tubers in dried or fermented form. Dry cassava leaves were higher in condensed tannin than dry peels and tuber (Table 3).

Table 3: Secondary metabolite concentrations reported in various cassava cultivars

Items	Cyanide mg/kg	Condensed tannins, %	Tannins (tannic acid), g/kg	Nitrate mg/10	Oxalate g/100 g	Phytate mg/100 g
Leaf meal, dry	16–1934	1–5	0.1	43–89	1.4–2.9	-
Leaf, fresh	2650–7200	-	26–156	-	33	705–1044
Peels, dry	33–1081	4	-	-	-	-
Peels, fresh	1300–2250	0.6	2–4	-	-	-
Peels, fermented	6–23	-	-	-	-	-
Tuber, dry	4–173	0.1	-	-	-	-
Tuber, fresh	233–1150	-	-	-	-	62,400

Source: *Heuzé and Tran (2012)*

2.2.1.4 Effects of feeding cassava by-products on cattle performance and methane emission

An additional advantage of cassava over cereal crops such as maize is that the foliage is a valuable source of bypass protein and the root is composed of highly digestible carbohydrate in the form of starch with little fiber as source of energy. The foliage is rich in protein which, allied with low levels of tannin (Bui Phan Thu Hang and Ledin, 2005; Netpana et al., 2001) 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 (Barry, 1999). The presence of cyanogenic glucosides which are converted to hydro-cyanide (HCN) in the rumen is a major problem but appears to be involved in a reduction in methanogenesis (Phuong et al., 2015).

Recently researches showed that cassava by-products have been fed successfully to improve performance and reduce methane emission in the cattle. Phanthavong et al., (2016) showed that the potential of the ensiled cassava pulp as a basis of an intensive fattening system supplemented with urea, fresh brewers' grains and rice straw, supported growth rates of over 600g/day with a DM feed conversion ratio of 6.67 in local yellow cattle. Other researchers reported that supplementation to a basal diet of ensiled cassava root-urea and fresh cassava foliage with rice distiller by-product with or without biochar improved the growth rate of local cattle by 37% and reduced methane emission by 36% (Sengsouly et al., 2016). Other results showed that live weight gain was increased by 25% by when adding biochar to the diet (in DM) and tended to be decreased when nitrate replaced urea as the source of NPN. Both biochar and nitrate reduced methane production by 22 and 29% respectively, when local yellow cattle were fed a basal diet with cassava root chips (Leng et al., 2012). However, the foliage of cassava has been shown to be an effective source of bypass protein for fattening cattle (Keo Sath et al., 2008; Wanapat et al., 1997; Ffoulkes and Preston, 1978). It is thus a local forage resource to provide additional protein from cassava foliage to diets rich in carbohydrate but low in protein. Cassava leaves are known to contain variable levels of condensed tannins (CT) about 3%

in DM according to Netpana et al., (2001) and Bui Phan Thu Hang and Ledin, (2005). Condensed tannins are also reported to decrease methane production and increase the efficiency of microbial protein synthesis (Makkar et al., 1995; Grainger et al., 2009). Reductions of methane production of 13-16% have been reported by Grainger et al., (2009); Carulla et al., (2005) and Waghorn et al., (2002) apparently through a direct toxic effect on methanogens.

2.2.2 Brewers' grains

2.2.2.1 Production

Brewers' grains are the solid residue left after the processing of germinated and dried cereal grains (malt) for the production of beer and other malt products (malt extracts and malt vinegar). In general, barley is most used for beer production, but brewing beer is also made from wheat, maize, rice, sorghum and millet (Mussatto et al 2006). In Laos, the beer production mostly used stricky rice, which obtained from local rice producers. The waste from beer brewering is brewer grains "Khi beer", with ingredients of beer brewing is malted barley, hops, yeast and water (LBC, 2017). The annual beer production capacity is 160 million litters. The by-product of brewers' grains is produced annually 96,000-150,000 tons/year (personal communication). The brewers' grains waste brewery by-products are prospected to use as animal feeds, mainly for cattle and pigs (Crawshaw, 2004; Ojowi et al., 1997; Preston et al., 1973).

2.2.2.2 Nutritive value

Brewers' grains are used to feed ruminant and monogastric animals. They are palatable and readily consumed when in good condition. Brewers' grains are also relatively rich in protein 25.9%, providing mineral array and high value of fiber with ADF of 17.2-24.8% DM (Heuzé et al., 2017). BG is suitable by-products for ruminant diets and also feeding to pigs and poultry in fresh or wet form. BG is a bulky feed with low energy content, which can limit their use.

Table 4: Nutritional table of fresh brewers' grains

Main analysis	Unit	Mean	Minimum	Maximum
Dry matter	% as fed	24.9	21.7	28.9
Crude protein	% DM	25.9	20.3	30.6
Crude fibre	% DM	16.4	7.8	21.2
NDF	% DM	49.6	34.3	62.5
ADF	% DM	20.8	17.2	24.8
Lignin	% DM	5.7	3.5	8.0
Ether extract	% DM	7.0	5.8	9.3
Ash	% DM	4.1	2.7	4.9
Starch (enzymatic)	% DM	5.7	3.3	9.6
Total sugars	% DM	1.0	0.7	1.3
Gross energy	MJ/kg DM	20.3	20.3	21.8

ADF: acid detergent fibre; NDF: neutral-detergent fibre

Sources: *Heuzé et al., (2017)*

2.2.3 Rice distillers' by-product

2.2.3.1 Production

Rice distillers' by-products is residuce stuffs after producing the alcohol, which was made from polished sticky rice. In rural smallholder, the rice distillers could be fed to livestock especially fed to fattening pigs (Oosterwijk and Vongthilath, 2003). The steps of processing to make alcohol are based on the procedure that firstly weigh 25kg of the sticky rice and put into the container, then soak it with clean water for 1 night. Secondly, steam the rice for 1:30 hours and then, the yeast is added 40g/jar (9kg/jar) and mixed together with the sticky rice. Thirdly, after harmonized, it is stored for 3days, then add water (8 liters/jar) and ferment it for 4-7days. Fourthly, distilling the alcohol by putting the fermented stuffs into large pot and boil, alcohol is evaporated and collected. About 19-21 liters of wine and rice distiller's residue around 52kg wet form are proced from 25kg of sticky rice (Taysayavong and Preston, 2010).

2.2.3.2 Nutritive value

The rice distillers' by-products is palatable and has a fairly high protein content 23% crude protein in dry matter of good quality with approximately 3.9g lysine/16g N. In addition it is a good source of B-vitamins (Manh Huu Luu et al., 2009). There are similar contained of CP ranging at 22.5-28.2% in DM of rice distillers' by-products (Manivanh et al., 2012; Taysayavong and Preston, 2010). Rice distillers' byproducts is potential rich in yeast and yeast cell walls are reported to contain 7.7% β-glucan (Waszkiewicz-Robak, 2013).

Table 5: Chemical composition and gross energy of rice distilles' by-products (% DM)

References	DM, %	CP	EE	Ash	CF	Ca	P	GE,MJ/Kg	pH
Luu Huu Manh et al., 2003	7.96	22.9	8.37	3.69	14.6	0.49	0.47	-	-
Luu Huu Manh et al., 2009	9.1	23.1	9.9	4.7	-	0.55	0.35	20	3.2
Taysayavong and Preston, 2010	14.6	28.2	-	1.97	2.3	-	-	-	-
Manivanh et al., 2012	7.8	24.9	-	1.4	-	-	-	-	-
Sivilai et al., 2017	7.9	22.5	-	13.5	3.6	-	-	-	3.4

Ca: calcium; CF: crude fiber; CP: crude protein; DM: dry matter; EE: ether extract;GE:gross energy; P:phosphate

2.2.3.3 Effects of feeding brewers' grains and rice distillers' by-products on cattle performance and methane emission

Sensouly et al., (2016) presented that supplementation of a basal diet of ensiled cassava root-urea and fresh cassava foliage with rice wine by-product improved the growth rate of local yellow cattle by 37% feed conversion by 21% and decreased the CH_4 emissions by 36%. Those authors reported that the potential source of protein for cattle fattening in Lao PDR as brewers' grians or rice distiller's by-product has been fed successfully to improve performance of local yellow cattle. Promoting beef production from the local cattle is one of the development activities ear-marked by the Ministry of Agriculture and Forestry (MAF, 2015) in Lao PDR. Emphasis is on technological upgrading and innovation to develop intensive fattening systems using the locally available feed resources, especially the brewers' grains or rice distiller by-products from the agro-industry.

III. METHANE PRODUCTION AND MITIGATION STRATEGIES

3.1 GREEHOUSE GAS PRODUCTION AND EMISSION FROM CATTLE PRODUCTION

3.1.1 Contribution of livestock production to global greenhouse gas emissions

Livestock plays an important role in global food production and in agricultural and rural economies in many developing regions, while the livestock sector is one of the fastest growing subsectors of agriculture; it is also an important contributor to anthropogenic greenhouse gas emissions. Enteric CH_4 production from ruminant livestock accounts for 17-37% of global anthropogenic CH_4 (Knapp et al., 2014; Cottle et al., 2011). With regard to CH_4, the global livestock sector is responsible for 37% of all human-induced CH_4 emissions, with 89% of these livestock-derived emissions arising from enteric fermentation (Steinfeld and Wassenaar, 2007). The importance of feeding the growing population while minimizing environmental impacts of livestock production has been given significant attention over the last decade (Eisler et al., 2014; Golub et al., 2012; Steinfeld et al., 2006). Cattle are considered to cause an increase in emissions with about 4.6Gt (gigatonnes) of CO_2, representing 65% of sector emissions. Average emission intensities are 2.8kg of CO_2 per kg of fat and 46.2 kg of CO_2 per kg of carcass weight for beef (Gerber et al., 2013).

3.1.2 Greenhouse gas production and emission from cattle production

3.1.2.1 Enteric fermentation

Ruminant animals particularly cattle, buffalo, sheep, goat and camels produce significant amount of CH_4 under the anaerobic conditions of the digestive processes (ASSAN, 2015; Sejian et al., 2011). Enteric CH_4 generated in the gastrointestinal tract of ruminants represents the greatest direct greenhouse gas released from the livestock sector and the single largest source of anthropogenic CH_4 at a global level (EPA, 2012). Enteric CH_4 production by cattle is one of the major sources of greenhouse gas emissions in the livestock sector. About 75% of total CH_4 emissions from livestock come from cattle and this is expected to increase in the next decades, especially in developing countries (Tubiello, 2013).

Abaout 87% enteric CH_4 is produced in the rumen, the remainder being released from fermentation in the large intestine (Lascano and Cárdenas, 2010). Although many factors influence CH_4 emissions from ruminants, the 03 major determinants are level of feed intake, type of carbohydrate fed, and manipulation of rumen microflora (Johnson and Johnson, 1995). In rumen function, the network of microbes act on feed particles to degrade plant polysaccharide and produce volatile fatty acids (VFAs, mainly acetate, propionate and butyrate) and gases (CO_2 and H_2) as main end products. The activity of hydrogen-utilizing methanogens in rumen reduces the end product inhibition of hydrogen, thereby allowing more rapid fermentation of feed. Even a small amount of hydrogen in rumen can limit the oxidation of sugar, VFAs conversion and hydrogenase activity, if alternative pathways for disposal are absent (McAllister and Newbold, 2008). Two methods utilized for disposal of reducing equivalents are the production of more highly reduced VFAs and hydrogen by membranebound hydrogenases. However, these hydrogenases have an acute sensitivity to an increased partial pressure of hydrogen (Russell, 1998).

Methane from ruminants is produced when feed macromolecules are fermented by microorganisms in the gastro-intestinal tract (GIT). The catabolism yields volatile fatty acids (VFAs), CO_2, ammonia (NH_3), H_2 and heat. Volatile fatty acid and NH_3 are absorbed via the rumen wall, where CO_2 is both absorbed and eructed (Preston and Leng, 1987). Methane production is the last step of the fermentation process and is carried out by methanogenic archaea (methanogens), which in the rumen predominately utilize H_2 as an energy source to reduce CO_2 to CH_4. Cattle produce about 150 to 420litres of CH_4 per day (107-300g CH_4/day) and sheep about 25-55litres per day (18-39 g CH_4/day), depending on intake (McAllister et al., 1996). Methane production from ruminant digestion not only contributes to the global greenhouse effect, but it also represents a substantial waste of feed energy as a percentage of gross energy (GE) consumed by ruminants during anaerobic fermentation in the rumen represents 2-12% gross energy loss and 15% of the total atmospheric CH_4 flux (Mahesh et al., 2013; Zhi-Hua et al., 2012). The loss of dietary energy in the form of methane has been extensively researched and reviewed (Cottle et al., 2011). Microorganisms called methanogens produce CH_4 (methanogenesis) in the digestive tract as a by-product of anaerobic fermentation. Briefly, the process of methanogenesis (Moss et al., 2000) consists of:

1. Glucose equivalents from plant polymers or starch (cellulose, hemicellulose, pectin, starch, sucrose, fructans and pentosans) are hydrolysed by extracellular microbial enzymes to form pyruvate in the presence of protozoa and fungi in the digestive tract:

$$\text{Glucose} \rightarrow 2\ \text{pyruvate} + 4H$$

2. The fermentation of pyruvate involves oxidation reactions under anaerobic conditions producing reduced co-factors such as NADH. Reduced co-factors such as NADH are then re-oxidised to NAD to complete the synthesis of volatile fatty acids (VFAs) with the main products being acetate, butyrate and propionate (anions of acetic, butyric and propionic VFAs):

$$\text{Pyruvate} + H_2O \rightarrow \text{acetate (C2)} + CO_2 + 2H$$

$$2C2 + 4H \rightarrow \text{butyrate (C4)} + 2H_2O$$

$$\text{Pyruvate} + 4H \rightarrow \text{propionate (C3)} + H_2O$$

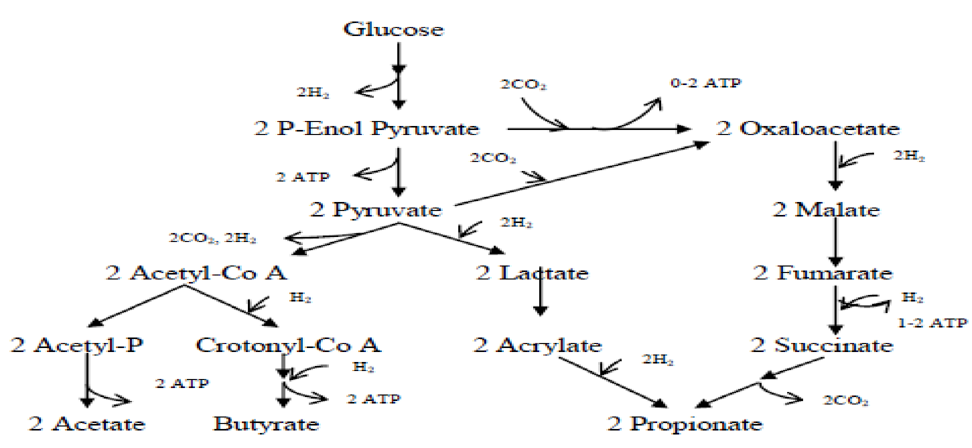

Diagram 1: Fermentation pathways in the rumen

Source: *Ungerfeld and Kohn, (2007)*

3. The VFAs are then available to be absorbed through the digestive mucosa into the animal's blood stream. The production of acetate and butyrate provides a net source of hydrogen or alternatively propionate can utilise any available hydrogen. Methanogens eliminate the available hydrogen by using carbon dioxide (CO_2) to produce methane:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

In ruminants, some 87-93% of methane production occurs in the foregut, with the highest rate of production being after eating (Kebreab et al., 2006). Reductions in enteric CH_4 production from ruminants can result from a reduction in rumen fermentation rate (suppression in microbial activity) or a shift in VFA production (Johnson et al., 1995). An inverse relationship exists between the production of CH_4 in the rumen and the presence of propionate. If the ratio of acetate to propionate was greater than 0.5, then hydrogen would become available to form methane (Joblin, 1999). If the hydrogen produced is not correctly used by methanogens, such as when large amounts of fermentable carbohydrate are fed, ethanol or lactate can form, which inhibits microbial growth, forage digestion, and any further production of VFAs (Murray et al., 1999). In practice, ethanol or lactate may form, but any excess hydrogen is simply eructated.

3.1.2.2 Manure management

In addition to enteric CH_4, excreta are another source of CH_4, especially when stored an aerobically (Klevenhusen et al., 2011). CH_4 generated from manure from ruminant and non-ruminant livestock contributes 2% and 0.4% of global CH_4 emissions, respectively. In regions with low input is enteric fermentation undoubtedly the main emission source. However, in an industrialized region with high production and food processing is manure important a source of emissions (Gerber et al., 2013).

During manure storage, CH_4 is generated through a reaction similar to that of enteric fermentation. Cellulose in the manure is degraded by microbes, with products of this process serving as substrates for methanogenesis (Chianese et al., 2009). Livestock manure contains portion of organic solids such as proteins, carbohydrates and fats that are available as food and energy for growth of anaerobic bacteria. Obvious benefit from methane production could be the energy value of the gas itself. But the gas production from manure depends mainly upon the efficiency of operating system for it. Gas yield can be a certain amount of gas produced per unit of solids degraded by the anaerobic bacteria (Song et al., 2011). Anaerobic digestion is a natural process in which the microorganisms consume organic matter under an oxygen-free environment. It results in production of microbial biomass and greenhouse gases (CO_2 and CH_4).

3.2 STRATEGIES OF GREENHOUSE GAS MITIGATION FOR CATTLE PRODUCTION

Methane production through microbial fermentation in ruminants such as cattle and buffalo has gained attention, both for its role as a greenhouse gas and because it represents a loss of feed energy to the animal. Much effort has focused on discovering methods to decrease CH_4 production in ruminants by developing management or nutritional mitigation strategies (Hristov et al., 2013). A recent review by Knapp et al., (2014) classified studies on enteric CH_4 mitigation strategies into 3 categories: (i) feeding and nutrient management, (ii) rumen modifiers, and (iii) increasing animal production through genetic and management approaches.

3.2.1 Mitigation options

3.2.1.1 Inhibitors

CH_4 inhibition alters the microbial community, H_2 production and fermentation response in the rumen of cattle (Martinez-Fernandez, 2016). Different CH_4 inhibitors have been studied due to their specific inhibitory effect on rumen archaea. Among the most successful compounds tested *in vivo* were bromochloro-methane (BCM), 2-bromo-ethane sulfonate (BES), chloroform and cyclodextrin. These CH_4 inhibitors statistically reduced CH_4 production by up to 50% *in vivo* in cattle (Mitsumori et al., 2011); Chloroform (Knight et al., 2011); Cyclodextrin (Lila et al., 2004). More recent reports also indicated that bromochloro-CH_4 may be an effective CH_4 inhibitor *in vivo*. In a series of experiments with Brahman cross steers, Tomkins et al., (2009) observed an up to 93% decrease in CH_4 production with bromochloro-methane fed at 0.3g/100kg body weight. In conclusion, methane inhibitors specifically bromochloro-methane and chloroform are effective methane inhibitors. It is apparent that a banned compound, such as bromochloro-methane, cannot be recommended as methane mitigating agent, but compounds with similar mode of action may be developed. The long-term effect of methane inhibitors is uncertain and more data are needed to establish their overall production effects.

Among these, fumarate, nitrates, sulphates and nitroethane (Brown et al., 2011) have been studied the most. Leng (2008) provided a comprehensive review of the earlier literature on nitrates. More recent research with cattle (Hulshof et al., 2012; Van Zijderveld et al., 2011a,b) has shown promising results with nitrates decreasing enteric CH_4 production by up to 50%. Additional issues with nitrates include potential increase in NH_3 production and potential toxicity from intermediate products (nitrite). The toxicity issue has been addressed in detail by Leng, (2008). This concluded that nitrite production from nitrate in the rumen may be prevented by feeding management.

3.2.1.2 Ionospheres

Ionophores are highly lipophilic ion carriers that modify ion transport through biological membranes. Monensin acts on the cell wall of the Gram-positive bacteria that produce H^+ and interferes with ion flux, which results in decreasing acetate to propionate ratio in the rumen and thus a reduction in enteric CH_4 emissions. Hristov et al., (2013) concluded that ionophores are likely to have a moderate CH_4 mitigating effect, but their effect appears to be inconsistent. Monensin has been the most studied ionophore and is routinely used in beef production and more recently in dairy cattle nutrition (Russell and Houlihan, 2003). In a meta-analysis of 22 controlled studies, monensin (given at 32 mg/kg DM) reduced CH_4 emissions and CH_4 conversion rate (Ym) in beef steers fed total mixed rations by 19±4g/animal per day and 0.33±16%, respectively (Appuhamy et al., 2013). Meta-analyses have shown monensin to produce improvement in feed efficiency in feedlot cattle by 7.5% (Goodrich et al., 1984), growing cattle on pasture by 15% (Potter et al., 1986), which may lead to reduced enteric CH_4 emission. Moreover, another meta-analysis has also shown a consistent decrease in acetate: propionate (Ac:Pr) ratio with monensin addition in high grain diets fed to beef cattle (Ellis et al., 2012), which may lead to a reduction in CH_4 emission per unit of feed.

3.2.1.3 Plant bioactive compounds

Plant-bioactive compounds form a large and heterogeneous group and vary in chemical structure. The category includes a variety of plant secondary compounds, specifically tannins, saponins, and essential oils and their active ingredients. Tannins and saponins have been extensively studied and show the most promising for mitigating potential within this category. Tannins as feed supplements or as tanniferous plants have often, but not always (Beauchemin et al., 2007), shown a potential for reducing enteric CH_4 emission by up to 20%. Condensed (and hydrolyzable) tannins are widely distributed in browse and warm climate forages and are usually considered anti-nutritional, although they can have good potential to reduce intestinal nematode numbers and allow acceptable production in the presence of a parasite burden (Niezen et al., 1995).

