# Impact of fermentation on the nutritive value of cassava root pulp and soybean pulp as a feed for growing pigs in Lao PDR

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Cover: Fermented cassava root pulp and soybean pulp as a feed for growing pigs.
(Figure: Lotchana Taysayavong, 2018)

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## Abstract

This thesis evaluated the impact of fermentation on the nutritive value of cassava root pulp (CRP) and soybean pulp (SBP) as feed for growing pigs. The evaluation was based on four studies (two fermentation and two animal studies). In these, the chemical composition of CRP aerobically fermented with four different yeast sources was evaluated; the digestibility, nitrogen balance, gut environment and visceral organ size were studied in growing Moo Lath and Large White pigs fed unfermented and fermented CRP and SBP; CRP and SBP were anaerobically fermented (ensiled) with or without rice bran; and the digestibility and nitrogen balance of the ensiled products was studied in growing Large White pigs.

Crude protein, true protein, neutral detergent fibre (NDF), starch and ash content in fermented CRP differed between yeast sources. Crude protein and ash content increased and starch content decreased with days of fermentation for all yeast sources and all levels of nitrogen addition (1.25, 2.5 %). The greatest increase in crude protein and ash content during fermentation was observed for Lao alcohol yeast. The greatest decrease in starch content was also found for Lao alcohol yeast, followed by *Schwanniomyces occidentalis*, *Saccharomyces cerevisiae* and *Rhodotorula toruloides*.

Organic matter and crude protein digestibility was higher for diets with SBP than for diets with CRP, and did not differ between pig breeds. Nitrogen retention, expressed as a proportion of nitrogen digested, was similar between breeds and was highest for fermented CRP and lowest for unfermented CRP. There were no effects of diet on gut environment or visceral organ size.

For both CRP and SRP, the pH decreased with days of ensiling and was elevated by inclusion of rice bran. Dry matter (DM) content increased with days of ensiling and with increasing inclusion of rice bran. In CRP, the crude protein content increased with days of ensiling, while there was a decrease in crude protein content in SBP. Increasing inclusion of rice bran elevated the crude protein content in CRP, but reduced it in SBP. Increasing inclusion of rice bran raised the ammonia-nitrogen ($NH_3$-N) content in both CRP and SBP, and the content within pulp source remained elevated during ensiling.

Ensiling CRP and SBP with rice bran produced in a feed product that was well digested and utilised in growing Large White pigs, but with differences in palatability. Ensiling both forms of pulp with inclusion of rice bran (10% for CRP and 20% for SBP) improved the digestibility of both organic matter and crude protein, suggesting synergistic effects in the porcine gastrointestinal tract.

*Keywords:* Smallholder farmer, digestibility, ensiling, rice bran, yeast

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# Dedication

To my parents, my husband Soukpasong, my daughter Soukvilay and all my cousins

# Contents

# List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Taysayavong, L., Bakeeva, A., Passoth, V. & Lindberg, J.E. 2018. Impact of aerobic yeast fermentation on the nutrient content of cassava root pulp (Manuscript).

II Taysayavong, L., Lindberg, J.E. & Ivarsson, E. 2018. Digestibility, nitrogen retention, gut environment and visceral organ size in Moo Lath and Large White growing pigs fed unfermented and fermented cassava root pulp and soybean pulp. *Livestock Research for Rural Development.* Volume 30, Article #4. Retrieved January 1, 2018. Available at: http://www.lrrd.org/lrrd30/1/jan_s30004.html

III Taysayavong, L., Ivarsson, E. & Lindberg, J.E. 2018. Ensiling of fresh cassava root pulp and fresh soybean pulp with or without rice bran. *Livestock Research for Rural Development.* Volume 30, Article #107. Retrieved August 20, 2018. Available at: http://www.lrrd.org/lrrd30/6/lotc30107.html.

IV Taysayavong, L., Lindberg, J. E. & Ivarsson, E. 2018. Nutritive value of ensiled cassava root pulp and ensiled soybean pulp as feed for growing pigs (Manuscript).

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# Abbreviations

| | |
|---|---|
| ADF | Acid Detergent Fiber |
| ATTD | Apparent total tract digestibility |
| CP | Crude protein |
| CRP | Cassava root pulp |
| CRPF0 | CRP fermented without rice bran + fish meal |
| CRPF5 | CRP fermented with 5% rice bran + fish meal |
| CRPF10 | CRP fermented with 10% rice bran + fish meal |
| CRPF20 | CRP fermented with 20% rice bran+ fish meal |
| DM | Dry matter |
| DMI | Dry matter intake |
| FCMP | Fermented cassava root pulp-medium protein |
| HCN | Hydrogen cyanide |
| NDF | Neutral Detergent Fiber |
| OM | Organic matter |
| SBP | Soybean pulp |
| SPHP | Soybean pulp-high protein |
| SPMP | Soybean pulp-medium protein |
| UCLP | Unfermented cassava root pulp-low protein |

# 1 Introduction

The tropics present great opportunities for sustainable development, thanks to the cultural and biological riches of these regions. Rational exploitation of local feed sources and local breeds of livestock will support sustainable production systems in the medium and long-term perspective. However, this has received insufficient attention in the past and has not been considered seriously because of the introduction of 'exotic' systems based on high inputs, high technology and breeds of 'high genetic merit'. As result, local breeds of pigs and cattle in many tropical countries have disappeared or their population is decreasing drastically (Rodríguez & Preston, 1997). Local breeds perform well in low-input systems, fulfilling multiple functions for smallholder households. They display poorer performance than exotic breeds, but have lower production input requirements (Le *et al.*, 2005).

In Lao PDR, more than 80% of pig herds are native breeds and belong to smallholders with combined rearing systems (Keonouchanh *et al.*, 2011). Local pigs are the main livestock raised and their feeds are made from vegetables collected from the forest, cultivated crops and crop residues and household leftovers. The main constraint in this low-input, low-output system is protein supply (Keo, 2000). Protein and mineral supply to local pig breeds reared in Central Lao PDR currently appears to be below requirements. This is partly due to low nutrient allowance, but also reflects the fact that obtaining animal feed is becoming an increasing challenge for smallholder farmers in developing countries. The main reason for this is that traditional feed ingredients for these animals (*i.e.* cereals, roots, tubers, soybeans and fish) are also important staple foods for humans (Sansoucy, 1995). As a result, these feed ingredients have become expensive and scarce. Thus, there is an urgent need to find alternative feed ingredients that can complement or replace the traditional feed ingredients.

The overall aim of this thesis was to evaluate fermented by-products from cassava and soybean as feed sources for pigs in smallholder systems in Lao PDR

*Objectives of the thesis:*

The main objectives of the work described in this thesis were:

- To investigate the impact of aerobic yeast fermentation of cassava root pulp on the nutrient content
- To determine the nutrient digestibility, nitrogen retention and nitrogen utilisation of diets with unfermented and yeast-fermented cassava root pulp and soybean pulp fed to growing Moo Lath and Large White pigs
- To study