Tannins are polyphenolic substances widely distributed in plants which are characterised by their ability to bind proteins in aqueous solutions. Tannin-protein complexes involve both hydrogen-bonding and hydrophobic interactions, causing a reduction in protein degradation in rumen conditions. Tannins are anti-nutritional factors when dietary crude protein concentrations are limiting production, because they reduce absorption of amino acids (Waghorn, 2008). The anti-methanogenic effect of hydrolysed tannins is caused by their inhibition of the rumen archaea, whereas condensed tannins act indirectly by inhibition of fibre digestion (Goel and Makkar, 2012). Tannins were reported to show good potential for reducing CH_4 emissions, by up to 20% (Hristov et al., 2013).

Saponins are glycoside compounds present in many plants in which the sugars units are linked to a triterpene or steroidal aglycone moiety. They modify ruminal fermentation by their toxic effect on ruminal protozoa. Therefore, saponins have the potential to enhance flow of microbial protein, which is an alternative H_2 sink in the rumen, and in this way increase the efficiency of feed utilisation and reduce enteric CH_4 production. However, their effect is not always consistent, since it has been reported that saponins can be inactivated by rumen bacterial populations and the saliva of adapted animals (Newbold et al., 1997). The potential of essential oils as inhibitors of CH_4 production has been studied extensively in *in vitro* experiments (Benchaar et al., 2011; Calsamiglia et al., 2008). These substances have an antimicrobial activity against rumen archaea by reducing H_2 availability. However, it is likely that the doses required for any substantial mitigation *in vivo* are not economically feasible.

Interestingly, in some studies protein degradation of tanniferous forages (38 sainfoin accessions measured in a short-term inhibitor *in vitro* system) could not be explained by any of the tannin assays (Lorenz et al., 2012), further emphasizing the need for accurate tannin assays (Makkar, 2003). Degradation of plant tannins during ensiling of the plant, although not supported by sufficient research (Theodoridou et al., 2012), might represent another issue with tanniferous forages. Oliveira et al., (2009), reported condensed tannin concentration in silage from a high-tannin sorghum hybrid to be about 17% of that in the original forage (1.0 vs 5.9g/kg DM). Zhang et al., (2012) indicated that tannins in *Leucaena leucocephala* were rapidly degraded during ensiling to about 40% of the initial concentration within 30days of ensiling. The effect of saponins and tannins on CH_4 production in ruminants examined mostly *in vivo* studies and concluded that the risk of impaired rumen function and animal productivity with tannins is greater than with saponins and, for decreasing enteric CH_4 production, the concentration range for tannins is narrower than for saponins. In some dietary situations, however, decreased protein degradability in the rumen, combined with a shift in protein digestion to the small intestine, may be beneficial even if there is a decreased supply of digestible ruminally-undegradable protein (RUP). Such a shift may also have the benefit of reducing urinary N losses (vs faecal N losses). According to Goel and Makkar, (2012), the antimethanogenic effect of tannins depends on the application rate and is positively related to the number of hydroxyl groups in their structure. Overall, hydrolyzable tannins tend to act by directly inhibiting rumen methanogens, while the effect of condensed tannins on rumen CH_4 production is more through inhibition of fibre digestion. As with other CH_4 mitigating agents, the long-term effects of tannins and saponins have not been established. In addition, as indicated by Goel and Makkar, (2012) a substantial reduction in CH_4 emission with these compounds, particularly tannins would be difficult without compromising animal production.

3.2.1.4 Direct-fed microbial

Direct-fed microbials are commonly used as supplements in animal production. Probably the most common Direct-fed microbials used in ruminant nutrition are yeast-based products (YPs). A variety of commercially-available products fitting the description of YP exists, including: live yeast (highly concentrated live yeast), yeast culture (yeast cells with varying viability and the fermentation medium on which they were grown; (Fonty and Chaucheyras-Durand, 2006) or yeast products (a general term representing both live yeast and yeast culture). This variability among YP is reflected in inconsistent animal responses to YP. Earlier analyses reported increased DMI and milk yield in dairy cows, no effect on rumen pH but decreased lactate concentration, and increased duodenal microbial protein and methionine flows (considered the first limiting amino acid in lactating dairy cows and other farm animals) suggesting enhanced microbial protein synthesis in the rumen (Poppy et al., 2012). Strains of *Aspergillus oryzae* and *Saccharomyces cerevisiae* have been the most commonly studied. Desnoyers et al., (2009) concluded that *S. cerevisiae*-based YP increased ruminal pH, increased total VFA and decreased lactate concentration, and increased OM digestibility, but had no effect on acetic:propionic (Ac:Pr) ratio. Hristov et al., (2010) showed that methanogen-specific DGGE (denaturing gradient gel electrophoresis) analysis of rumen methanogens did not reveal any yeast-based products (YPs) specific banding patterns and this result was supported by the lack of effect of yeast-based products (YPs) on ruminal CH_4 production and overall ruminal fermentation (pH, protozoal counts, VFA). The yeast-based products (YPs), however, slightly decreased NH_3 and CH_4 emissions from manure measured in a steady-state gas emission system.

McGinn et al., (2004) reported no effect of commercial YP on CH_4 production in beef cattle. Thus, the potential for mitigating rumen CH_4 production with YP appears to be through increased production, feed efficiency and overall ruminal health. Other DFM interventions of ruminal fermentation include inoculation with lactate-producing and lactate-utilizing bacteria to promote more desirable intestinal microflora and stabilize pH and promote rumen health, respectively. Krehbiel et al., (2003) reported a generally positive trend for improved health in young, growing dairy or beef cattle treated with various direct-feed microbials (Based on *Lactobacillus* and *Streptococcus* and in some cases *Propionibacterium* spp). Overall, feeding direct-feed microbials (DFM) to feedlot cattle resulted in a 2.5 to 5.0% increase in average daily grain (ADG) and around a 2% improvement in feed efficiency, whereas the response in DMI was inconsistent (Krehbiel et al., 2003).

3.2.1.5 Defaunation

Association and cross-feeding between ruminal protozoa and archaea have been established (Finlay et al., 1994) and are the basis for suggesting defaunation as a CH_4 mitigation strategy (Hristov and Jouany, 2005). However, the response in methane to partial or complete defaunation has been variable.

Morgavi et al., (2010) calculated an average decrease in CH_4 production of about 10% due to defaunation, but the data were extremely variable. Moreover, all responses were attributed to loss of protozoa without accounting for depressed ruminal fibre digestibility, which promotes acetate/CH_4 fermentation pathways and typically accompanies defaunation (Eugène et al., 2011). Popova et al., (2011) showed that beef cattle has no effect on rumen methanogen abundance despite a 65% difference in protozoal numbers between a high-forage and a high-starch, lipid-supplemented diet. With such variability and uncertainty in the response (Morgavi et al., 2011), defaunation cannot be recommended as a CH_4 mitigation practice. In addition, apart from lauric acid and coconut oil (Hristov et al., 2011) and some vegetable oils high in unsaturated fatty acids (FAs) such as linseed (Doreau and Ferlay, 1995), which can severely depress DMI in cattle, there has been no effective and, more importantly, practical defaunating agent tested rigorously *in vivo*.

3.2.1.6 Dietary lipids

There is a large body of evidence that lipids (vegetable oil or animal fat) suppress methane production in the rumen. The effects of lipids on rumen archaea are not isolated from their overall suppressive effect on bacteria and protozoa. Rabiee et al., (2012) reported that a consistent decrease in DMI with all types of dietary fat examined (tallow, various calcium salts of fatty acid (FA), oilseeds, prilled fat). Beauchemin et al., (2007b) compared animal fat (tallow) and sunflower oil (48% higher unsaturated FA concentration in the diet) both supplemented at 3.4% of dietary DM, and reported no effect on DM and NDF digestibility, feed intake and ADG in cattle. CH_4 production reduced by about 12% both of lipid sources and there was no effect of level of saturated fatty acid (FA).

Janssen (2010) expected that methanogens would adjust to higher hydrogen to maintain population density with the feeding of lipids and decreasing ruminal pH (typically through feeding higher grain or less effective fibre diets). Presumably, these events would be additive, so lipids plus lower pH would potentially combine to thermodynamically stimulate propionate and decrease methanogenesis. Combining lipids with higher concentrate, presumably through lower ruminal pH, has been suggested to decrease protozoal counts (Firkins, 1996) but, unfortunately, it is also expected to decrease lipolysis and inhibit the terminal step of biohydrogenation (Jenkins et al., 2008). That discussed protozoal lipids being highly enriched in polyunsaturated and trans-11 fatty acid (FA), and both polyunsaturated fats and biohydrogenation intermediates are removed from the ruminal biohydrogenation pool through growth and ruminal passage of protozoa. Therefore, CH_4 mitigation strategies combining lipids in dietary situations that limit rumination or enhance ruminal acidity can decrease methanogenesis. It can be concluded that inclusion of lipids in ruminant diets will likely produce CH_4 mitigating effect, but it may also depress feed intake and consequently, animal productivity. Thus, at least part of the mitigation effect reported in the dataset is a result of decreased intake of dietary carbohydrate, which is a consequence of decreased dry matter intake as a result of lipids replacing carbohydrate in the diet.

3.3 FEEDING STRATEGIES FOR METHANE MITIGATION FROM CATTLE PRODUCTION

3.3.1 Feed intake

Feed intake is an important variable in predicting CH_4 emission. Johnson and Johnson, (1995) stated that as feed intake increases, the Ym (CH_4 energy estimated as percent of gross energy ingested) factor decreases by 1.6% units per each level of intake above maintenance. However, these authors also noted that a strong relationship among diet digestibility, intake and CH_4 production could not be demonstrated. They suggested no relationship between dietary gross energy (GE) digestibility and the proportion of gross energy intake (GEI) lost as CH_4. Increasing intake increases fractional passage rate and decreases digestibility and the decrease in digestibility will depend on diet quality.

Hegarty et al., (2010) proposed the following relationships among feed intake, digestibility (55-85%) and CH_4 production for growing small ruminants on pasture: "(i) an increase in dry matter intake is associated with a linear increase in average daily grain, with the rate of ADG being greater for feeds of greater digestibility; (ii) increased DMI is associated with increased CH_4 production. For diets of low to moderate digestibility, such as those consumed in extensive grazing systems, the CH_4 released per unit additional intake is greater than when high intakes of high digestibility feed are consumed; (iii) CH_4 production per unit of metabolizable energy (ME) intake is lowest for diets with high-energy densities; (iv) although an increase in the intake of any diet reduces the emissions intensity of growth (g CH_4 produced per kg ADG), emissions intensity at any given DMI is less for high-digestibility feeds than for low digestibility feeds; and (v) small changes in energy intake result in small changes in CH_4 output, but in large changes in animal performance. Despite the obvious relationships among digestibility, intake and enteric CH_4 production (absolute or per unit of DMI), the Ym factor used by IPCC, (2006) is calculated on a GEI basis only. Ellis et al., (2010) evaluated nine empirical CH_4 prediction equations and observed the Ym factor model to perform adequately, compared with other equations. However, these authors argued that because it is based simply on GEI, Ym does not have the capacity to fully describe changes in composition of the diet and has limited use when estimating the impact of varying nutritional strategies on CH_4 emissions. Increased DMI usually decreases digestibility, which may increase excretion of fermentable OM with manure and thus, CH_4 or N_2O emissions depending on the type of manure handling system. Huhtanen et al., (2009) pointed out that diets which had high digestibility at maintenance exhibited greater depression in digestibility with increasing DMI.

3.3.2 Inclusion of concentrates

It is important to remember that dietary variables are not independent. Increasing or decreasing the concentration of one entity will decrease or increase concentration of another. As discussed earlier, mitigation options aimed at reducing urinary N excretion may well result in elevated enteric CH_4 emission (Dijkstra et al., 2011). Decreasing dietary concentration of CP will result in an increasing concentration of other nutrients such as starch or NDF, and these changes may affect enteric and manure CH_4 and N_2O emissions. Thus, effects on greenhouse gas emissions as a result of changes in one nutrient have to be interpreted in the context of potential effects resulting from changes in other dietary constituents.

According to Eugène et al., (2011), addition of starch and lipid combination to the diet of feedlot cattle reduced greenhouse gas emissions per unit of feed intake and body weight gain. It was concluded that total CH_4 emissions were lowest for the high grain diet, but N_2O and CO_2 emissions were greatest. There are some studies have not reported a decrease in CH_4 production (absolute or per unit of feed DM intake) by increasing the proportion of concentrate feeds (Popova et al., 2011). In some cases, the opposite effect was observed with enteric CH_4 production increased (per unit of DM intake) with increasing concentrate inclusion in the diet (McGinn et al., 2006; Islam et al 2000).

In general, concentrate will have a higher amount of fermentable OM (per unit feed) than roughage, which helps to explain an increased in CH_4 production. Sauvant and Giger-Reverdin, (2009) showed that small and moderate variation in dietary concentrate proportion is unlikely to affect enteric CH_4 emission. Feedlot cattle are typically fed high grain diets (> 90% grain on a DM basis) to achieve maximum profit. Beauchemin et al., (2011) estimated that extended grain finishing of cattle from 170-210 days (shortened back grounding period: 110 vs 40 days) would reduce greenhouse gas emission intensity of beef production by 2%, mainly due to lower enteric CH_4 emissions of grain versus forage based diets. There would also be less CH_4 emission from manure because its production would be reduced by 11.3% due to the greater digestibility of grain (Beauchemin et al., 2011). However, the authors indicated that intensifying ruminant production by feeding less forage should be promoted as a greenhouse gas mitigation strategy only after careful assessment using life cycle assessment (LCA) because these results may not be applicable to all beef production systems.

Overall, the inclusion of concentrate feeds in the diet of ruminants will likely decrease enteric CH_4 emission intensity, particularly when inclusion is above 35-40% of dry matter intake, but the effect will depend on inclusion level, production response, effects on fibre digestibility, rumen function, and plane of nutrition, type of grain and grain processing and supplementation with small amounts of concentrate feeds will likely increase animal productivity and thus decrease greenhouse gas emission intensity, although absolute CH_4 emissions may not be reduced. In spite of these potential gains, concentrate supplementation cannot be a feasible substitute for high-quality forage for ruminants. In addition, in many parts of the world, this may not be an economically feasible and socially acceptable mitigation option.

3.3.3 Forage type, quality and management

An important feed characteristic that can impact enteric CH_4 production is forage quality, specifically its digestibility. Blaxter and Clapperton, (1965) indicated that increased intake of poor quality; less digestible feeds has little effect on CH_4 production when expressed on a DM intake basis. Forages are the feed ingredients with the largest variability in composition and more impact on diet digestibility such as plant species, variety, maturity at harvest and preservation can all affect forage quality and digestibility.

Lignin is the key element that limits forage cell wall digestibility; as the plant matures, phenolic acids and lignin are deposited and cross linkage of lignin and cell wall polysaccharides are formed by ferulic acid bridges limiting polysaccharide digestibility by the animal (Jung and Allen, 1995). Dietary fibre concentration is one of the factors determining feed intake in ruminants. Feed intake is a critical factor for improving animal productivity, feed efficiency and greenhouse emissions both CH_4 and N_2O (Allen, 2000). However, a factor that control feed intake included from physical such as palatability and fill limitation and physiological (rumen propionate production). Another factors that feed intake is affected by managements (such as animal handling, feed accessibility, frequency of feeding, method of feed presentation), feed (feed palatability, feed physical properties, such as processing, chemical composition, nutrient availability, plant species) and animal (capacity to ingest food, appetite and energy demand) factors (Mertens, 1994).

In general, enteric CH_4 reductions are correlated with greater nutrient quality and digestibility, two attributes for which forage type and maturity might be indicators. Grazing management might be used as a potential mitigate through optimizing pasture maturity, allowing for adequate pre-grazing herbage mass or intensive grazing. Boadi and Wittenberg, (2002) noted that impact on enteric CH_4 mitigation, when scaled per unit of animal product should be greater when animals consume higher quality forage. While harvesting forage at an earlier stage of maturity increases its soluble carbohydrate content and reduces lignification of plant cell walls thereby increasing its digestibility and decreasing enteric CH_4 production per unit of digestible (in DM) (Van Soest, 1994).

3.3.4 Feed processing

In ruminants, small forage particle size through mechanical processing or chewing is an important component of enhancing forage digestibility and providing greater microbial access to substrate, reducing energy expenditures and increasing passage rate, feed intake and animal productivity. However, forage processing must be balanced between enhancing passage rate to increase intake and utilization of easily digestible nutrients, which may not be easy to achieve for lower quality feeds. Based on processing, through its effect on digestibility, energy losses and passage rate can also be effective enteric CH_4 mitigation practices that although it may be economically infeasible in some production systems. Another processing of grain feed is a key factor in an improving feed efficiency and reducing greenhouse gas emissions from livestock operations (Theurer et al., 1999). Firkins et al., (2001) concluded that grain processing to enhance starch digestibility in the rumen will have a negative effect on NDF digestibility. Ruminal degradability of starch may be up to 75-80% (Harmon et al., 2004) of the starch entering the duodenum may be potentially digestible in the small intestine. Another 35-50% of the starch that escapes small intestinal digestion may be further degraded in the large intestine (Harmon et al., 2004). The capacity to digest starch in the intestine, however, is limited by the supply of pancreatic amylase and increasing starch flow to the small intestine may result in decreased total tract starch digestibility (Theurer, 1986). However, the processing of grain to increase its digestibility is likely to reduce enteric CH_4 production per unit of animal product and the minimal processing is recommended, so the grain energy is better utilized for animal production.

Literature review provides information on (i) cattle production in Lao PDR; (ii) the use of available feed resources for cattle production; (iii) introduction to some main local feed resources such as cassava and their by-products, brewers' grains and rice distiller's grains; (iv) methane emissions and its mitigation strategies in the production; and (v) feed and feeding strategies for methane mitigation from cattle production. The literature review shows a potential to use local feed resources for cattle production for the two purposes of increasing animal performance and reducing methane emission.

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CHAPTER 2:

EFFECT OF EITHER ENSILED OR DRIED CASSAVA ROOT (*Manihot esculenta,* Crantz) ON METHANE PRODUCTION IN AN *IN VITRO* RUMEN FERMENTATION USING CASSAVA LEAVES AND WATER SPINACH (*Ipomoea aquatic*) AS A PROTEIN SOURCE

ABSTRACT

The hypothesis of the present study was that in an *in vitro* rumen fermentation methane production would be reduced when cassava leave meal replaced water spinach as the protein source and when the carbohydrate was from ensiled rather than dried cassava root. The treatments in a 2*3 factorial design were (i) carbohydrate source: ensiled or dried cassava root meal and (ii) protein sources: cassava leaf meal, water spinach meal or mixture of equal proportions of cassava leaf meal and water spinach meal. Urea was included in all incubations at 2% of the dry matter (DM) substrate. The quantity of substrate in each fermentation bottle was 12g DM to which was added 240 ml of rumen fluid (from slaughtered cattle) and 960ml of buffer solution. The incubations were done in a simple *in vitro* system using 1.5 liters of water bottles with gas collection by water displacement and methane was measured by an infra-red methane gas detector. The incubation was for 24hours with measurements of total gas production and methane percentage in the gas at intervals of 06, 12, 18 and 24hours, with determination of residual unfermented substrate at the end.

The results showed that there were consistent effects at each fermentation interval for a decrease in gas production and methane content of the gas when: (i) ensiled cassava root replaced the dried root as carbohydrate source; and (ii) when cassava leaf meal replaced water spinach meal as protein source. Methane concentration in the gas increased linearly from 10-12 to 26-30% of the gas as the fermentation interval advanced from 0-6hours to 18-24hours. Over the overall 24hours fermentation the methane production per unit of substrate DM mineralized was decreased by 18% by the combination of ensiling versus drying of the cassava root and replacement of water spinach by cassava leaf meal.

This research implicated that methane production can be reduced when ensiled cassava root replaced dried cassava root and when cassava leaf meal was used as a source of protein in stead of water spinach.

Keywords: *cassava, carbohydrate, drying, ensiling, methane, water spinach*

INTRODUCTION

Greenhouse gas emissions play an important role in increasing global temperature (IPCC, 2007). Agriculture produces 10-12% of total anthropogenic greenhouse gas emissions with the livestock sector contributing 44% of these emissions in the form of methane; the remaining sources are estimated to be 29% as N_2O and 27% as CO_2 (Gerber et al., 2013). Ruminants are estimated to produce up to 95 million tonnes of methane annually and are implicated as a major source of greenhouse gas production (Patra, 2014).

Enteric methane 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. Kebreab et al., (2008) showed that methane emissions inventories were more accurately estimated through diet-specific mechanistic models. Chagunda et al., (2010) indicated that, in order to mitigate methane emissions in a way acceptable for both the environment and animal welfare, it was important to quantify the effects of different diets on methane emissions. Other results suggest that a major difference is needed in dietary starch concentration in order to alter ruminal methanogenesis (Hassanat et al., 2013).

Cassava (*Manihot esculenta,* Crantz) is grown in over 90 countries and is a most important food crop worldwide. It is the primary staple for more than 800 million people in the world (Lebot, 2009). Of importance in a warming world appears that cassava is potentially highly resilient to future climatic changes and according to Jarvis et al., (2012) “could provide Africa with options for adaptation whilst other major food staples face challenges”. Roots of cassava have a high levels of energy (75 to 85% of soluble carbohydrate) and minimal levels of crude protein (2 to 3% CP); they have been used as a source of readily-fermentable energy (Kang et al., 2015; Polyorach et al., 2013). Cassava foliage is considered to be a good source of bypass protein for ruminants (Ffoulkes and Preston, 1978; Wanapat, 2001; Keo Sath et al., 2008). It has been fed as a major component of the diet for sheep (Hue et al., 2008), goats (Ho Quang Do et al., 2002; Dung et al., 2005; Phengvichith and Ledin, 2007; Seng Sokerya and Preston, 2003) and cattle (Wanapat et al., 2000; Thang et al., 2010) in fresh, wilted or dried form.

The potential of cassava foliage as a protein source in ruminant feeds has not been fully exploited, probably because of the risk of toxicity resulting from the content of precursors of hydrogen cyanide (Wanapat, 2001). However, it is known that the capacity to liberate HCN from cassava foliage is reduced by processing such as sun drying or ensiling (Khieu Borin et al., 2005; Phengvichith and Ledin, 2007). The role of cyanide as inhibitor of methanogenesis in sludge fermentation has been discussed by Gijzen et al., (2000). Cassava leaves are known to contain variable levels of condensed tannins; about 3% in DM according to Netpana et al., (2001) and Bui Phan Thu Hang and Ledin, (2005). Condensed tannins at moderate levels are known to have positive effects on the nutritive value of the feed by forming insoluble complexes with dietary protein, resulting in "escape" of the protein from the rumen fermentation (Barry and McNabb, 1999). Numerous studies have also shown the potential of the tannin content in cassava leaves to play an anthelminthic role for the control of nematode parasites in ruminants (Seng Sokerya and Preston, 2003; Seng Sokerya et al., 2009; Netpana et al., 2001; Khoung and Khang, 2005). Condensed tannins are also reported to decrease methane production and increase the efficiency of microbial protein synthesis (Makkar et al., 1995; Grainger et al., 2009). Reductions of methane production due to presence of tannins of up to 13-16% were reported by Carulla et al., (2005), Waghorn et al., (2002), Grainger et al., (2009) and Woodward et al., (2004), apparently through a direct toxic effect on methanogens.

Water spinach (*Ipomoea aquatica*) plays an important role for farmers in rural areas; it is easy to cultivate and has a very high yield of biomass with a short growth period (Kean Sophea and Preston, 2001). The crude protein content in the leaves and stems can be as high as 32 and 18% in dry basis (Ly Thi Luyen, 2003). Water spinach is widely used for human food, but at the same time this vegetable can serve as feed for all classes of livestock. The objective of this study to study effects of carbohydrate sources from ensiled or dried cassava roots supplemented with sources of protein from cassava leave meal; water spinach meal and cassava leave meal plus water spinach meal in an *in vitro* rumen fermentation on gas and methane production.

MATERIALS AND METHODS

Location and duration

This experiment was done in the laboratory of the Animal Science department, the Faculty of Agriculture and Forest Resource, Souphanouvong University.

Experimental design

The experiment was arranged in a 2*3 factorial in a completely randomized design (CRD), 6 treatments combinations, each with 4 replicates. The experimental factors were:

Source of carbohydrate:

- ECR: Ensiled cassava root meal
- DCR: Dried cassava root meal

Source of protein:

- CLM: Cassava leaf meal
- WSM: Water spinach meal
- CLM-WS: Cassava leaf meal plus water spinach meal (50:50)

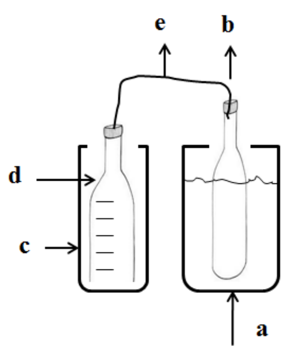
Table 1: The proportions of ingredients (% DM basis) in the substrates

Items	Ensiled cassava root			Dried cassava root		
	CLM	WS	CLM-WS	CLM	WS	CLM-WS
Ensiled cassava root	72	71	72			
Dried cassava root				72	71	72
Cassava leave meal	26		13	26		13
Water spinach meal		27	13		27	13
Urea	2	2	2	2	2	2
CP in DM, %	13.2	13.1	13.1	13.2	13.1	13

CLM: cassava leave meal; WS: water spinach meal; CLM-WS: cassava leave meal with water spinach

In vitro rumen fermentation system

The *in vitro* rumen fermentation system was as described by Sangkhom Inthapanya et al., (2011). The water bottles (1.5liters each) were used for the fermentation and collection of the gas. A hole was made in the lid of each of the bottles, which were interconnected with a plastic tube (id 4mm). The bottle receiving the gas had the bottom removed and was suspended in a larger bottle (3liters capacity) partially filled with water, so as to collect the gas by water displacement. The bottle that was suspended in water was calibrated at 50ml intervals to indicate the volume of gas (Diagram 1).



a. Water bath
b. Fermentation bottle (1.5liters)
c. Water storage reservoir (3liters)
d. Gas collection bottle (1.5liters)
e. Plastic tube (0.4m of length and 4mm of diameter)

Diagram 1: A schematic view of apparatus to measure in an in vitro rumen fermentation

Experimental procedure

The cassava root; cassava leaves and water spinach foliage (leaves and petioles) were collected from Souphanouvong University's farms. The fresh cassava root was chopped into small pieces of around 1-2cm long and ground in a liquidizer, and then stored anaerobically in a plastic bag for ensiling over 7days. The other fresh cassava root; cassava leaves and water spinach were chopped into small pieces of around 1-2cm long and dried in an oven at 80ºC for 24hours before being ground through a 1mm sieve by machine.

The source of carbohydrate from ensiled cassava root or dried cassava root meal were offered at 72% of dry matter (DM). Source of protein from cassava leaf meal and water spinach meal were offered at 26% of dry matter (DM) substrate and was added 2% of urea in dry matter (DM) substrate into the incubation bottle. Amounts of the substrates equivalent to 12g dry matter (DM) were put in the incubation bottle, followed by 960 liters of buffer solution and 240 ml of rumen fluid obtained from cattle immediately after being slaughtered. The bottles were then filled with carbon dioxide and incubated at 38 ^{0}C in a water bath for 24 hours.

Table 2: Ingredients of the buffer solution

Ingredients	**$CaCl_2$**	**$NaHPO_4.12H_2O$**	**NaCl**	**KCl**	**$MgSO_4.7H_2O$**	**$NaHCO_3$**	**Cysteine**
(g/liter)	0.04	9.30	0.47	0.57	0.12	9.80	0.25

Source*: Tilly and Terry, (1963)*

Data collection and measurements

After incubation, the gas volume was recorded for the periods of 0-6, 6-12, 12-18 and 18-24hours. After each time interval, the methane concentration in the gas was measured with a Crowcon infra-red analyser (Crowcon Instruments Ltd, UK). At the end of the incubation, the contents of the incubation bottle were filtered through cloth to determine the mineralization of the substrates.

Photo 1: Measurement of CH_4 concentration in the gas by Crowcon infra-red analyser (Crowcon Instruments Ltd, UK)

Chemical analyses

Samples of ensiled cassava root; dried cassava root meal, cassava leave meal and water spinach meal were analyzed for dry matter (DM), crude protein (CP), crude fiber (CF) and ash following AOAC, (1990) methods. The cassava root (ensiled and dried) and the cassava leaves (fresh and dried) were analyzed for hydrogen cyanide (HCN) and condense tannin (CT) content according to AOAC, (1990) methods.

Statistical analyses

The data were analyzed with the General Linear Model (GLM) option in the ANOVA program of the Minitab software (version 16.0). In the model the sources of variation were treatments, treatment interaction and random error. Turkey's pair-wise comparisons was used to determine the differences between source of protein when the P value of F test P<0.05. The statistical model used was:

$$Y_{ijk} = \mu + c_i + p_j + (c*p)_{ij} + e_{ijk}$$

Where: Y_{ijk} is dependent variables; μ is overall mean; c_i is the effect of carbohydrate source; p_j is the effect of protein source; $(c*p)_{ij}$ is the interaction between source of carbohydrate and source of protein and e_{ijk} is random error.

RESULTS AND DISCUSSION

Chemical composition

The water spinach foliage had less crude fiber (CF) and more ash than the cassava leaves (Table 3). Crude protein (CP) contents were similar for both cassava leaf meal and water spinach meal.

Table 3: Chemical composition of substrate components

Items	DM	As % of DM		
		CP	CF	Ash
Ensiled cassava root	38.2	2.04	1.13	0.84
Dried cassava root	90.6	1.98	2.86	2.47
Cassava leaf meal	91.3	23.0	15.2	6.18
Water spinach meal	92.4	22.0	9.47	10.9

CP: crude protein; CF: crude fiber; DM: dry matter

Hydrogen-cyanide (HCN) was higher in fresh than in dried cassava leave; and in ensiled rather than dried cassava root. Condensed tannin content was lower in the roots than in the cassava leave (Table 4).

Table 4: Hydro cyanide and tannin values in the substrates (DM basis)

Items	HCN (mg/kg)	Condensed tannin (%)
Ensiled cassava root	119	0.95
Dried cassava root	94.0	0.87
Fresh cassava leave	485	2.80
Dried cassava leave	369	1.50

HCN: hydrogen-cyanide

Gas production

At all incubation times, the gas production was lower in treatments with ensiled cassava root than in those with dried root (Table 5).

Table 5: Mean values of gas production, percent of methane in the gas and dry matter (DM) digestibility in an in vitro rumen fermentation using ensiled cassava root or dried cassava root supplement with cassava leaf meal and/or water spinach meal

Items	Carbohydrate source		SEM (df=12)	Prob	Protein source			SEM (df=8)	Prob	Interaction (C*P)
	Ensiled	Dried			CLM	CLM-WS	WS			Prob.
Gas production, ml										
0-6hr	592	638	15.4	0.053	563[a]	625[ab]	656[b]	18.9	0.010	0.729
6-12hr	813	879	16.4	0.012	825	825	888	20.1	0.068	0.609
12-18hr	688	746	12.4	0.005	713[a]	681[ab]	756[b]	15.1	0.011	0.008
18-24hr	533	629	12.3	<0.001	531[a]	575[b]	638[b]	15.1	0.001	0.011
Total gas, ml	2625	2892	36.8	<0.001	2631[a]	2706[b]	2938[b]	45.1	0.001	0.036
Methane in the gas, %										
0-6hr	9.67	11.7	0.18	<0.001	9.9[a]	10.6[b]	11.5[b]	0.22	<0.001	0.134
6-12hr	16.5	20.1	0.27	<0.001	17.0[a]	18.3[b]	19.6[c]	0.33	<0.001	0.323
12-18hr	20.9	25.6	0.13	<0.001	22.0[a]	23.3[b]	24.5[c]	0.16	<0.001	0.095
18-24hr	25.6	30.0	0.13	<0.001	26.3[a]	27.8[b]	29.4[c]	0.16	<0.001	0.027
Total CH_4, ml	473	632	7.58	<0.001	498[a]	536[b]	624[c]	9.28	<0.001	0.004
DM digested, %	67.3	73.7	0.43	<0.001	67.5[a]	70.5[b]	73.5[c]	0.52	<0.001	0.034
Methane, ml/g DM substrate	59.6	72.8	0.96	<0.001	62.1[a]	64.5[b]	72.0[b]	1.18	<0.001	0.029

[a,b,c] values on the same row and within each treatment with different superscripts differ (P<0.05); CLM: cassava leaf meal; CLM-WS: cassava leaf meal with water spinach meal; WS: water spinach meal

There was lower for treatments with cassava leave meal alone, and for cassava leave meal combined with water spinach meal, than for those with water spinach meal as the only protein source (Figures 1 to 4). Gas production increased from the 0-6hours to the 6-12hours interval and then decreased linearly to the lowest values at 18-24hours (Figures 5-6).

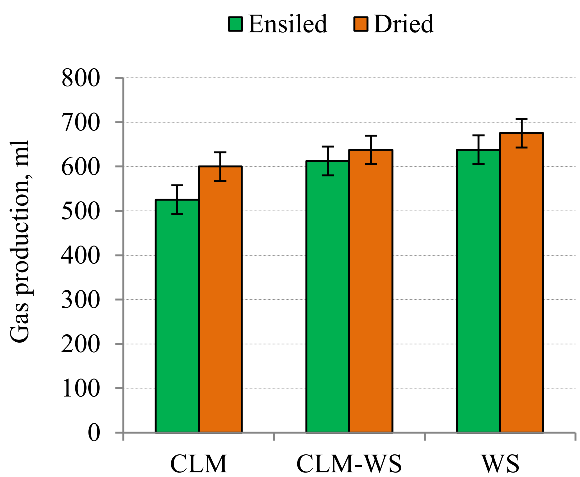


Figure 1: Gas production (0-6h) from ensiled or dried cassava root supplemented with cassava leaf meal (CLM) or water spinach meal (WS) and cassava leaf meal combined with water spinach meal (CLM-WS)

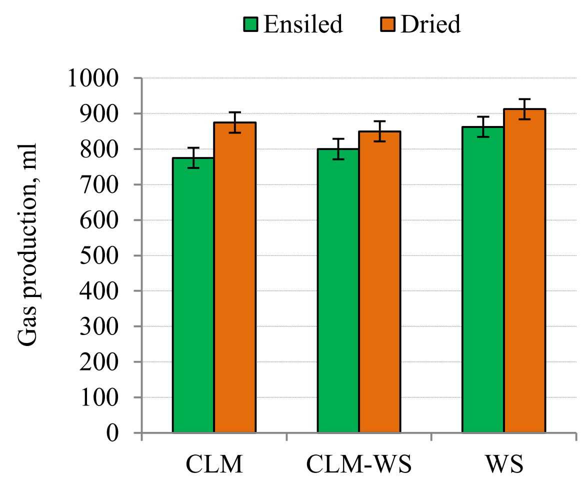


Figure 2: Gas production (6-12h) from ensiled or dried cassava root supplemented with cassava leaf meal (CLM) or water spinach meal (WS) and cassava leaf meal combined with water spinach meal (CLM-WS)

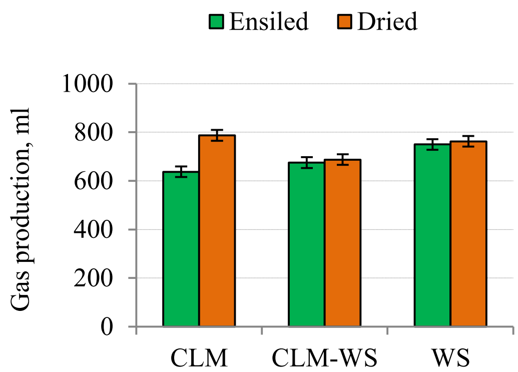


Figure 3: Gas production (12-18h) from ensiled or dried cassava root supplemented with cassava leaf meal (CLM) or water spinach meal (WS) and cassava leaf meal combined with water spinach meal (CLM-WS)

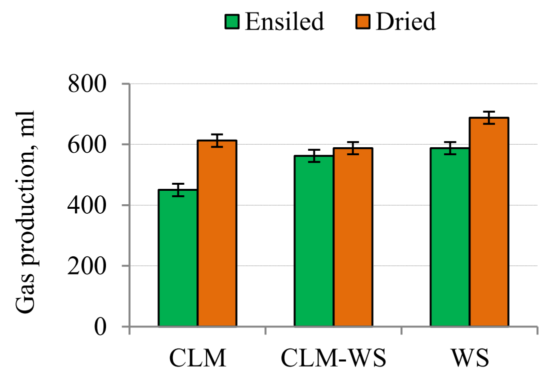


Figure 4: Gas production (18-24h) from ensiled or dried cassava root supplemented with cassava leaf meal (CLM) or water spinach meal (WS) and cassava leaf meal combined with water spinach meal (CLM-WS)

At all incubation periods of time, the percentage of methane in the gas was lower for cassava leaf meal or cassava leave meal combined with water spinach meal than for water spinach meal; and for ensiled compared with dried cassava root (Figures 7-10).

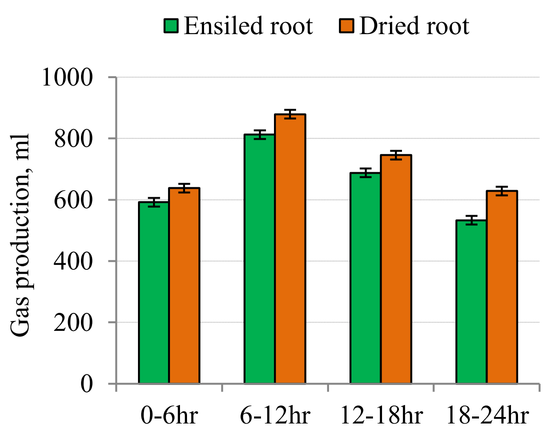


Figure 5: Gas production from ensiled or dried cassava root at each fermentation interval

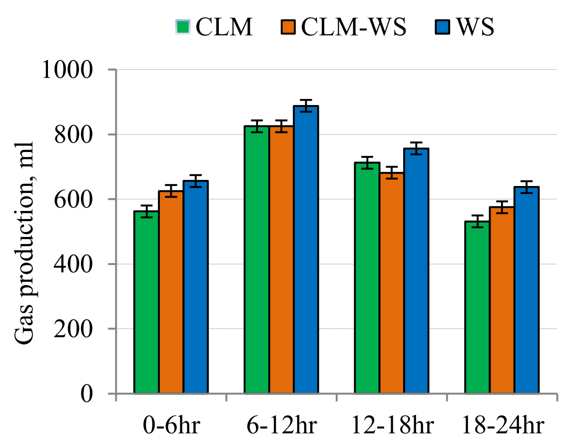


Figure 6: Gas production from cassava leaf meal; water spinach meal and cassava leaf meal combined with water spinach meal at each fermentation interval

Methane in the gas was lower for ensiled than for dried cassava root and increased as water spinach replaced cassava leaf meal (Figures 7-10).

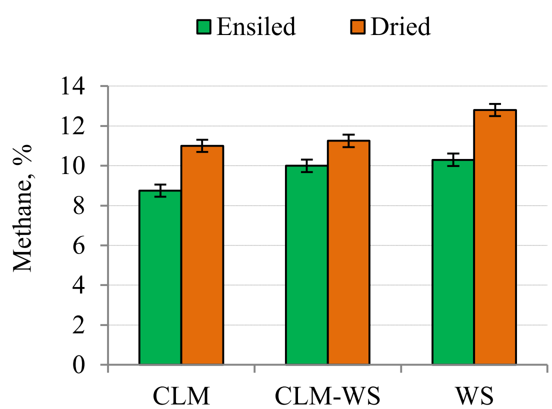


Figure 7: Methane in the gas (0-6h) for substrates with ensiled or dried cassava root, and those with cassava leaf meal (CLM) or water spinach meal (WS) and cassava leaf meal combined with water spinach meal (CLM-WS)

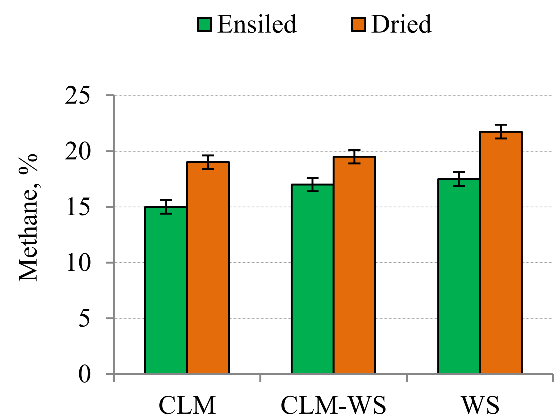


Figure 8: Methane in the gas (6-12h) for substrates with ensiled or dried cassava root, and those with cassava leaf meal (CLM) or water spinach meal (WS) and cassava leaf meal combined with water spinach meal (CLM-WS)

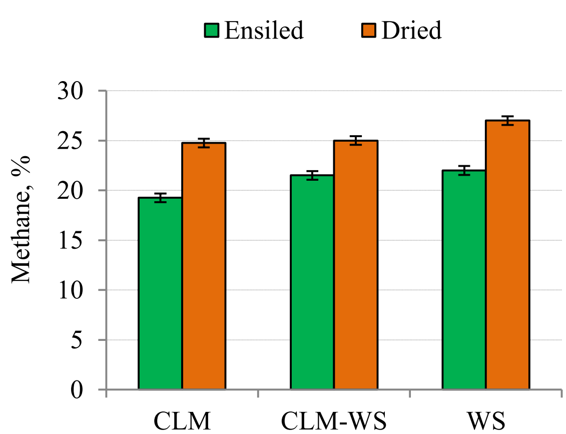


Figure 9: Methane in the gas (12-18h) for substrates with ensiled or dried cassava root, and those with cassava leaf meal (CLM) or water spinach meal (WS) and cassava leaf meal combined with water spinach meal (CLM-WS)

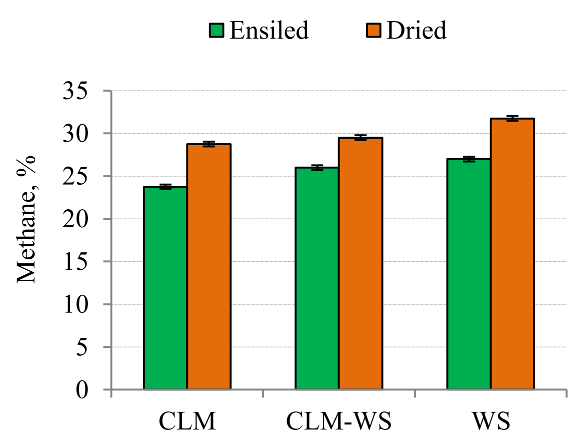


Figure 10: Methane in the gas (18-24h) for substrates with ensiled or dried cassava root, and those with cassava leaf meal (CLM) or water spinach meal (WS) and cassava leaf meal combined with water spinach meal (CLM-WS)

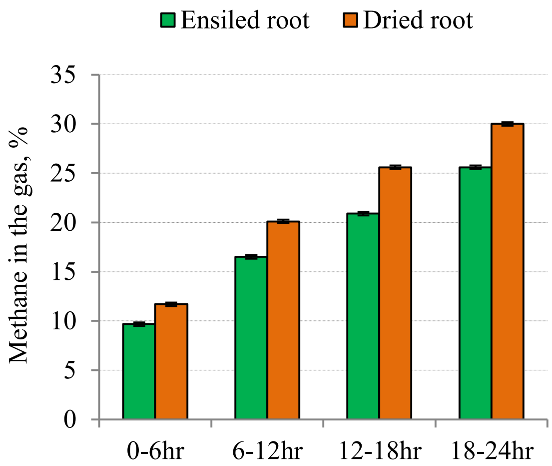


Figure 11: The percentage of methane in the gas at each fermentation interval for substrates with dried or ensiled cassava root

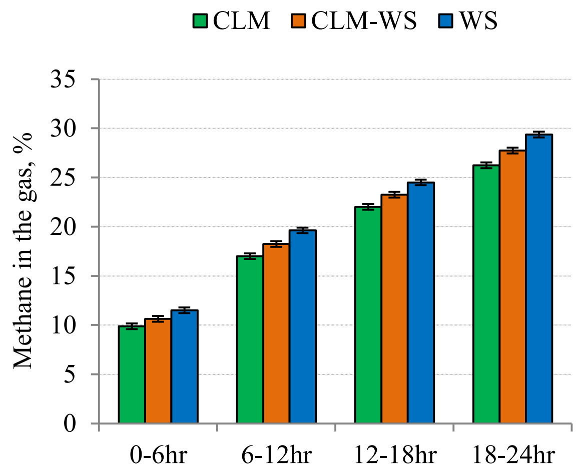
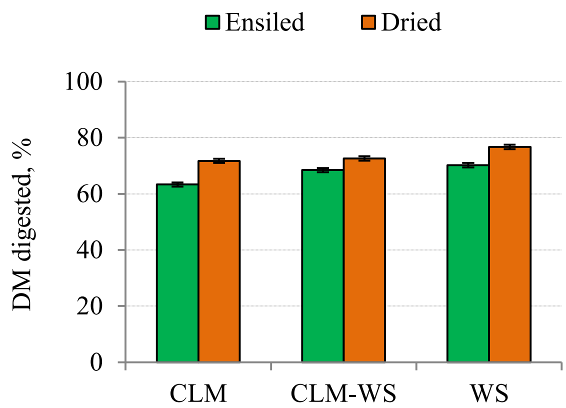


Figure 12: The percentage of methane in the gas at each fermentation interval for cassava leaf meal; water spinach meal and cassava leaf meal combined with water spinach meal

The methane concentration in the gas increased linearly with fermentation interval (Figure 11-12). The proportion of the DM that was mineralized was greater with dried than with ensiled cassava root and increased as cassava leaf meal was replaced by waster spinach meal (Figure 13). Methane production per unit DM mineralized was greater with dried than ensiled root and increased as water spinach replaced cassava leaf meal (Figure 14).



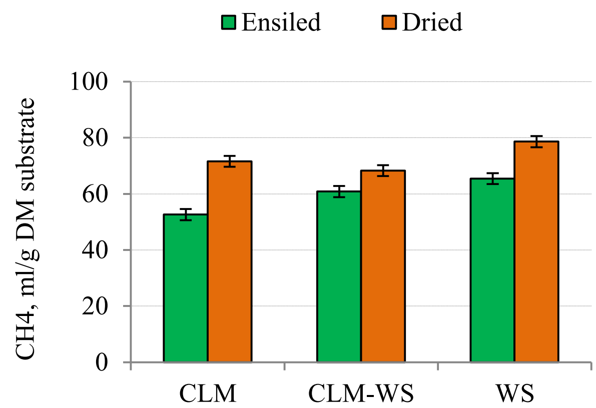


Figure 13: DM digested after 24h for substrates with ensiled or dried cassava root, and those with cassava leaf meal (CLM) or water spinach meal (WS) and cassava leaf meal combined with water spinach meal (CLM-WS)

Figure 14: Methane produced per unit DM mineralized for substrates with ensiled or dried cassava root, and those with cassava leaf meal (CLM) or water spinach meal (WS) and cassava leaf meal combined with water spinach meal (CLM-WS)

The increase in methane production with duration of incubation, indicative of the transition to a secondary fermentation of the volatile fatty acid (VFA) to methane, supports the findings of Sangkhom Inthapanya et al., (2011); Le Thi Binh Phuong et al., (2011); Thanh et al., (2011); Outhen et al., (2011) and Marin et al., (2014). Replacing cassava leaf meal with water spinach meal led to linear increases in gas production of higher methane content. This effect is similar to that reported in *in vitro* rumen fermentation when water spinach replaced cassava leaf meal with urea treated rice straw as the substrate (Inthapanya and Preston., 2014) and when water spinach meal was added to substrates of Bauhinia and Guazima leaf meals (Silivong and Preston, 2015). The lower production of methane when the carbohydrate (CHO) substrate was ensiled rather than dried cassava root meal can be ascribed to the higher level of hydro-cyanide (HCN) precursors in the former, and the resultant toxic effect of the HCN on methanogens as reported by Rojas et al., (1999) and Smith et al., (1985). The increase in methane production as water spinach replaced cassava leaf meal could similarly be linked to decreasing concentration of hydro-cyanide (HCN) precursors and of condensed tannins in the substrates as water spinach replaced cassava leave meal, as there are no reports of either cyanogenic glucosides or of condensed tannins in water spinach. Decreased methane production due to condensed tannins in the diet has been described by several authors (Makkar et al., 1995; Grainger et al., 2009).

The mechanisms by which enteric methane is decreased by ensiling rather than drying of the cassava roots, and by supplementation with cassava leaves rather than water spinach, are thought to be related to the toxic effects on methanogens from cyanogenic precursors that are in greater concentration in the ensiled compared with dried cassava roots and in cassava leaves rather than water spinach. These apparent benefits in methane mitigation from low dietary concentrations of cyanogenic precursors have still to be verified in *in vivo* studies.

CONCLUSIONS

- There were consistent decreases in gas production and methane content of the gas when: ensiled cassava root replaced the dried root; and when cassava leaf meal replaced water spinach meal.
- Over the overall 24hour fermentation the methane production per unit of substrate DM mineralized was decreased by 18% by the combination of ensiling versus drying of the cassava root and replacement of water spinach by cassava leaf meal.

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CHAPTER 3:

EFFECT OF BREWERS' GRAINS ON FEED INTAKE, DIGESTIBILITY AND NITROGEN RETENTION IN LOCAL YELLOW CATTLE FED ENSILED CASSAVA ROOT SUPPLEMENTED WITH FRESH CASSAVA FOLIAGE OR WATER SPINACH AS A PROTEIN SOURCE

ABSTRACT

The experiment was carried out to study effects of brewers' grains and supplemented with sources of protein: cassava foliage and water spinach on feed intake, digestibility and nitrogen (N) balance in local yellow cattle fed ensiled cassava root, urea and straw as a basal diet. There were four local yellow male cattle assigned to 4 treatments combination in a 4*4 Latin square arrangement (2*2 factorial arrangement within a 4*4 Latin Square arrangement): BG-CSF: brewers' grains with cassava foliage, BG-WS: brewers' grains with water spinach; NBG-CSF: no brewers' grains with cassava foliage; NBG-WS: no brewers' grains with water spinach. Experimental periods were of 14days: 9 days for adaptation, 5 days for collecting feeds offered and refused to determine fed intake, and for collecting daily feces and urine to determine N balance and the last day of each periods for taking the rumen fluid from each animal in the morning to measure the pH and ammonia.

The results showed that adding small amount of brewers' grains at 5% dry matter (DM) to the diet of ensiled cassava root; urea and rice straw supplemented with either cassava foliage or water spinach as the main protein source increased the dry matter (DM) feed intake, the apparent DM digestibility and increased by 42% of nitrogen (N) retention. Similar but smaller benefits were found when water spinach replaced cassava foliage as the main source of (true) supplementary dietary protein. This research implicated that adding brewers' grains to cattle diet can be considered as an option to increase N retention and thus local yellow cattle performance.

Key words: *Bypass protein, by-product, soluble protein, rumen ammonia*

INTRODUCTION

Livestock production in tropical areas plays a crucial role, which extends beyond its traditional supply of meat and milk. Livestock are used for multiple purposes as means of transportation, capital, credit, meat, milk, social value, hides, and a source of organic fertilizer for seasonal cropping (Wanapat and Kang, 2013a; Jetana and Bintbihok, 2013). Cattle and buffaloes are important on smallholder farms in most developing countries to provide meat, milk, traction power and manure in integrated crop and livestock farming systems (Preston and Leng, 2009).

Cassava (*Manihot esculenta* Crantz) is an annual crop grown widely in the tropical and subtropical regions. Roots of cassava have high levels of energy (75-85% of soluble carbohydrate) and minimal levels of crude protein (2-3% CP); they have been used as a source of readily-fermentable energy (Kang et al., 2015; Polyorach et al., 2013). Cassava foliage is a by-product, considered to be a good source of bypass protein for ruminants (Ffoulkes and Preston, 1978; Wanapat et al., 2001; Promkot and Wanapat, 2003; Sath et al., 2008). It has been fed successfully to improve performance of sheep (Hue et al., 2008), goats (Ho Quang Do et al., 2002; Phengvichith and Ledin, 2007) and cattle (Wanapat et al., 2000; Thang et al., 2010) in fresh, wilted or dried form. Cassava leaves are known to contain variable levels of condensed tannins; about 3% in dry matter (DM) according to Netpana et al., (2001) and Bui Phan Thu Hang and Ledin, (2005).

Water spinach (*Ipomoea aquatica*) plays an important role for farmers in rural areas. It is easy to cultivate and has a very high yield of biomass with a short growth period; it can be harvested in dry or flood period (Sophea and Preston, 2001). The crude protein content in the leaves and stems can be as high as 32 and 18 % in dry basis, respectively (Ly Thi Luyen, 2003). Water spinach is widely used as human food, but at the same time this vegetable can be given to animals such as rabbits, pigs, poultry and small ruminants. Kongmanila et al., (2011) reported positive responses in feed intake and N retention when foliage from the Mango tree, which is rich in tannins and of low digestibility (Kongmanila et al., 2007) was supplemented with water spinach (*Ipomoea* *aquatica*), the protein which is considered to be highly degradable by rumen microbes (Kongmanila et al., (2007).

Brewers' spent grains (BG) are the major by-product of the brewing industry, representing around 85% of the total by-products generated (Mussato et al., 2006). It is a lignocelluloses material available in large quantities throughout the year. It is considered to be a good source of bypass protein (Promkot and Wanapat, 2003). The purpose of the present study was to identify adding small quantity of brewers' grains at 5% dry matter (DM) to diet and supplementary dietary protein from cassava foliage or water spinach on feed intake, digestibility and nitrogen (N) balance in local yellow cattle fed ensiled cassava root; urea and rice straw as a basal diet.

MATERIALS AND METHODS

Location and duration

The experiment was carried out at the farm area of the Department of Animal Science, Faculty of Agriculture and Forest Resource, Souphanouvong University, Luang Prabang province, Lao PDR.

Experimental design

The following treatments were applied in 2*2 factorial design within a 4*4 Latin square with 4 treatments. The treatment were:

- BG-CSF: Brewers' grains with cassava foliage
- BG-WS: Brewers' grains with water spinach
- NBG-CSF: No brewers' grains with cassava foliage
- NBG-WS: No brewers' grains with water spinach

The basal diets were fed ensiled cassava root ad-libitum, urea at 2% of diet DM and rice straw at 1% of live weight. Experimental periods (Table 1) were of 14 days: 9 for adaptation and 5 for collection the data.

Table 1: Layout of experimental design

Periods	Cattle			
	1	**2**	**3**	**4**
1	BG-WS	BG-CSF	NBG-WS	NBG-CSF
2	BG-CSF	NBG-WS	NBG-CSF	BG-WS
3	NBG-WS	NBG-CSF	BG-WS	BG-CSF
4	NBG-CSF	BG-WS	BG-CSF	NBG-WS

Animals and housing

The four local yellow male cattle with initial body weight of 90±1.0kg were confined in metabolism cages with the floor area of 100*130cm. The cage was designed in such a way that can separate feces and urine. Before the commencement of the experiment, the cattle were vaccinated against epidemic diseases and drenched to control internal parasites.

Feeding and management

The fresh cassava root was sliced by hand then ground, and stored anaerobically in a sealed plastic bag over 7days. Rice straw was chopped into small pieces (3-5cm) before feed to animals. Brewers' grains were bought from Beer Lao factory in Vientiane capital in Lao PDR. Water spinach and cassava foliage were brought from the farm of Souphanouvong University. Animals were adapted gradually over a 2 weeks period to the cages and the experimental diets before starting the experiment.

The ensiled cassava root was fed ad-libitum; urea offered at 2% of dry matter (DM) diet and rice straw was offered at 1% of live weight. Brewers' grains (BG) were fed at 5% of diet DM. Cassava foliage and water spinach were offered at 30% of dry matter (DM) intake. The feeds were offered two times a day at 7.00 am and 4.30 pm and water was always available.

Data collection and measurements

The cattle were weighed in the morning before feeding at the beginning of the trial and after finishing each experiment period of 14days. Feeds offered and refused were weighed and samples were collected daily to determine feed intake. Feces and urine were collected daily of each period (5days). There was added 100ml of a solution of 10% H_2SO_4 daily to the urine collector to maintain the pH below 4.0. At the end of each period (days 6), the samples of rumen fluid were taken 03 hours after feeding in the morning using a stomach tube and was measured the pH; and 10 ml were preserved with H_2SO_4 for determination of ammonia.

Chemical analyses

The samples of ensiled cassava root, cassava foliage, water spinach, brewers' grains were analyzed for the dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) in feed offered and refused according to standard methods (AOAC, 1990). The feces was analyzed for the dry matter (DM) and ash the urine and feces were analyzed for the nitrogen (N) according to AOAC, (1990) methods. The rumen fluid was analyzed for the pH and ammonia. The soluble nitrogen was determined by extraction with Molar NaCl according to the method as outlined in Whitelaw et al., (1963).

Statistical analyses

The data were analyzed with the general linear model (GLM) option of the ANOVA program in the Minitab software (Minitab 2010). Sources of variation were: period, animal, treatment effects and their interaction and random error. The statistical model used was:

$Y_{iljk} = \mu + b_i + p_l + (b*p)_{il} + C_j + R_k + e_{iljk}$

Where: Y_{iljk} is dependent variables; μ is overall mean; b_i is the effect of brewers' grains supplements (i= 1-2); p_l is the effect of cassava foliage or water spinach (l= 1-2); $(b*p)_{il}$ is the interaction between source of brewers' grains and source of protein; C_j is the effect of animal (j=1-4); R_k is the effect of period (j=1-4) and e_{iljk} is random error.

RESULTS AND DISCUSSION

Chemical composition of diet ingredients

The values for neutral detergent fiber (NDF) and acid detergent fiber (ADF) were lower, but ash and crude protein (CP) were similar, in water spinach compared to cassava foliage (Table 2). Brewers' grains had highest level of crude protein. N solubility in water spinach was twice that in cassava foliage with intermediate values for brewers' grains.

Table 2: Chemical characteristics of diet ingredients

Items	ECR	CSF	WS	RS	BG
DM, %	28.6	26.0	15.5	90.1	25.5
As % of DM					
Ash	4.28	9.79	11.2	13.1	5.93
CP	2.86	19.6	20.8	3.10	28.6
NDF	34.3	41.5	39.7	65.8	32.1
ADF	29.7	33.8	25.2	44.1	22.2
N solubility[#]	10.8	30.3	59.9	9.46	33.9

[#] % N soluble in Molar NaCl; ADF: acid detergent fiber; BG: brewers' grains; CSF: cassava foliage; CP: crude protein; DM: dry matter; ECR: ensiled cassava root; NDF: neutral detergent fiber; RS: rice straw; WS: water spinach;

Feed intake

Total daily intake of dry matter (DM) and intake per unit live weight (g/kg live weight) were higher when brewers' grains were fed and when water spinach replaced cassava foliage (Table 3; Figure 1a;b). The level of 11-12% crude protein in dry matter (DM) would appear to be adequate to maximize the feed intake on a basal diets.

Table 3: Mean values of DM feed intake of diets with or without brewers' grains supplemented with cassava foliage or water spinach in local yellow cattle fed a basal diet with ensiled cassava root; urea and rice straw

Items	By-product		Prob	Protein source		Prob	SEM
	Brewers' grains	No brewers' grains		Cassava foliage	Water spinach		
DM intake, g/day							
Ensiled cassava root	1538	1485	0.001	1521	1502	0.146	8.98
Rice straw	828	698	<0.001	744	781	0.057	13.4
Brewers' grains	242	-		126	116	0.008	2.53
Cassava foliage	249	225	0.076	462	-		
Water spinach	249	250	0.707	-	499		
Urea	33.4	33.3	0.131	33.5	33.3	0.003	0.04
Total, g	3138	2690	<0.001	2886	2943	0.070	21.9
g/kg LW	32.9	28.3	<0.001	30.2	31.0	0.020	0.24
CP, % in DM	12.1	11.1		11.5	11.7		

CP: crude protein; DM: dry matter; P: probability; SEM: standard error of the mean with df_{error} =6

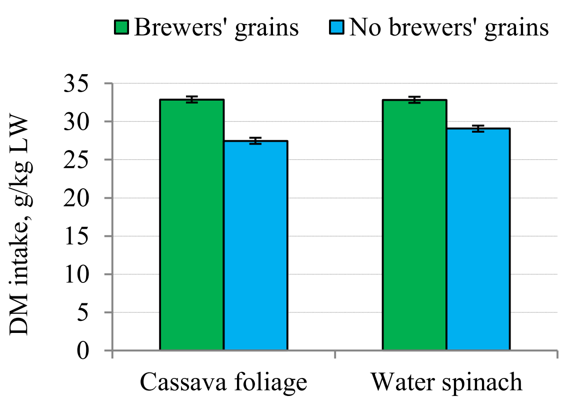

Figure 1a: Effect of with or without the brewers' grains on DM intake in local cattle fed a basal diet of ensiled cassava root, urea, rice straw and either fresh cassava foliage or water spinach of supplements

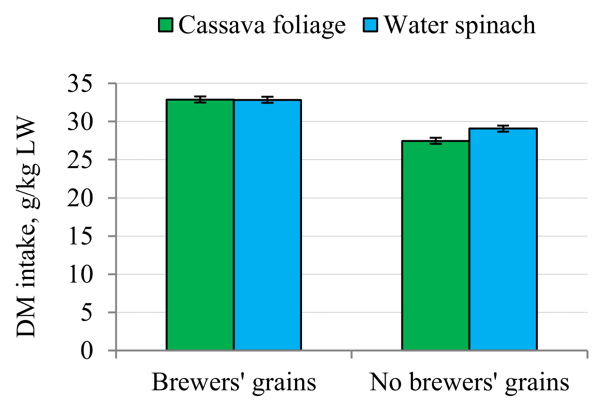

Figure 1b: Effect of supplements of cassava foliage or water spinach on DM intake of local cattle fed a basal diet of ensiled cassava root, urea and rice straw and with or without brewers' grains

Apparent digestibility, N balance and rumen ammonia

Apparent digestibility of dry matter (DM), organic matter (OM) and protein (CP) were higher when brewers' grains were fed and when the main protein source was water spinach rather than cassava foliage (Table 4; Figure 2a;b). N retention was higher when brewers' grains were fed and when water spinach rather than fresh cassava foliage was the main protein source (Table 4; Figure 3a;b).

Table 4: Mean values on apparent digestibility and N balance with or without brewers' grains supplemented with cassava foliage or water spinach in local yellow cattle fed a basal diet with ensiled cassava root; urea and rice straw

Items	By-product		Prob	Protein source		Prob	SEM
	Brewers' grains	No brewers' grains		Cassava foliage	Water spinach		
Apparent digestibility, %							
Dry matter	72.6	68.3	<0.001	68.5	72.4	0.001	0.79
Organic matter	73.8	69.7	<0.001	70.0	73.5	0.002	0.76
Crude protein	83.0	77.7	<0.001	79.1	81.6	<0.001	0.58
N balance, g/day							
Intake	50.3	37.1	<0.001	42.2	45.2	<0.001	0.36
Feces	10.2	10.7	0.228	10.9	10.0	0.005	0.24
Urine	8.51	7.69	0.001	7.98	8.22	0.311	0.10
N retention							
g/day	41.8	29.4	<0.001	34.2	37.0	<0.001	0.41
% of N intake	69.0	61.5	<0.001	64.0	66.5	0.003	0.59
% of N digested	83.1	79.2	<0.001	80.8	81.5	0.267	0.44
Rumen pH and ammonia							
pH	6.96	6.95	0.224	6.94	6.97	0.003	0.01
NH_3, mg/liter	234	230	<0.001	232	233	0.427	0.54

N: nitrogen; NH_3 : ammonia; P: probability; SEM: standard error of the mean with df_{error} =6

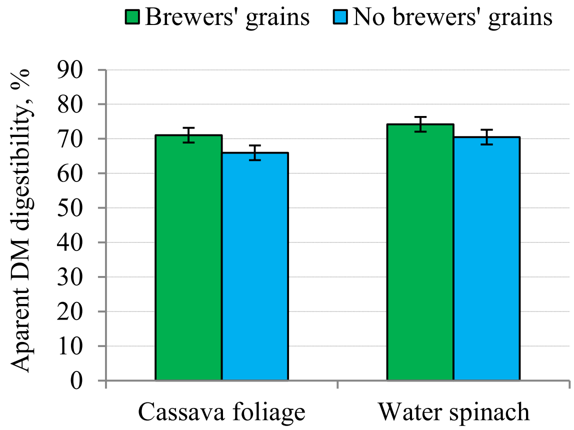

Figure 2a: Effect of with or without brewers' grains on DM digestibility in local cattle fed a basal diet of ensiled cassava root, urea, rice straw and either fresh cassava foliage or water spinach supplements

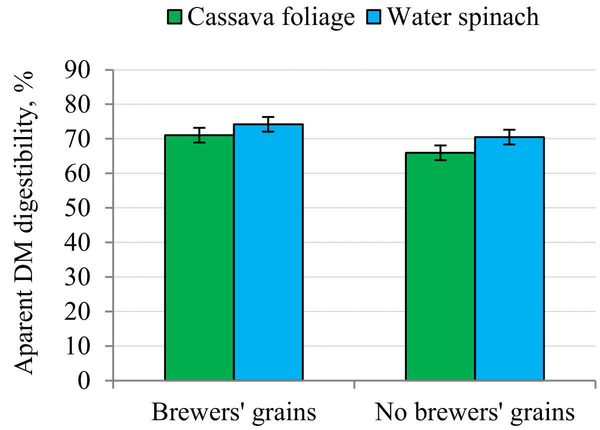

Figure 2b: Effect of supplements of cassava foliage or water spinach on DM digestibility of local cattle fed a basal diet of ensiled cassava root, urea and rice straw with or without brewers' grains

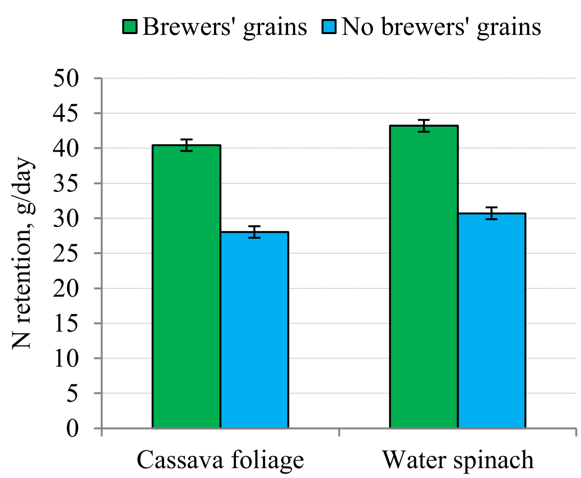

Figure 3a: Effect of with or without brewers' grains on N retention in local cattle fed a basal diet of ensiled cassava root, urea, rice straw and either fresh cassava foliage or water spinach supplements

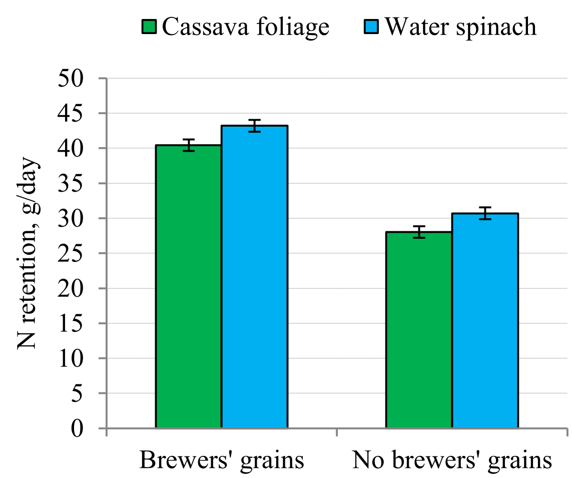

Figure 3b: Effect of supplements of cassava foliage or water spinach on N retention in local cattle fed a basal diet of ensiled cassava root, urea, rice straw with and without brewers' grains

The stimulation of growth in ruminants with small inputs of brewers' grains (BG) into a low true protein diet has been demonstrated in many publications (Preston and Leng, 1987) and has usually been attributed to the "escape" properties of the protein where ammonia levels in the rumen are adequate for optimal microbial growth. In the case reported here the large response in nitrogen (N) retention to a very small input of protein as brewers grains may be explained by a high level of protection (escape characteristics) or a high level of essential amino acids in the escape component. Brewers' grains are also rich in phenolic compounds, particularly ferulic acid and p-coumaric acid. These soluble secondary plant compounds could precipitate protein in cassava and water spinach foliage, thus enhancing their "escape protein" properties. In other words, the brewers' grains enhance the protein to energy ratio in the metabolisable protein arising in the intestines to digested and absorbed. This is a similar effect to that of fish meal added to a diet of molasses-urea fed to ruminants (Preston, 1971).

However, the dramatic increase in the nutritive value of the experimental diet (42% increases in nitrogen retention) as a result of supplementation with small quantities of brewers' grains (5% of diet DM) implies that the effect of this supplement was more than its contribution in enhancing the supply of escape protein. As a result of the process of fermentation by yeast in the production of beer, it can be expected that the residual "spent" grains would contain a range of "B" vitamins and other fermentation products such as the lactobacilli bacteria as well as the yeast per se. Beneficial effects on milk production and composition in dairy cattle have been reported from replacement of soybean meal by brewers' grains (Belibasakis and Tsirgogianni, 1996). Finally there are the known health benefits from soluble phenolic compounds, including ferulic acid and p-coumaric acid, manifested in anti-oxidant, anti-cancer, anti-atherogenic and anti-inflammatory effects. Thus potential prebiotic and probiotic benefits may also be expected from supplementation of cattle diets with small amounts of brewers' grains.

CONCLUSIONS

- Adding 5% of brewers' grains to a diet of ensiled cassava root; urea and rice straw supplemented with either cassava foliage or water spinach as the main protein source, increased the DM intake, the apparent dry matter (DM) digestibility and nitrogen (N) retention in local yellow cattle. Similar but smaller benefits were found when water spinach (WS) replaced cassava foliage (CFS) as the main source of (true) protein.

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CHAPTER 4:

EFFECT OF BREWERS' GRAINS AND RICE DISTILLERS' BYPRODUCT ON METHANE PRODUCTION IN AN *IN VITRO* RUMEN FERMENTATION USING ENSILED OR FERMENTED CASSAVA ROOT (*Manihot esculenta*, Crantz) AS A CARBOHYDRATE SOURCE

ABSTRACT

An *in vitro* rumen fermentation was carried out to study effects on methane production of ensiled cassava root compared with fermented cassava root and brewers' grains or rice-wine distillers' by-product or nor supplement. The treatments in a 2*3 factorial arrangement were (i) processing sources: ensiled cassava root or fermented cassava root and (ii) by-product sources: brewers' grains, rice distiller or no supplement. The quantity of substrate in each fermentation bottle was 12g DM to which was added 240 ml of rumen fluid (from slaughtered cattle) and 960 ml of buffer solution. The incubations were done in a simple *in vitro* system using 1.5liters bottles with gas collection by water displacement and methane measured by an infra-red methane gas detector. The incubation was for 48 hours with measurements of total gas production and methane percentage in the gas at intervals of 03, 06, 24 and 48hours.

The results showed that fermented cassava root with yeast, urea and DAP increased the true protein content from 1.8 to 7.6% in DM. Gas production was lower for fermented than ensiled cassava root but was increased by supplementation with brewers' grains and rice distillers' by-product. The concentration of methane in the gas increased with the duration of the incubation and was lower for the fermented rather than the ensiled cassava root. The DM mineralized during the incubation was less for the fermented than the ensiled cassava root but was not affected by supplementation with brewers' grains or rice distillers' by-product when supplemented. Methane production per unit substrate DM fermented was less for the fermented compared with the ensiled cassava root and was reduced by supplementation with brewers' grains or rice distillers' by-product. Cassava root fermented with yeast, urea and DAP had a positive effect on digestion in the intestines

and fermentation in the cecum-colon as disposal of H_2 in fermentative degradation in the cecum-colon appears to be dominated by acetogenesis. This research implicated that methane production can be reduced when fermented cassava root was used instead of ensiled cassava root as source of carbohydrate and and when supplemented with brewers' grains or rice distillers' by-product.

Keywords: *acetogenesis, di-ammonium phosphate, DAP, mineralization, urea, yeast*

INTRODUCTION

Global warming, caused by increasing atmospheric concentrations of greenhouse gases, is major worldwide environmental, economic, and social threat, and it is well documented that livestock production contributes to this problem (O'Mara, 2011). Ruminants are estimated to produce up to 95 million tonnes of methane (CH_4) annually and are implicated as a major source of greenhouse gas production (Patra, 2014). Enteric methane 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. Kebreab et al., (2008) showed that methane emissions inventories were more accurately estimated through diet-specific mechanistic models. Chagunda et al., (2010) indicated that, in order to mitigate methane emissions in a way acceptable for both the environment and animal welfare, it was important to quantify the effects of different diets on methane emissions. Other results suggest that a major difference is needed in dietary starch concentration in order to alter ruminal methanogenesis (Hassanat et al., 2013).

Brewers' grains are the major by-product of the brewing industry, representing around 85% of the total by-products generated (Mussato et al., 2006). It is a lignocellulosic material available in large quantities throughout the year. It is considered to be a good source of bypass protein (Promkot and Wanapat, 2003). Many factors are known to affect the microbial ecology of wine production and yeasts, as both lactic acid and bacteria are involved in different ways in winemaking. Several studies showed the positive effects of spontaneous fermentations on the organoleptic complexity of wine as a consequence of the growth of different species and/or strains together at high levels (Le Jeune et al., 2006; Wondra and Boveric, 2001). Rice distillers' by-product is the residue from production of "rice wine" which is an alcoholic drink made from sticky rice, maize, sweet potato, cassava or bananas (Oosterwijk and Vongthilath, 2003). Studies in Vietnam by Luu Huu Manh et al., (2000) and Luu Huu Manh et al., (2009) reported 23% of high quality protein of rice distillers' by-product in the DM. These authors suggested that this by-product was appropriate for supplementing feeds of lower nutritional density such as rice bran and forages. More recently, Taysayavong et al., (2010) in a study in Lao PDR reported increased growth rates from the feeding of rice distillers' by-product to pigs.

Cassava (*Manihot esculenta,* Crantz) is an annual crop grown widely in the tropical and subtropical regions. Roots of cassava have high levels of energy (75 to 85% of soluble carbohydrate) and minimal levels of crude protein (2 to 3% CP); they have been used as a source of readily-fermentable energy in cattle diets (Kang et al., 2015; Polyorach et al., 2013). Cassava foliage is an agricultural by-product, considered to be a good source of bypass protein for ruminants (Ffoulkes and Preston, 1978; Wanapat et al., 2001; Promkot and Wanapat, 2003; Keo Sath et al., 2008). It has been fed successfully to improve performance of sheep (Hue et al., 2008), goats (Do et al., 2002; Phengvichith and Ledin, 2007) and cattle (Wanapat et al., 2000; Thang et al., 2010) in fresh, wilted or dried form. Cassava leaves are known to contain variable levels of condensed tannins; about 3% in DM according to Netpana et al., (2001) and Bui Phan Thu Hang and Ledin, (2005). Cassava contains cyanogenic glucosides, mainly linamarin, which release hydrogen cyanide after hydrolysis in the rumen by an endogenous linamarase (Butler et al., 1965). Anaerobic digestion can be inhibited by cyanide, because of the high sensitivity of methanogenic bacteria to this compound (Eikmanns and Thauer, 1984; Smith et al., 1985). Cuzin and Labat, (1992) showed that additions of 5, 10 and 25 mg/litre of cyanide (HCN or linamarin) temporarily inhibited methanogenic bacteria.

A recent development in Lao PDR is the industrial production of starch for export using cassava roots as the feedstock. There are presently 5 factories in operation with a yearly demand of 200,000 tones of cassava roots. The extracted starch accounts for some 60% the root DM, the remainder being a by-product known as cassava pulp. Recent research has demonstrated that the pulp is of similar nutritive value to the original cassava root (Phanthavong et al., 2016) and can be used as the basis of an intensive system of cattle fattening. This development has led to increased interest in the use of the whole cassava roots as the basis of a feeding system for cattle. The roots contain 60 to 70% moisture and are usually sun-dried or ensiled for long-term storage. The fresh roots can also be fermented (Manivanh and Preston, 2016) with the objective of enhancing the protein content which in the fresh roots is only of the order of 2-3% in DM. The purpose of the present study was to compare ensiled versus fermented cassava roots as the source of processing in an *in vitro* rumen fermentation and to investigate the effect of incorporating small quantities of brewers' grains based on the observations by Phanthavong et al., (2016) that the former appeared to have "probiotic" effects in counteracting the apparent toxicity caused by feeding cattle with fresh cassava foliage when this was derived from a bitter variety with known high levels of cyanogenic glucosides. Rice distillers' by-product was included as an additional treatment in view of its similarity to brewers' grains in terms of being a by-product from the fermentation of a cereal grain (rice) to make rice "wine". Therefore, the objective of this study to study effects on gas and methane production of ensiled cassava root compared with fermented cassava root and brewers' grains or rice distillers' by-product or nor supplement in an *in vitro* rumen fermentation.

MATERIALS AND METHODS

Location and duration

The experiment was conducted in the laboratory of the Animal Science department, Faculty of Agriculture and Forest Resource, Souphanouvong University, Lao PDR.

Experimental design

The experimental design was a 2*3 factorial arrangement in a completely randomized design (CRD), 6 treatments combinations, and each with 4 replicates. The experimental factors were:

Cassava root processing:

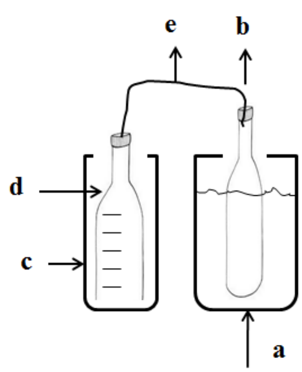
- ECR: Ensiled cassava root
- FCR: Fermented cassava root

Brewers' grain and rice distiller supplement:

- No: No supplement
- BG: Brewers' grains
- RDB: Rice distiller

***In vitro* rumen fermentation system**

The *in vitro* rumen fermentation system was as described by Sangkhom Inthapanya et al., (2011). The water bottles (capacity 1.5liters) were used for the fermentation and collection of the gas. A hole was made in the lid of each of the bottles, which were interconnected with a plastic tube (id 4mm). The bottle receiving the gas had the bottom removed and was suspended in a larger bottle (3liters capacity) partially filled with water, so as to collect the gas by water displacement. The bottle that was suspended in water was calibrated at 50ml intervals to indicate the volume of gas (Diagram 1).



a. Water bath
b. Fermentation bottle (1.5liters)
c. Water storage reservoir (3liters)
d. Gas collection bottle (1.5litrers)
e. Plastic tube (0.4m of length and 4mm of diameter)

Diagram 1: A schematic view of apparatus to measure in an in vitro rumen fermentation

Experimental procedure

The cassava root and leaves were collected from farm of the Faculty of Agriculture and Forest Resource, Souphanouvong University, Lao PDR. The fresh cassava root was chopped into small pieces around 1-2cm of length and ground in a liquidizer, and then stored anaerobically in a plastic bag for ensiling over 7days. For the process of fermenting the cassava root, the additives (% DM basis) were 3% of yeast, 1% of di-ammonium phosphate (DAP), 3% of urea and 1% of sulphur-rich minerals (Table 1). These were mixed with the ground cassava root and the mixture stored anaerobically in a sealed plastic bag for fermenting over 7 days.

Table 1: Ingredients used in the fermentation of cassava root

Items	DM basis, %
Cassava root	92
Yeast	3
DAP (Di-ammonium phosphate)	1
Urea	3
Mineral#	1
Total	100
Crude protein, % in dry matter (DM)	13.4

Cassava leaves were chopped into small pieces around 1-2cm of length and dried in an oven at 80ºC for 24hours before grounding through a 1mm sieve by machine. Brewers' grains are bought from Lao beer factory in Vientiane, Lao PDR and the rice distillers' was collected from farmers who make "Lao Kao" wine in Luang Prabang province.

Amounts of the substrates equivalent to 12g DM were put in the incubation bottle, including the cassava leaf meal was offered to all the substrate at 30% of DM, the urea at 2% of DM, and the sulphur-rich minerals was added to all the substrates at 1% of DM. Both of brewers' grains and rice distillers supplement were offered at 4% of DM substrate. They were added 0.96 liters of buffer solution into the bottles and 240 ml of rumen fluid obtained from a cow immediately after being slaughtered. The bottles were then filled with carbon dioxide and incubated at 38 ^{0}C in a water bath for 48 hours.

Table 2: Ingredients of the buffer solution

Ingredients	$CaCl_2$	$NaHPO_4.12H_2O$	NaCl	KCl	$MgSO_4.7H_2O$	$NaHCO_3$	Cysteine
(g/liter)	0.04	9.30	0.47	0.57	0.12	9.80	0.25

***Source**: Tilly and Terry (1963).*

Data collection and measurements

During the incubation the gas volume was recorded at each period of 0-3, 3-6, 6-12, 12-24 and 24-48 hours. After each time interval, the methane concentration in the gas was measured with a Crowcon infra-red analyser (Crowcon Instruments Ltd, UK). At the end of the incubation, the contents of the incubation bottle were filtered through cloth to determine the mineralization of the substrates.

Chemical analyses

Samples of ensiled cassava root, fermented cassava root, brewers' grains, rice distillers and cassava leave meal were analyzed for dry matter (DM), crude protein (CP) and ash following AOAC, (1990). The soluble protein was determined by extraction with Molar NaCl according to the method as outlined in Whitelaw et al., (1963). True protein (TP) in the ensiled cassava root and fermented cassava root was measured after precipitation with Trichlor-acetic acid according to AOAC, (1990) methods.

Statistical analyses

The data were analyzed with the General Linear Model (GLM) option in the ANOVA program of the Minitab software (version 16.0). In the model the sources of variation were treatments, treatment interaction and random error. Turkey's pair-wise comparison was used to determine the differences between means. The statistical models used as:

$$Y_{ijk} = \mu + p_i + b_j + (p*b)_{ij} + e_{ijk}$$

Where: Y_{ijk} are dependent variable; μ is overall mean; p_i is effect of cassava root processing (either ensiled or fermented); b_j is effect of supplement brewers' grain or rice distiller or no supplement; $(p*b)_{ij}$ is the interaction between the two factors; e_{ijk} is random error.

RESULTS AND DISCUSSION

Chemical composition

The true protein content of the cassava root fermented with urea, DAP and yeast increased from 1.8 to 7.6% in DM, similar to that reported for the same procedure by Vanhnasin and Preston, (2016) and Manivanh and Preston, (2016).

The protein content and the solubility of the protein were similar in the brewers' grains and in the rice distillers (Table 3). However, as the proportions in the substrate were low (4% of the DM) their role as sources of protein would be small as compared to the protein derived from cassava leaf meal.

Table 3: The chemical composition of the feed ingredients

Items	DM, %	Ash	CP	TP	CP
		As % of DM			solubility, %
Ensiled cassava root	31.7	4.24	2.84	1.79	
Fermented cassava root	32.1	4.27	13.4	7.56	
Cassava leaf meal	90.3	9.82	19.8		29.3
Brewers' grains	26.3	5.95	26.4		33.6
Rice distiller	8.66	5.42	24.2		37.8
Yeast	90	5.74	50		31.2
Urea	100		280		
DAP	100		113		

DAP: di-ammonium phosphate; DM: dry matter, CP: crude protein, TP: true protein

Gas production

Gas production was higher for ensiled than fermented cassava root in the first 12 hours and for the total incubation over 48 hours. Both brewers' grains and rice distillers were increased the gas production compared to the control that was not supplemented (Table 4; Figure 1).

Table 4: Mean values of gas production, percent of methane in the gas and dry matter (DM) digestibility in an in vitro rumen fermentation using ensiled or fermented cassava root supplement with brewers' grains or rice distiller or no supplement.

Items	**Processing**		**SEM (df=12)**	**Prob**	**By-product**			**SEM (df=8)**	**Prob**	**Interaction (P*B)**
	Ensiled	**Fermented**			**No**	**BG**	**RBD**			**Prob**
Gas production, ml										
0-3hr	196	167	0.094	150^{b}	188^{ab}	206^{a}	14.28	0.036	196	0.647
3-6hr	504	421	<0.001	438^{b}	463^{ab}	488^{a}	11.41	0.021	504	0.676
6-12hr	571	492	<0.001	500^{b}	538^{ab}	556^{a}	13.98	0.032	571	0.936
12-24hr	1029	1038	0.799	969^{b}	1063^{a}	1069^{a}	27.95	0.036	1029	0.319
24-48hr	771	733	0.146	700^{b}	788^{a}	769^{ab}	21.35	0.023	771	0.559
Total gas, ml	196	167	0.094	150^{b}	188^{ab}	206^{a}	14.28	0.036	196	0.27
Methane production, %										
3-6hr	9.0	8.8	0.608	10.0^{a}	8.5^{b}	8.3^{b}	0.276	0.001	9.0	0.764
6-12hr	18.0	14.6	<0.001	16.9^{a}	16.8^{a}	15.3^{a}	0.473	0.047	18.0	0.238
12-24hr	28.9	24.3	<0.001	28.1^{a}	26.5^{b}	25.1^{b}	0.400	<0.001	28.9	0.458
24-48hr	38.0	34.4	<0.001	38.0^{a}	36.0^{b}	34.6^{b}	0.410	<0.001	38.0	0.807
Total methane, ml	3071	2850	<0.001	2756^{b}	3038^{a}	3088^{a}	42.24	<0.001	3071	0.697
DM mineralized, %	737	613	<0.001	668^{a}	695^{a}	662^{a}	13.62	0.222	737	0.939
Methane,ml/g substrate	85.1	75.9	0.77	<0.001	82.8^{a}	80.5^{ab}	78.1^{b}	0.95	0.009	0.614

^{a,b,c} *values on the same row with different superscripts differ (P<0.05); BG: brewers' grains; No: no supplement; P: probability; RDB: rice distiller*

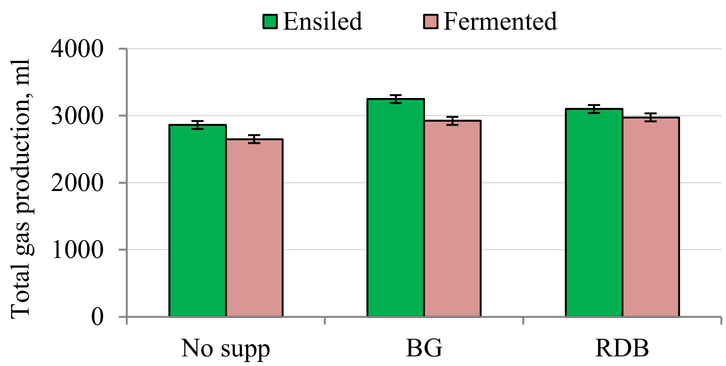


Figure 1: Total gas production from ensiled or fermented cassava root and different supplements of no supplement (No supp) or brewers' grains (BG) or rice distillers (RDB)

On all the treatments, the concentration of methane in the gas increased with the duration of the incubation (Table 4), was lower values being recorded for the fermented rather than the ensiled cassava root; lowest values for rice distiller rather than brewers' grains or no supplement (Figures 2 and 4). The DM digested during the incubation was less for the fermented than the ensiled cassava root but was not affected by supplementation with brewers' grains or rice distillers' by-product (Figure 4). Methane production per unit substrate was less for fermented than for ensiled cassava root and was reduced by supplementation with brewers' grains and rice distillers' by-product (Figures 5 and 6).

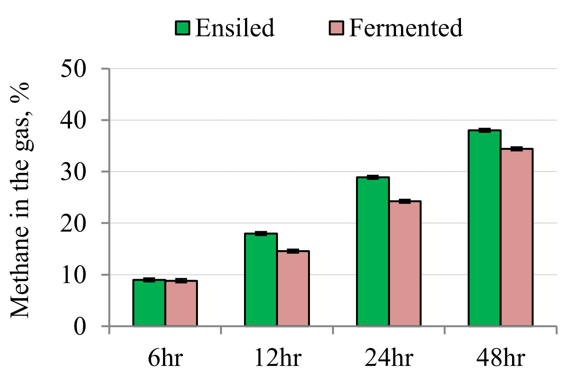


Figure 2: The percentage of methane in the gas at each fermentation interval for substrates with ensiled or fermented cassava root

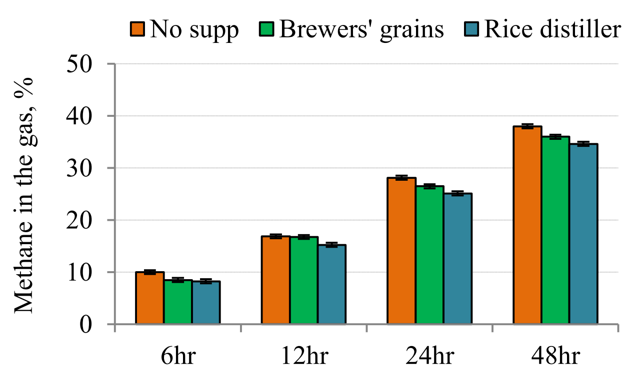


Figure 3: The percentage of methane in the gas at each fermentation interval for no supplement (No supp), brewers' grains (BG) or rice distillers (RDB)

The increases in methane concentration in the gas with fermentation time are similar to the findings by several researchers (Inthapanya et al., 2011; Binh Phuong et al., 2011; Thanh et al., 2011; Outhen et al., 2011) who used a similar *in vitro* system but with different substrates.

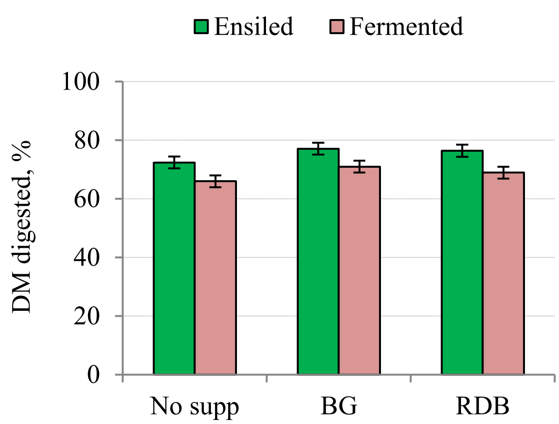


Figure 4: DM digested after 48h from ensiled or fermented cassava root and different supplements of no supplement (No supp) or brewers' grains (BG) or rice distillers' by-product (RDB)

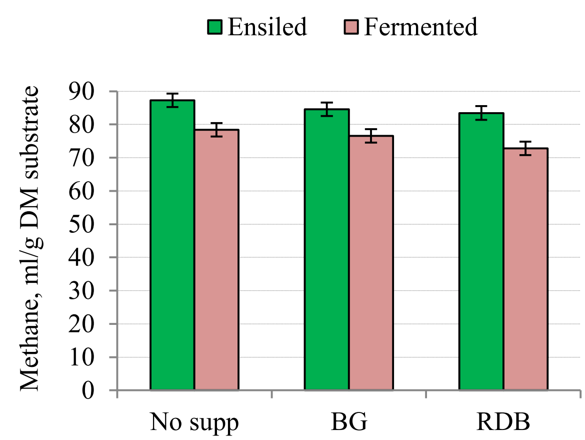


Figure 5: Methane per unit substrate from ensiled or fermented cassava root and different supplements of no supplement (No supp) or brewers' grains (BG) or rice distillers' by-product (RDB)

Methane production per unit substrate

The lower values for DM mineralized with fermented compared with ensiled cassava root are in line with the values for gas production which was lower for fermented than ensiled cassava root. This can be explained the fact that yeast fermentation results in part of the carbohydrate being converted to protein. Yeast protein is of low solubility and thus will be fermented to only a small extent in the in vitro rumen, the overall effect being to decrease the gas production and percentage DM mineralized. The effect of fermentation is thus to change the balance of the site of digestion with less nutrients being fermented in the rumen relative to those digested in the intestines and fermented in the cecum-colon. As disposal of hydrogen in fermentative degradation in the cecum-colon appears to be dominated by acetogenesis (Demeyer, 1991; Immig, 1996; Popova et al., 2013; Leng, 2016). This would account for the decreased methane production for the fermented compared with the ensiled cassava root.

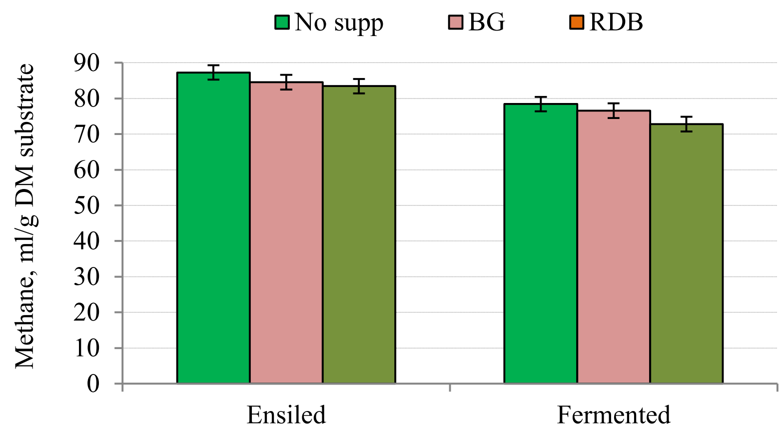


Figure 6: Methane per unit substrate from ensiled or fermented cassava root and different supplements of no supplement (No supp) or brewers' grains (BG) or rice distillers' by-product (RDB)

The reduction in methane production due to supplementation with brewers' grains and rice distillers' solubles is supported by research in growing goats fed cassava foliage when 4% brewers' grains in the diet led to decreased production of methane (Vor Sina, 2016, personal communication). A similar response was reported by Sengsouly and Preston, (2016) in cattle fed on ensiled cassava root, the methane production was decreased when the diet was supplemented with 4% of rice distillers' by-product.

CONCLUSIONS

- Fermenting cassava root with yeast, urea and DAP increased the true protein content from 1.8 to 7.6% in DM.
- Gas production was lower for fermented than ensiled cassava root but was increased by supplementation with brewers' grains and rice distillers' by-product.
- The concentration of methane in the gas increased with the duration of the incubation and was lower for the fermented rather than the ensiled cassava root.

- The DM mineralized during the incubation was less for the fermented than the ensiled cassava root but was not affected by supplementation with brewers' grains or rice distillers' by-product.
- Methane production per unit substrate was less for the fermented compared with the ensiled root and was reduced by supplementation with brewers' grains and rice distillers' by-product.

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CHAPTER 5:

EFFECT OF SUPPLEMENTS OF YEAST (*Saccharomyces cerevisiae*), RICE DISTILLERS' BY-PRODUCT AND FERMENTED CASSAVA ROOT ON METHANE PRODUCTION IN AN *IN VITRO* RUMEN INCUBATION OF ENSILED CASSAVA ROOT, UREA AND CASSAVA LEAF MEAL

ABSTRACT

An *in vitro* rumen incubation was carried out to determine effects on methane production of supplementing ensiled cassava root, urea and cassava leaf meal with rice distillers' by-product, fermented cassava root and yeast (*Saccharomyces cerevisiae*). The four treatments in a completely randomized design were: CTL: No supplement; RDB: 4% (in DM) rice distillers' by-product; FCR: 4% (in DM) urea-fermented cassava root; Yeast: 1% (in DM) commercial yeast. The quantity of substrate in each fermentation bottle was 12g DM to which was added 240 ml of rumen fluid (from slaughtered cattle) and 960 ml of buffer solution. The incubations were done using water bottles with gas collection by water displacement. Measurements of total gas production and methane percentage in the gas were made at intervals of 0-3, 3-6, 6-12, 12-18 and 18-24hours.

The hourly rate of gas production increased to a maximum in the 3-6hours of incubation interval and then decreased linearly. In contrast, the proportion of methane in the gas increased linearly with incubation interval from the beginning until the final 18-24hours interval. Total gas production was highest for the fermented cassava root additive, followed by the rice distillers' byproduct with lowest values for the control and yeast treatments. The methane content of the gas was highest for the control treatment, followed by the fermented cassava root and yeast with the lowest value for rice distillers' by-product, for which the overall reduction in methane was of the order of 25%. Methane production per unit DM digested showed a similar trend as the methane percentage in the gas. It is suggested that the benefits from brewers' grains and rice distillers' by-product, in reducing methane production in the rumen fermentation, both *in vitro* and *in vivo,* are the

indirect effects of these additives increasing the proportions of propionic acid in the rumen VFA.

Key words: *β-glucan, greenhouse gas, hydrolysis, propionate, urea*

INTRODUCTION

Agriculture is an important source of greenhouse gas mainly through emissions of methane from enteric fermentation in ruminants and decomposition of manure (Gerber et al., 2013). Ruminants are estimated to produce up to 95 million tonnes of methane annually, mainly from enteric fermentation and to a lesser extend from decomposition of manure (O'Mara, 2011; Patra, 2014).

Strategies to reduce these emissions should first address the need to increase productivity of ruminant livestock. This will reduce methane emissions per unit of livestock product-meat and milk (Diagram 1); secondly, the feeding systems that lead to increased ruminant productivity are those that lead to increased proportions of propionic acid; and thirdly, the escape of protein from the rumen contributes amino acids directly to the animal through enzymic digestion of protein in the intestines. The additional advantage of this process is that fibrous feed particles that escape the rumen attached to the protein will still be fermented to useful end products but in the cecum-colon section of the ruminant digestive tract in which the fermentation process does not produce methane (Demeyer, 1991).

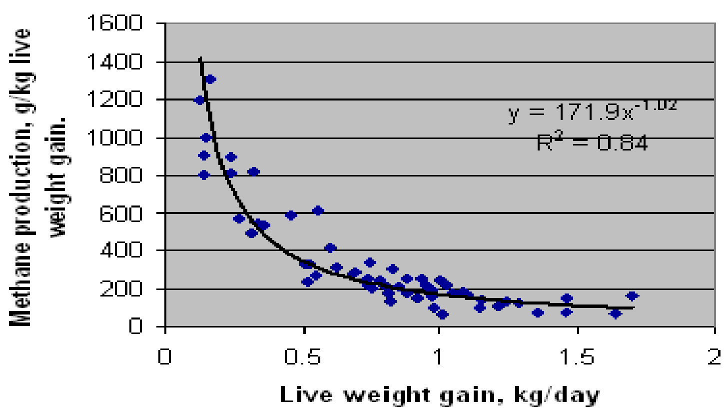


Diagram 1: Relationship between growth rate of cattle (kg/d) and the production of methane (g/kg live weight gain) (Klieve and Ouwerkerk, 2007)

In countries located in temperate latitudes, cereal crops such as maize and barley are the choice of feeds for intensifying ruminant production. Maize is grown in tropical latitudes but yields do not compete with those in temperate climates. By contrast, cassava (*Manihot esculenta* Crantz) is a crop that originated in the tropics (in the Caribbean) and is now grown in over 90 countries world-wide (Lebot, 2009). Of importance in a warming world is that cassava is potentially highly resilient to future climatic changes and according to Jarvis et al., (2012) "could provide Africa with options for adaptation whilst other major food staples face challenges".

Regarding the use of cassava for cattle production, these developments have been the driving force for a series of researches directed at optimising the use of both the pulp and the fresh cassava root as the basis of intensive systems of livestock production, especially the fattening of local cattle (Phanthavong et al., 2014; 2015; 2016; 2017; Inthapanya et al., 2016). An additional advantage of cassava over cereal crops such as maize is that the foliage has proved to be a valuable source of bypass protein such that the cassava plant becomes a source both of highly digestive carbohydrate (from the root) as well as protein (from the foliage). The only additional features needed are a source of fermentable nitrogen (available locally as fertilizer grade urea) and minerals.

The development of cattle feeding systems based on cassava has stimulated an important outcome, namely how to manage the potential toxicity linked with the presence throughout the plant of cyanogenic glucosides that give rise to hydrocyanic acid when exposed to favorable conditions (eg: appropriate enzymes) in the plant itself or within the digestive of animals that consume it. Recent research, much of it in the laboratory of the senior author of this paper, has shown that the potentially toxic cyanogenic glucosides in cassava can be neutralized by supplementing the animal diet with small quantities of locally available by-products from fermentation industries, specifically from the production of beer which gives rice to "brewer' grains"; and the artisan distillation of fermented rice to make an alcoholic wine, which produces a by-product known in Lao PDR as "Quilao", in Vietnam as "Hem" and in Cambodia as "Bar Ran". Brewers' grains have been shown to aid directly in the detoxification of HCN in cases where forage of a "bitter" (high HCN potential variety) was fed (Binh et al., 2017). Both brewers' grains and rice distillers' by-product, fed at less than 5% of the diet, have resulted in reduced production of rumen methane and improved growth rates in cattle fed ensiled cassava pulp-urea of ensiled cassava root-urea as basal diet (Keopaseuth et al., 2016; Sengsouly and Preston, 2016).

Access to brewers' grains is limited to farmers living in close proximity to the beer factory. Rice distillers' by-product is more widely available in rural areas, but supplies are limited. For these reasons, research to identify alternatives to both brewers' grains and rice distillers' by-product are considered to be of high priority. As both brewers' gains and rice distillers' by-product are products of fermentation by yeast (specifically *Saccharomyces cerevisiae*), it was decided to evaluate: (i) a commercial source of yeast commonly available in local markets; and (ii) cassava root enriched by yeast fermentation with additional sources of nitrogen (urea) and phosphorus (diammonium phosphate-DAP).

The purpose of the present study was to determine effects on methane production in an *in vitro* rumen fermentation when yeast, rice distillers' by-product, or fermented cassava root, were added in small amounts (4% DM basis) to a basal substrate of ensiled cassava root supplemented with urea and cassava leaf meal.

MATERIALS AND METHODS

Location and duration

The experiment was conducted in the laboratory of the Department of Animal Science, Faculty of Agriculture and Forest Resources, Souphanouvong University, Luang Prabang province, Lao PDR.

Experimental design

The experimental design was completely randomized (CRD), 4 treatments, and each with 5 replicates. The treatments were:

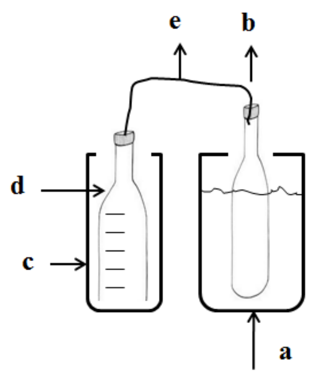
- CTL: Ensiled cassava root
- RBD: CTL + rice distillers' by-product at 4% of DM

- FCR: CTL+ fermented cassava root at 4% of DM
- Yeast: CTL+ yeast at 1% of DM

All the diets included urea (2% of root DM), cassava leaf meal from a bitter variety (25% of substrate DM) and S-rich minerals (1% of DM substrate).

***In vitro* rumen fermentation system**

The *in vitro* rumen fermentation system (Diagram 2) was as described by Sangkhom et al (2011). The water bottles (1.5liters) were used for the fermentation and collection of the gas. A hole was made in the lid of each of the bottles, which were interconnected with plastic tube (id 4mm). The bottle receving the gas had the bottom removed and was suspended in a larger bottle (3liters capacity) partially filled with water, so as to collect the gas by water displacement. The bottle that was suspended in water was calibrated at 50ml intervals to indicate the volume of gas.



a. Water bath
b. Fermentation bottle (1.5liters)
c. Water storage reservoir (3liters)
d. Gas collection bottle (1.5litrers)
e. Plastic tube (0.4m of length and 4mm of the diameter)

Diagram 2: A schematic view of apparatus to measure in an in vitro rumen fermentation

Experimental procedure

The cassava roots and leaves were collected from the Souphanouvong Universitys' farm; the roots were chopped into pieces around 1-2cm of length, ground in a liquidizer, and then stored in a plastic bag for ensiling over 7days. Cassava leaves were chopped into small pieces around 1-2cm in length, then dried in the oven at 80ºC for 24hours before grinding. The rice distillers' by-product was collected from a farmer accustomed to produce "rice wine alcohol". The procedure for producing "fermented cassava root" was as follows: the roots were chopped and steamed for 30 minutes, allowed to cool for

15mintutes and then mixed with di-ammonium phosphate (DAP) at 0.8%; 3% of yeast (*Saccharomyces cerevisiae*) and 2% of urea (all on DM basis) prior to being fermented in a closed plastic bag for 7days. Representative samples (12g DM) of the substrates was put in the incubation bottle to which was added 0.96 liters of buffer solution (Table 1) and 240ml of rumen fluid (obtained from a slaughtered cow) prior to filling each bottle with carbon dioxide. The bottles were incubated at 38 ^{0}C in a water bath for 24hours.

Table 1: Ingredients of the buffer solution

Ingredients	**$CaCl_2$**	**$NaHPO_4.12H_2O$**	**NaCl**	**KCl**	**$MgSO_4.7H_2O$**	**$NaHCO_3$**	**Cysteine**
(g/liter)	0.04	9.30	0.47	0.57	0.12	9.80	0.25

Source: *Tilly and Terry, (1963).*

Data collection and measurements

The gas volume was recorded over intervals of 0-3hours, 3-6hours, 6-12hours, 12-18hours and 18-24hours. The methane concentration in the gas collected over each interval was measured with a Crowcon infra-red analyser (Crowcon Instruments Ltd, UK). At the end of the incubation, the remaining substrate was filtered through cloth and the solid residue dried at 100C to determine the DM digested.

Chemical analyses

Samples were analyzed for DM, ash, crude protein and crude fiber according to AOAC (1990) methods. The solubility of the protein in diet ingredients was determined by extraction with M NaCl according to the method as outlined in Whitelaw and Preston (1963).

Statistical analyses

The data were analyzed by the general linear model option of the ANOVA program in the Minitab software (version 16.0). In the model the sources of variation were: treatments, and error. Tukey's pair-wise comparisons was used to determine the differences between treatments when the P value of F test <0.05. The statistical model used was:

$Y_{ij} = \mu + T_j + e_{ij}$

Where: Y_{ij} was the dependent variable, μ the overall mean; T_j the effect of treatment, and e_{ij} was random error.

RESULTS AND DISCUSSION

Chemical composition

The values for DM, crude protein and solubility of the protein in the rice distillers' by-product (Table 2) were similar to those reported for the same product by Luu Huu Manh et al., (2009) and Sangkhom et al., (2017). The cassava root after fermentation with yeast, urea and DAP had a similar level of crude protein as reported for the same procedure by Vanhnasin et al., (2016) and Manivanh et al., (2016).

Table 2: Chemical composition of substrate components

Items	DM, %	CP	CF	Ash	Soluble CP
		As % of DM			% of total CP
Ensiled cassava root	32.1	2.51	1.12	0.88	9.22
Fermented cassava root	39.9	10.4	1.13	0.87	11.6
Bitter cassava leaf meal	90.3	19.0	15.2	6.14	31.3
Rice distillers' by-product	7.46	24.2	2.29	4.45	37.9
Yeast		45.0			32.4

CP: crude protein; CF: crude fiber; DM: dry matter

Gas production

Gas production was higher for fermented cassava root than rice distillers' by-product and yeast or control treatment (Table 3), whereas the methane content of the gas was differed among treatments with highest values for the control treatment, followed by the fermented cassava root, yeast and rice distillers' by-product, respectively.

Table 3: Mean values for gas production, methane in the gas, digestibility and methane per unit substrate digested

Items	CTL	FCR	Yeast	RDB	SEM	Prob
Gas production, ml						
0-3hr	360^a	420^b	370^a	380^a	11.7	0.012
3-6hr	510^a	700^b	520^a	580^a	36.7	0.008
6-12hr	760^a	1070^b	820^a	950^c	36.7	<0.001
12-18hr	640^a	940^b	690^{ac}	720^c	20.6	<0.001
18-24hr	470^a	630^b	480^a	600^b	19.0	<0.001
Methane, %						
0-3hr	8.4^a	7.8^a	6.4^b	6.4^b	0.28	0.001
3-6hr	11.4^a	10^b	8.4^c	7.6^c	0.35	<0.001
6-12hr	18.6^a	17.6^a	15^c	12.6^b	0.70	<0.001
12-18hr	22^a	21.2^a	19.6^c	17^b	0.39	<0.001
18-24hr	24.2^a	23.6^a	21.2^c	19^b	0.37	<0.001
Total gas, ml	2740^a	3760^b	2880^a	3230^c	62.7	<0.001
Total methane, ml	484^b	637^c	427^a	426^a	11.5	<0.001
Methane, % total gas	17.7^d	16.9^c	14.8^b	13.2^a	0.227	<0.001
DM digestibility, %	59.2^a	69.4^b	61.1^a	65.2	0.83	<0.001
CH4, ml/ g DM digested	68.4^c	76.5^d	58.2^b	54.4^b	1.76	<0.001

^{a,b,c} values on the same row with different superscripts differ (P<0.05)

Effect of incubation interval

The rate of gas production increased to a maximum in the interval 3-6hour of incubation then decreased linearly (Table 4; Figure 1). In contrast, the proportion of methane in the gas increased linearly with incubation interval (Table 4; Figure 2).

Table 4: Mean values for percent methane in the gas, and gas production per hour, during successive intervals of the fermentation

Items	Interval, hour					SEM	Prob
	0-3	3-6	6-12	12-18	18-24		
Methane, %	7.25	9.35	16.0	20.0	22	0.203	<0.0001
Gas, ml/h	128	193	150	125	91	3.8	<0.0001

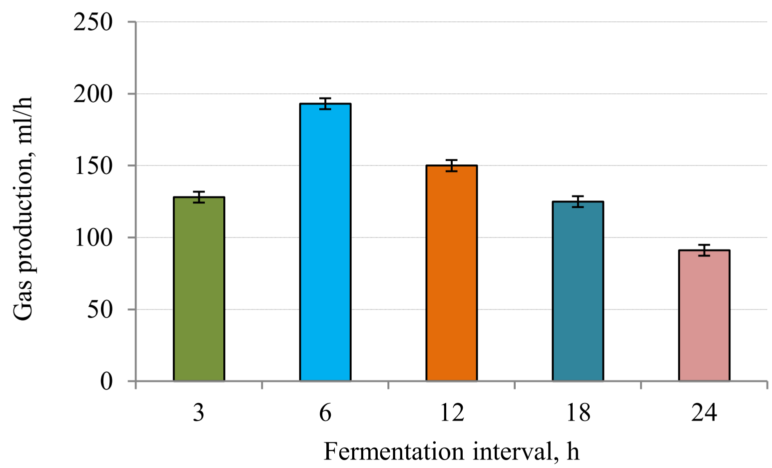


Figure 1: Effect of fermentation interval on rate of gas production in an in vitro fermentation of ensiled cassava root supplemented with urea and cassava leaf meal and different additives

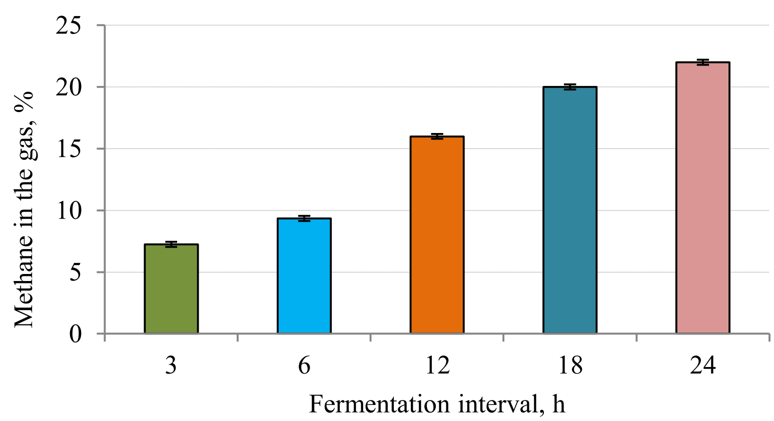


Figure 2: Effect of fermentation interval on the methane content of the gas in an in vitro fermentation of ensiled cassava root supplemented with urea and cassava leaf meal

Effect of additives

Total gas production was highest for fermented cassava root, followed by RDB with lowest values for the yeast and control treatments (Figure 3). The methane content of the gas (Figure 4) differed among treatments with highest values for the control treatment, followed by the fermented cassava root, yeast and RDB. On the RDB treatment the overall reduction in percent methane was of the order of 25%. Methane production per unit DM digestibility (Figure 5) showed the same trend as the methane percent in the gas.

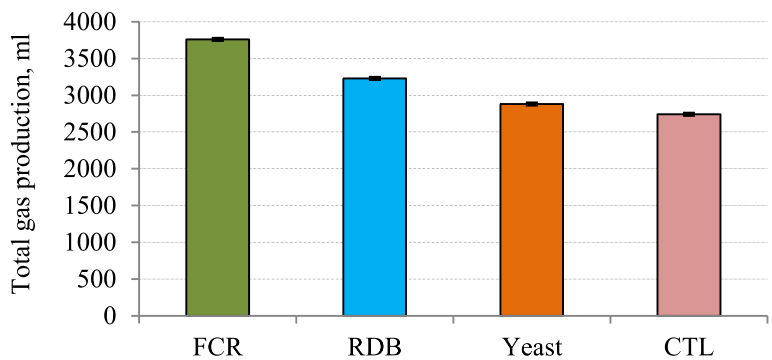


Figure 3: Effect of addition (% in DM) of fermented cassava root (4%), yeast (1%) or rice distillers' by-product (4%) on the gas production in 24h in an in vitro fermentation of ensiled cassava root supplemented with urea and cassava leaf meal

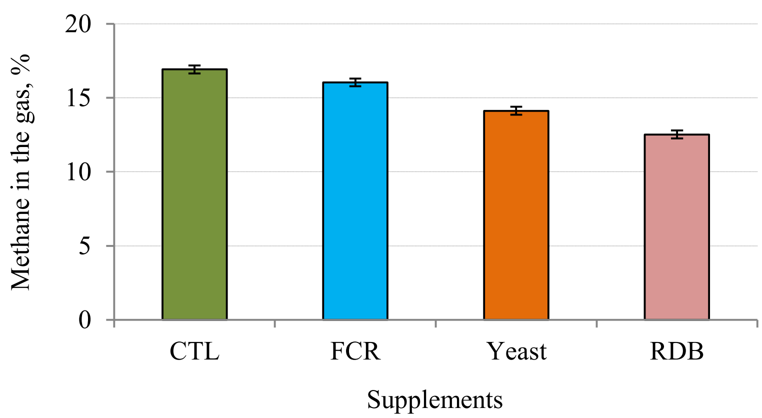


Figure 4: Effect of addition (% in DM) of fermented cassava root (4%), yeast (1%) or rice distillers' by-product (4%) on the methane content of the gas in an in vitro fermentation of ensiled cassava root supplemented with urea and cassava leaf meal

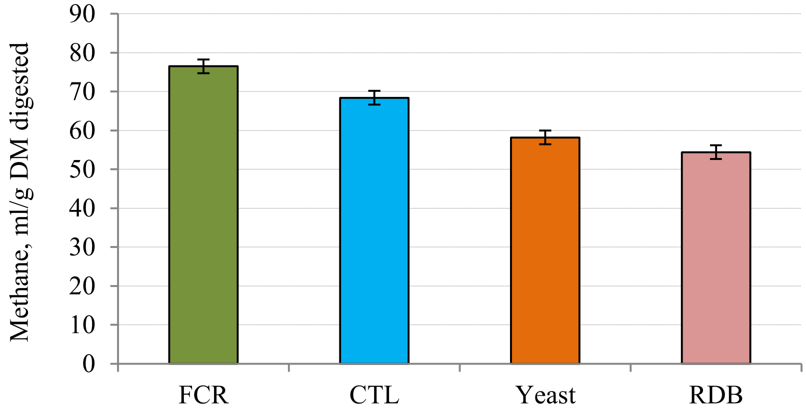


Figure 5: Effect of addition (% in DM) of fermented cassava root (4%), yeast (1%) or rice distillers' by-product (4%) on the methane produced per unit substrate DM digested

The increases in methane production in the gas with duration of fermentation time, indicative of the transition to a secondary fermentation of the VFA to methane, supports earlier findings using a similar *in vitro* incubation system but with different substrates (Le Thy Binh Phuong et al., 2011; Outhen et al., 2011; Sangkhom et al., 2011; Thanh et al., 2011).

The beneficial effect of the small quantity (4% of substrate DM) of rice distillers' by-product in reducing methane production from rumen fermentation is similar to the response reported by Sangkhom and Preston, (2016) when brewers' grains and rice distillers' by-product were added to *in vitro* incubations of ensiled and fermented cassava roots. Addition of yeast at 1% of the substrate also reduced methane production but was slightly less effective than the rice distillers' by-product. By contrast, an attempt to simulate some of the features of RDB by fermenting cassava root with yeast, urea and diammonium phosphate had no effect on methane production in the *in vitro* fermentation. It has been postulated that the benefits of yeast-based additives in improving human health and growth rates of animals are related to the β-glucan present in the yeast cell wall and their effect in stimulating the immune system (eg: Dritz et al.,1995; Hanh et al., 2006; Novak and Vetvicka, 2002; Waszkiewicz-Robak, 2013). Increases in growth rate of weanling pigs and improved resistance to *E coli infections* were reported by Thuy and Ha, (2017) when pure β-glucan, isolated from spent brewers' yeast was included in the diet.

In ruminant systems it is probable that their effects are modulated through effects on microbial ecosystems in the rumen and/or lower down the digestive tract. Thus alleviation of hydrocyanic acid toxicity, in cattle fed cassava foliage from a variety rich in HCN precursors, has been suggested as being due to the β-glucans in brewers' grains supporting biofilm-based fermentations in the rumen that favored detoxification of the HCN (Inthapanya et al., 2017). A shift in the microbial fermentation towards propionate at the expense of acetate production increases the overall yield of metabolisable energy. It also increases availability of glucogenic substrate which is often critically low in some feeds (Preston and Leng, 1987), Hydrogen produced when acetate is the end product of organic matter fermentation is converted to methane and thus reduces overall metabolisable energy. The magnitude of the inverse relationship is illustrated in Diagram 3, which shows the concentration of propionic acid and that of methane in the rumen of cattle that was achieved by varying the levels of rumen protozoa (Whitelaw et al., 1984).

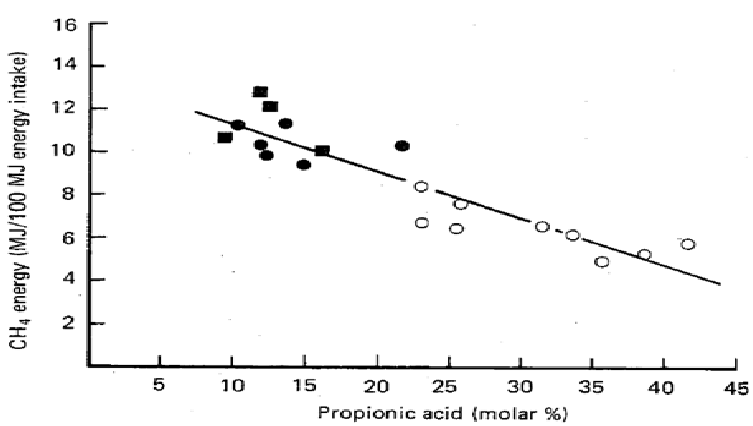


Diagram 3: Relationship between energy in eructed methane and the proportion of propionic acid n the rumen VFA from cattle. Closed symbols are data from faunated animals; open symbols are from ciliate-free animals (Whitelaw et al., 1984)

A similar relationship between rumen levels of methane and propionic acid was observed by Inthapanya et al., (2017). Improvements in growth rate and feed conversion in cattle supplemented with brewers' grains or rice distillers' product are thus to be expected from the combined benefits of increased availability of both glucose precursors from propionic acid and increased total metabolisable energy as a result of decreased methane production in the rumen. We therefore suggest that the consistently reported benefits of brewers' grains and rice distillers' by-product in reducing methane production in rumen fermentations, both *in vitro* (Sangkhom and Preston, 2016) and *in vivo* (Sengsouly and Preston, 2016; Sangkhom et al., 2017; Binh et al., 2017) are the indirect effects of these additives in increasing the proportions of propionic acid in the rumen VFA. The explanation of why small proportions in the diet of brewers' grains, or rice distillers' by-product, result in more propionic acid in the rumen VFA is less obvious. Common to both brewers' grains and rice distillers' by-product is the presence of yeast which has been subjected to heating (100°C) under acid conditions during the process of distilling off the ethanol as is standard practice in production of beer from barley (brewers' grains) and from polished/broken rice (rice "wine"). It is then assumed that it is the β-glucan derived from the cell walls of the yeast (and of the barley), which modifies microbial activities in the rumen biofilms (Leng, 2014) favoring higher proportions of propionic acid in the rumen VFA. β-glucans are present in the cell wall of barley (Havrlentová and Kraic, 2006) and yeast (Waszkiewicz-Robak, 2013).

An important issue is the need, or otherwise, to isolate the β-glucan which, as described by Nguyen Thi Thuy and Nguyen Cong Hanh, (2016), required high pressure homogenization to break the yeast cell wall followed by acid, then alkaline hydrolysis. The results from our experiment, in which there were no effects on methane by supplementing the substrate with fermented cassava root, partially support the hypothesis that breakage of the yeast/barley cell walls, followed by acid hydrolysis, are necessary first steps in facilitating the action of the β-glucan. The positive effect of the commercial yeast "starter" in decreasing methane production implies that some of the β-glucan in this product may have been in the freestate. However, the greater impact of the rice distillers' by-product in reducing methane production would probably have been facilitated by the degree of hydrolysis of the yeast cells that were likely to have occurred in the process of distilling the ethanol. The positive effect of rice distillers' by-product in reducing methane production *in vitro* mirrors the results obtained *in vivo* when local cattle were fed basal diets of ensiled or fermented cassava root supplemented with urea and fresh cassava foliage (Sengsouly et al., 2016; Sangkhom et al., 2016). In both these experiments the reduction in methane emissions were directly linked with improved growth rates and better feed conversion.

CONCLUSIONS

- The rate of gas production increased to a maximum in the 3-6hours incubation interval and then decreased linearly. In contrast, the proportion of methane in the gas increased linearly with incubation interval from the beginning until the final 18-24hours interval.
- Total gas production was highest for the fermented cassava root additive, followed by the rice distillers' by-product and lowest values for the yeast and control treatments
- The methane content of the gas was highest for the control treatment, followed by the fermented cassava root and then yeast and the lowest value for rice distillers' by-product, for which the overall reduction in methane was of the order of 25%.
- Methane production per unit DM digested showed the same trend as the methane percentage in the gas.
- It is suggested that the reported benefits from brewers' grains and rice distillers' by-product in reducing methane production in the rumen fermentations, both *in vitro* and *in vivo*, are the indirect effects of these additives increasing the proportions of propionic acid in the rumen VFA.

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CHAPTER 6:

RICE DISTILLERS' BY-PRODUCT IMPROVED GROWTH PERFORMANCE AND ENTERIC METHANE FROM "YELLOW" CATTLE FED A FATTENING DIET BASED ON CASSAVA ROOT AND FOLIAGE (*Manihot esculenta*, Crantz)

ABSTRACT

The aim of the study was to evaluate the effect of rice distillers' by-product (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 67.5±3.5kg (mean±standard deviation) were used for the experiment. The treatments were rice distillers' by-product at a predicted level of 4% of diet DM (RDB) or none (No RDB). 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' by-product 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' by-product. Rice distillers' by-product 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. This research implicated that rice distillers' by-product supplementation to cattle diet can improve growth performance and reduce enteric methane emissions from local yellow cattle.

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

INTRODUCTION

Livestock plays 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 1.3-1.7 million head from 2005-2013; while the buffalo population increased from 1.1-1.19 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 methane 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. Increased live weight grain and reduced methane production per unit of live weight grain (Klieve and Ouwerkerk, 2007). Thus, on any diet and particularly diets based on agro-industrial by-products, improving live weight gain and feed conversion efficiency by supplementation leads to a significant decrease in methane production per unit of production.

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 years 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 by-product (Quilao) from rice wine production could replace the brewers' grains (Sengsouly et al., 2017).

The objective of the study reported in this research was to determine if rice distillers' by-product 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 of 67.5±3.5kg (mean±standard deviation) were used. They were vaccinated against epidemic diseases and drenched against internal parasites. The treatments were:

- RDB: Rice distillers' by-product at 4% of predicted DM intake
- No RDB: No rice distillers' by-product

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

Items	DM basis, %	CP in DM, %
Cassava root	93	2.32
Yeast	3	1.35
DAP	1	1.30
Urea	2	5.60
S-rich mineral	1	
Total	100	10.6

CP: Crude protein ; DAP: Di-ammonium phosphate; DM: Dry matter; S: Sulphur (it is a component of amino acids methionine and cystine, as well as B-vitamins biotin and thiamine and a number of other organic compounds; Yeast: Saccharomyces cerevisiae

Feeding and management

The cassava roots bought from local farmers was 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' by-product 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 perennial forage with daily harvesting of the 2-3-month-old re-growth. Rice straw was collected from nearby farmers. Rice distillers' by-product 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 at all times.

Data collection and measurements

The offer level of rice distillers' by-product 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 carbon dioxide and methane 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 1) 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 minutes period, using a GASMET 4030 meter (Gasmet Technologies Oy, Pulttitie 8A, FI-00880 Helsinki, Finland). The methane and carbon dioxide 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).

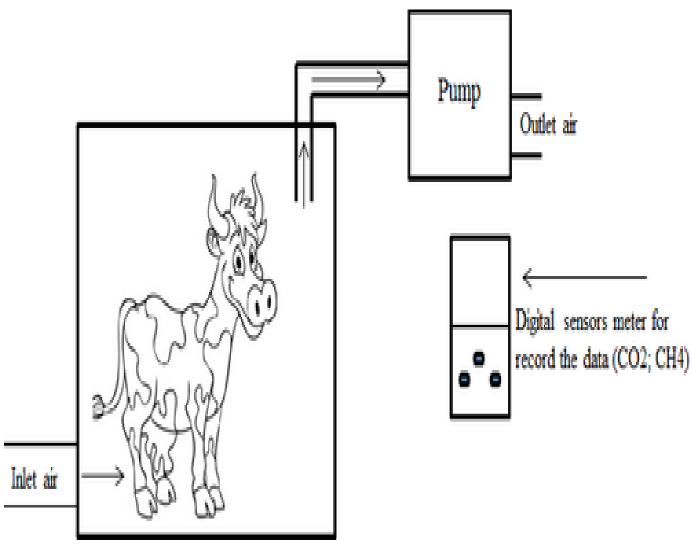

Diagram 1: A schematic view of the method for measuring CH_4 and CO_2 from cattle using GASMET infra-red analyzer

Photo 1: Wooden crates enclosed in plastic used to house the cattle during the 10 minute period of adaptation/ measurement using the GASMET infra-red analyser

Chemical analyses

Samples of feeds offered and residues were collected over a 24 hours period every 15days 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 3hours, filtering through Whatman No.4 filter paper and determining nitrogen in the filtrate.

Statistical analyses

Live weight gains were calculated from the linear regression of live weight (Y) on days in the experiment (X). The growth data, rumen ammonia and VFA, and methane/carbon dioxide ratios in mixed eructed gas and air were analyzed by the general linear model option of the ANOVA program in the Minitab software (version 16). The model the sources of variation were: treatments and error. The statistical model used was:

$Y_{ij} = \mu + T_i + e_{ij}$

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

RESULTS AND DISCUSSION

Composition of diet ingredients

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

Table 2: The chemical composition and N solubility of the feed ingredients (%)

Items	DM, %	Ash	CP	N solubility#
		….As % in DM….		
Fermented cassava root	28.5	4.26	11.4	
Cassava foliage	28.2	9.75	19.2	30.1
Rice distillers' by-product	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' by-product (Table 3; Figure 1). The average intake of rice distillers' by-product was 2.75% of the DM consumed on the "RDB" treatment. This was less than the intended concentration 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 distillers' by-product (RDB)

Items	No RDB	RDB	SEM	Prob
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
Rice distiller's by-product		1068		
DM intake, g/day				
Fermented cassava root	1796	1979	19	0.02
Rice straw	258	270	2.3	0.069
Cassava foliage	652	708	5.8	0.021
Rice distiller's by-product		83		
Total	2706	3041	26.5	0.012
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' by-product; RDB: With rice distillers' by-product; SEM: Standard error of the mean with df_{error} = 14

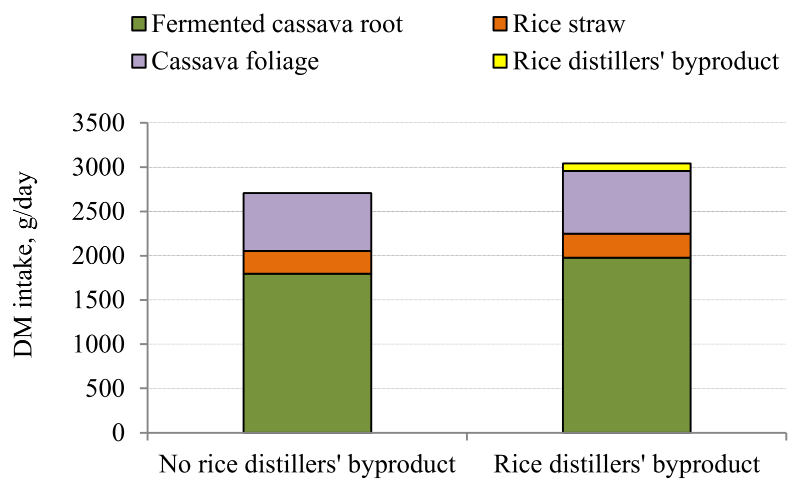

Figure 1: Effect of rice distillers' by-product intake of feed ingredients (as DM)

Growth performance

The cattle growth rate was uniform (Figure 2) and was increased by 40% supplementation to rice distillers' by-product (Table 4; Figure 3). DM feed intake (Figure 4) and DM feed conversion (Figure 5) followed the same response pattern as for growth rate with 20% improvement in DM feed conversion for supplementation with rice distillers' by-product.

Table 4: Mean values for initial 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' by-product (RDB)

Items	No RDB	RDB	SEM	Prob
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 ratio (FCR)	9.05	7.27	0.14	0.013

No RDB: Without rice distillers' by-product; RDB: With rice distillers' by-product; SEM: Standard error of the mean with $df_{error} = 14$

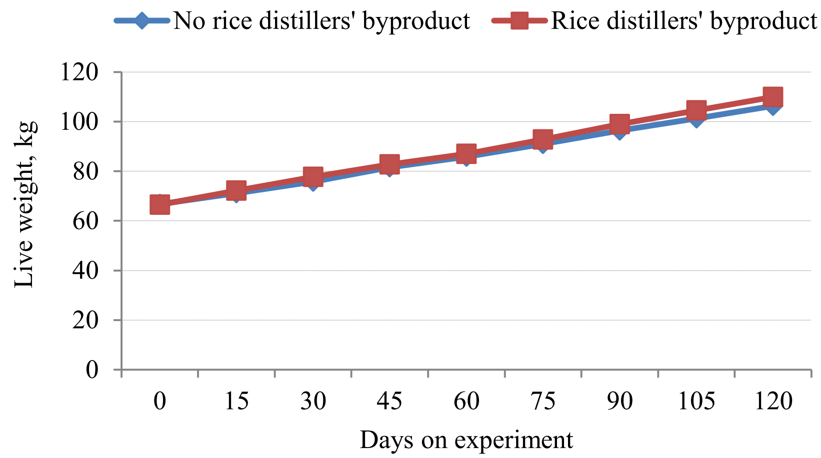

Figure 2: Effect of rice distillers' by-product on growth curves of yellow cattle fed fermented cassava root, fresh cassava foliage and rice straw

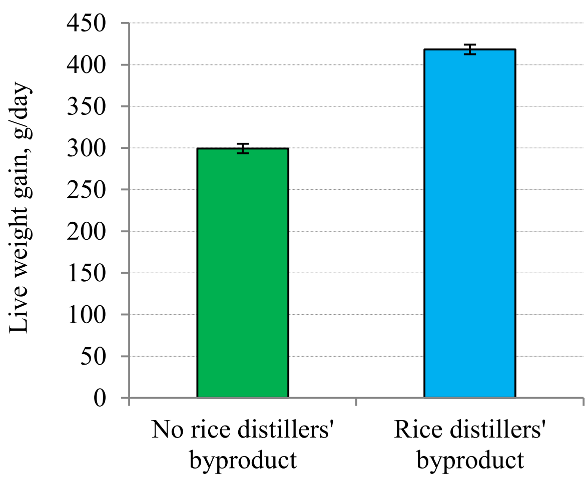

Figure 3: Effect of rice distillers' by-product on live weight gain of yellow cattle fed fermented cassava root, fresh cassava foliage and rice straw

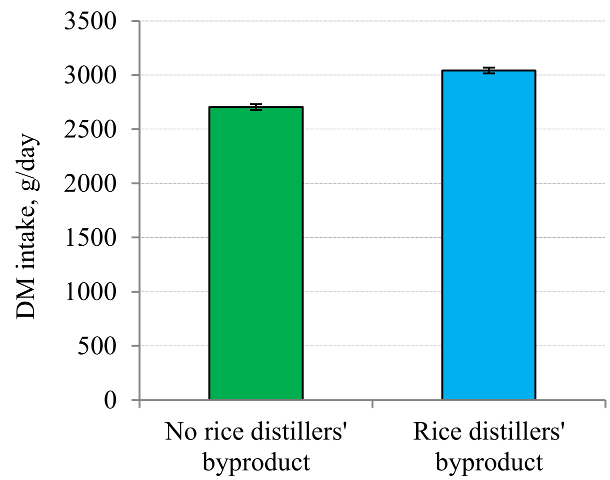

Figure 4: Effect of rice distillers' by-product on DM intake of yellow cattle fed fermented cassava root, fresh cassava foliage and rice straw

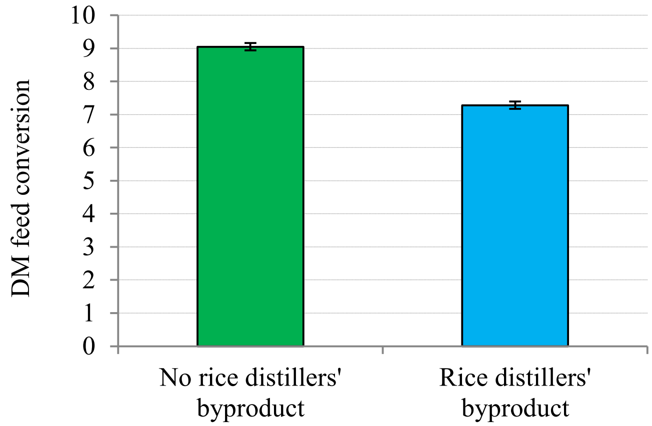

Figure 5: Effect of rice distillers' by-product supplements on DM feed conversion of yellow local cattle fed a basal diet of fermented cassava root, fresh cassava foliage and straw

Rumen parameters and ratio of methane and carbon dioxide

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

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

Items	No RDB	RDB	SEM	Prob
Rumen pH	7.02	6.98	0.029	0.376
NH_3 (mg/L)	246	312	5.438	<0.001
Rumen total 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

No RDB: Without rice distillers' by-product; RDB: With Rice distillers' by-product; SEM: Standard error of the mean with $df_{error} = 14$*; VFA: Volatile fatty acid*

Supplementation with rice distillers' by-product increased the proportion of propionic acid in the rumen VFA and decreased the ratio of acetic to propionic acid (Table

5; Figure 6). The ratio of methane to carbon dioxide in the mixed eructed gas and air was decreased 26% by supplementation with rice distillers' by-product (Table 6; Figure 7).

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

Items	No RDB	RDB	SEM	Prob
CO_2, ppm	3790	4334		
CH_4, ppm	144	127		
CH_4/CO_2	0.0384	0.0285	0.003	0.01

CO_2: Carbon dioxide; CH_4: Methane; No RDB: Without rice distillers' by-product; RDB: With rice distillers' by-product; SEM: Standard error of the mean with df_{error} = 14

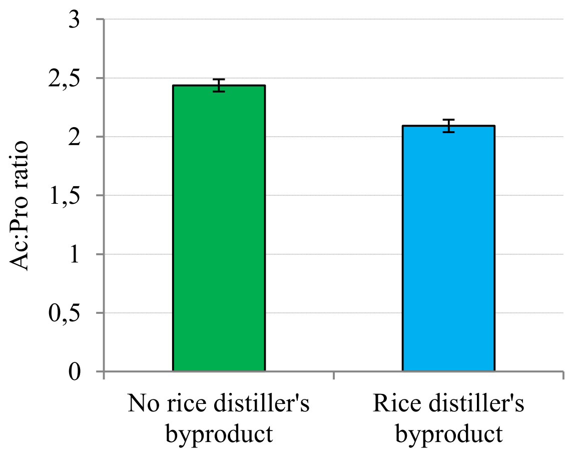

Figure 6: Effect of rice distillers' by-product on acetic: propionic acid ratio in rumen fluid of yellow cattle fed fermented cassava root, fresh cassava foliage and rice straw

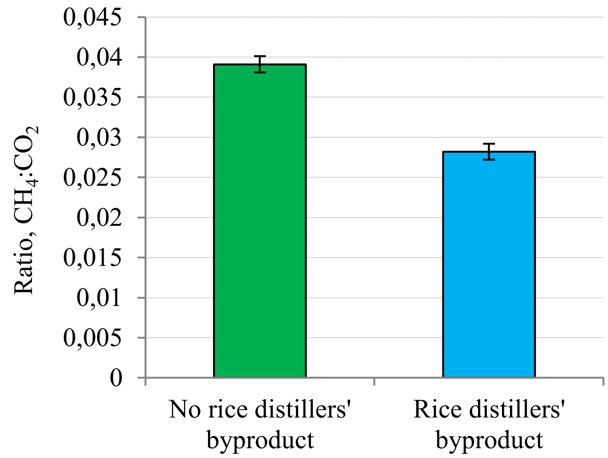

Figure 7: Effect of rice distillers' by-product on ratio of methane to carbon dioxide in mixed air: expired breath of yellow cattle fed fermented cassava root, fresh cassava foliage and rice straw

The positive effect of the low level (2.75% of diet DM) of rice distillers' by-product 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' by-product 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 7days after mixing with yeast-urea-DAP. Live weight responses to rice distillers' by-product were similar in both experiments: from a weight gain of 300g/day in non-supplemented animals to 440g/day for those that were supplemented (Sengsouly and Preston, 2016), compared with 299-418g/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) (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' by-product (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' by-product (perhaps β-glucan or related compounds) support biofilm formation which in turn increases the efficiency of microbial growth (Leng, 2014). Rice distillers' by-product is rich in yeast which in turn is rich in β-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 were 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' by-product or brewers' grains are suggested to be due to:

- They supply essential amino acids as bypass protein
- The yeast cells provide β-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' by-product.
- Rice distillers' by-product 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' by-product 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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CHAPTER 7:

GENERAL DISCUSSION AND CONCLUSIONS

7.1 GENERAL DISCUSSION

7.1.1 Livestock production and the environment

Population growth is challenging for agricultural systems around the world because it means producing more food to support an increasing population in a more efficient manner within the constraints of available natural resources, without compromising the future of coming generations. In developing countries, the demand for food will increase as a consequence of increasing population and net income, which are usually associated with increases in demand for animal products (FAO, 2016).

Livestock production has been criticised in recent decades for reasons such as: use of agriculture land that could be used for human food production, water consumption, deforestation, environmental pollution, animal welfare and human health, and concerns from eating animal products. Since feedstuff production is what links livestock production to land use, both directly via grazing and indirectly via traded grain or forage, environmental sustainability is an issue of major importance in the feed industry (Herrero et al., 2013). Livestock supply 13% of the energy in human diets, consume around 50% of the world's production of grains (Smith et al., 2014) and, at the same time, are responsible for about 14.5% of total anthropogenic greenhouse gas emissions (7.1 Gt CO_2-equivalents per year) (Gerber et al., 2013). Livestock play an important role in global food production and in agricultural and rural economies in many developing regions, while the livestock sector is one of the fastest growing subsectors of agriculture; it is also an important contributor to anthropogenic greenhouse gas emissions. Major culprit is methane produced by enteric fermentation and from decomposing manure from ruminants (IPCC, 2014). Reducing greenhouse gas (GHG) emissions from agriculture and especially from ruminant livestock should therefore be a top priority since it could help to curb global warming (Sejian et al., 2010). Successful mitigation of ruminant greenhouse gas emission is challenging technically but is made even more difficult because of the rising demand for milk and meat (Steinfeld et al., 2006). The challenge is to increase the production of meat and milk from ruminants without increasing, and preferably reducing methane emissions.

7.1.2 Strategies for reducing enteric methane from cattle

Methane is produced from feed fermentation in the rumen. Therefore, methane production can be mitigated by modifying the rumen fermentation. In addition, 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 by-products, improving live weight gain and feed conversion efficiency by supplementation leads to a significant decrease in methane production per unit of production.

7.1.3 Cassava as the means of intensifying ruminant production

In developed countries with temperate climates, particularly USA and Europe, the increase in ruminant productivity is based on use of maize and barley as the major source of feed. In the tropics, this role could be played by cassava (*Manihot esculenta* Crantz). Cassava is a perennial woody shrub of the family Euphorbiaceae. It originated in South America and is extensively cultivated as an annual crop in the tropics and sub-tropics for the dual purpose production of tuberous roots as a source of energy for humans and animals and foliage as a protein source for animals. In Laos, cassava is currently the third most important crop after rice and maize. It has become a major crop in Lao PDR mainly because of the export of starch that is extracted from the cassava root (MAF, 2014). Cassava products are a major source of income for rural households and also for use in livestock production such as in cattle diets. The root is composed of highly digestible carbohydrate in the form of starch with little fiber (Kang et al., 2015; Polyorach et al., 2013). The foliage is considered a good source of bypass protein for ruminants (Ffoulkes and Preston, 1978; Wanapat, 2001; Keo Sath et al., 2008).

The proximate composition, cassava root is a minimal level of protein content 2-3% in DM (Wanapat et al., 2013). The nutritive value of cassava roots can be improved by fermenting or ensiling with additive ingredients. In the present studies, the CP content was low for dried cassava roots (1.98%; Chapter II); meanwhile, this value was 2.56% for ensiled cassava root (Chapter II to V). However, the CP of cassava root could be improved by fermenting with urea, di-ammonium phosphate (DAP) and yeast (*Saccharomyces cereviceae*). Results in this thesis (Chapter IV and VI) indicated that CP content in fermented cassava root was 5 times higher than that in dry form (11.7 vs 1.98%). These confirmed by other findings reported by Watnapat et al. (2016); Poungchompu et al. (2009); Polyorach et al. (2012); Wanapat et al. (2013). On the other hand, results of the present study (Chapter IV) showed that the fermenting cassava root with yeast, urea and DAP increased the true protein content from 1.8 to 7.6% in DM. This finding supported by the findings of Vanhnasin et al. (2016) and Manivan and Preston (2016).

Processing methods affected hydrogen cyanide (HCN) and condensed tannin (CT) concentration. Results in this thesis (Chapter II) showed that HCN was higher in fresh cassava leaves (485mg/kg) than in dried form (369mg/kg), and in the ensiled rather than dried cassava root (119 vs 94mg/kg). The CT was also higher in fresh cassava leaves than in the dried form and in the ensiled cassava root rather than dried. These findings supported the findings of Heuzé and Tran (2012). Condensed tannins at moderate levels are known to have positive effects on the nutritive value of the feed by forming insoluble complexes with dietary protein, resulting in "escape" of the protein from the rumen fermentation (Barry and McNabb, 1999). It has been fed successfully to improve performance of cattle (Wanapat et al., 2000; Thang et al., 2010). The presence of cyanogenic glucosides in roots and leaves, which are converted to HCN in the rumen, is a problem that can be resolved according to recent findings in Vietnam (Phuong et al., 2015).

7.1.4 Modifying the rumen fermentation to reduce methane production and to improve cattle performance

In this thesis, a series of experiments have been carried out to examine ways of reducing enteric methane emissions in cattle. These studies were done employing *in vitro* rumen incubations (Chapters II, IV and V), a digestibility and nitrogen balance trial (Chapter III), and a feeding trial (Chapter VI). The basal diets consisted of ensiled and/or fermented or dried cassava root as a source of soluble carbohydrate; urea as a source of rumen ammonia; and cassava foliage and/or water spinach as a source of protein. Brewers' grains, rice distillers' by-product, yeast fermented and protein-enriched cassava root were used as additives predicated on the concept that they were potential sources of prebiotics/probiotics.

In the first *in vitro* rumen fermentation (Chapter II), the reduction in methane production with ensiled compared with dried cassava roots was ascribed to the higher concentration of glucogenic precursors in the former, and the toxic effect of the HCN derived from these compounds on rumen methanogens, which supported by Rojas et al. (1999) and Smith et al. (1985). Phuc et al. (1995) showed that drying cassava roots was more effective than ensiling them as a means of reducing the level of HCN. However, the presence of HCN in the leaf of cassava was considered to be the explanation for the reduced rumen methane production when using protein cassava leaf meal compared with water spinach in diet. The finding of the present study (Chapter II) supported the findings of Makkar et al. (1995) and Grainger et al., (2009), who reported that there are decreases in methane production when diets have contained HCN concentration and CT in the substrates.

Present study (Chapter III) was predicated on the observation by Phanthavong et al. (2016) that when cattle were fed foliage from bitter cassava, rich in HCN precursors, they had a craving to eat brewers' grains. The experiment in chapter III, it was hypothesized that the brewers' grains were acting as a "prebiotic" providing habitat enabling the evolution of rumen microbial communities capable of detoxifying the HCN when the cassava foliage was consumed by the cattle. To test this hypothesis, fresh brewers' grains were fed at 5% of the diet DM of local yellow cattle which were fed ensiled cassava roots and supplemented with either sweet cassava foliage or fresh water spinach. The 42% increase in N retention when the cattle were fed the low level of brewers' grains was considered to be evidence that the brewers' grains were having a positive "prebiotic" effect on overall animal wellbeing rather than being simply an additional source of "bypass" protein. It was notable that the effect of the brewers' grains was more pronounced when cassava foliage was the source of dietary protein rather than water spinach. This confirmed the result by Phuong et al., (2017), who reported major benefits of cattle increased growth performance when small brewers' grains (4% of the diet DM) were added to the diet of cassava pulp-urea-cassava foliage. Here the implication is that the cassava foliage was a superior source of bypass protein (solubility of the protein was 30% for brewers' grains compared with 67% for water spinach) but this potential advantage was constrained by the negative effect of the HCN precursors (which was ameliorated by the addition of 5% of brewers' grains to the diet).

The finding of this study (Chapter III) confirmed that the 42% improvement in N retention, resulting from supplementation with 5% brewers' grains, would be reflected in reduced methane production. The present study (Chapter IV) showed that rice distillers' by-product, the residue after yeast fermentation of rice and alcohol distillation, would have similar 'prebiotic" effects as brewers' grains, was also tested. Methane production was reduced by both additives with the effect being more pronounced with rice distillers' by-product than with brewers' grains. The experiment also included a comparison of ensiled versus fermented cassava root with yeast, urea and di-ammonium phosphate (DAP), providing further proof that the former treatment resulted in lower gas production and an associated reduction in the methane percentage in the gas. These results can be explained the fact that yeast fermentation results in part of the carbohydrate being converted to protein. Yeast protein is of low solubility and thus will be fermented to only a small extent in the in vitro rumen, the overall effect being to decrease the gas production, as suggested by Demeyer, (1991); Immig, (1996); Popova et al., (2013); Leng, (2016). The another results showed that yeast could stimulated the growth and methabolism of rumen microorganism especially lactase utilized bacteria, such as *Megasphaera elsdenii* or *Selenomonas* ruminantium and supply different growth factors, such as amino acids, peptides, vitamins and organic acids, essential for the ruminanal bacteria growth, hence, enhancing VFA concentratation and reducing C2:C3 proportion, thus fermented cassava root affect reduce methane than ensiled cassava root or dried cassava root (Chapter IV), this confirmed other findings report by Lynch et al., (2002); Chuacheryras et al., (2008); Polyorach et al., (2014).

Present study (Chapter V) reported the results of an *in vitro* experiment to test a range of potential "prebiotic" additives in their capacity to reduce methane production. These were selected according to their origin and/or their method of preparation (ie: having been produced by some form of yeast fermentation). Only the rice distillers' by-product produced the expected reduction in methane. Addition of 1% live yeast reduced methane slightly but there was no benefit from adding protein-enriched cassava root following yeast fermentation. These findings support the idea that the process of developing prebiotic activities is conditional on a final acid-hydrolysis treatment as occurs when the fermented product is subjected to distillation as in both beer and rice wine manufacture.

The data of the present study (Chapter VI) confirmed that the reduction in methane resulting from supplementation with rice distillers' by-product was reflected in improved growth and feed conversion when local yellow cattle were fed cassava roots fermented with urea and yeast and supplemented with cassava foliage. This confirmed the results by Hanh et al., (2006); Novak and Vetvicka, (2008); Waszkiewicz-Robak, (2013) who postulated that the benefits of yeast-based additives in improving human health and growth rates of animals are related to the β-glucan present in the yeast cell wall and their effect in stimulating the immune system. Therefore, this present study showed that growth rate and feed conversion were improved by 40 and 20%, respectively, while the ratio of methane (CH_4) to carbon dioxide (CO_2) in eructed gas were reduced by 26%. The expected gain in energy metabolism from methane reduction was manifested in a 14% increase in molar propionate relative to acetate. This present study supported the result of Sengsouly and Preston, (2016), who reported the rice distillers' by-product was added at 4% to a basal diet of ensiled cassava root-urea and fresh cassava foliage of fattening cattle.

7.2 GENERAL CONCLUSIONS

- There were consistent decreases in gas production and methane content of the gas when ensiled cassava root replaced the dried root; and when cassava leaf meal replaced water spinach meal. Over 24hours fermentation, the methane production per unit of DM mineralized substrate was decreased by 18% by the combination of ensiling versus drying of the cassava root and replacement of water spinach by cassava leaf meal.
- Adding 5% of brewers' grains to a diet of ensiled cassava root, urea and rice straw supplemented with either cassava foliage or water spinach as a main protein source, increased the DM intake, the apparent DM digestibility and N retention in local yellow cattle.
- Gas production was lower for fermented than ensiled cassava root but was increased by supplementing with brewers' grains and rice distillers' by-products. The methane content in the gas was lower for fermented than for ensiled cassava root. Methane production per unit substrate was less in fermented compared with ensiled cassava root and was reduced by supplementing with brewers' grains and rice distillers' by-product.
- Total gas production was highest for fermented cassava root, followed by rice distillers' by-product and lowest for ensiled cassava root and yeast fermentation. The methane content of the gas was highest in ensiled treatment, followed by fermented cassava root than yeast and the lowest value in rice distillers' by-product, for which the overall reduction in methane was of the order of 25%. Methane production per unit DM digested showed the same trend as the methane content in the gas.
- 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' by-product.

Rice distillers' by-product supplementation increased the concentration of propionic acid in the rumen and reduced by 26% the ratio of methane to carbon dioxide in the eructed rumen gas.

7.3 IMPLICATIONS AND FURTHER RESEARCH

7.3.1 Implications

The strategy underlying the research developed in this thesis for reducing methane emissions from ruminants was based on two concepts: (i) the higher animal productivity, the lower methane production per unit of edible/useable product; and (ii) enteric methane production per unit of fermentable feed DM can be reduced when the nature of the diet facilitates the escape of nutrient-rich substrate to the lower digestive tract and/or the habitat for microbial communities in the digestive tract is enhanced by feeding of natural products (prebiotics) derived from fermentation followed by acid digestion of cell walls of cereals (barley and rice) and/or yeasts (principally *Saccharomyces cerevisiae*).

Increasing ruminant productivity requires feeding systems based on highly fermentable carbohydrate that favour propionate-rich rumen fermentation and sources of true protein that escape ("bypass") the rumen. The research developed in this thesis has shown that the cassava plant can be basis for intensification of ruminant production in tropical countries such as Lao PDR. The cassava plant provides highly fermentable energy-rich roots, easily preserved by ensiling, and foliage which is rich in bypass protein, and thus much superior to the residual foliage (straw and stove) from cereal crops. The potential disadvantages inherent in the feeding of cassava, namely the cyanogenic glucosides – precursors of the highly toxic hydrocyanic acid - instead of being risk factors can become an advantage as a means of reducing rumen methane through their toxic effects on methanogens.

Finally, the study in this thesis has confirmed the valuable role of a by-product from making rice wine, common in rural communities throughout SE Asia, namely "Kilao" (in Laos), "Hem" (in Vietnam) and "Bar Ra" (in Cambodia) as a "probiotics/prebiotic" effective in enhancing livestock growth and feed conversion, reducing rumen methane and providing protection against HCN toxicity.

7.3.2 Further research

Addressing the implications of the issues raised in the previous section, requires that future research should be concentrated in a number of related areas: (i) modifying the fermentation of rice (or cassava root) to produce at farm level the equivalent of "Kilao" but without the alcohol; and (ii) using the residual stems after cassava root harvest as a potential source of fuel enabling smoke-free cooking in rural households and production of biochar – another prebiotic that facilitates development of habitat for biofilms hosting microbial communities and their associated nutrients in symbiotic relationships beneficial to the host animal.

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I. Inthapanya, S., Preston, T.R., Leng, R.A., Ngoan, L.D. and Phung, L.D., 2017. 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). *Livestock Research for Rural Development*. Volume 29, Article #131. http://www.lrrd.org/lrrd29/7/sang29131.html

II. Inthapanya, S., Preston, T.R., Phung, L.D. and Ngoan, L.D., 2017. Effect of supplements of yeast (Saccharomyces cerevisiae), rice distillers' by-product and fermented cassava root on methane production in an *in vitro* rumen fermentation of ensiled cassava root, urea and cassava leaf meal. *Livestock Research for Rural Development.* Volume 29, Article #220. http://www.lrrd.org/lrrd29/12/sang29220.html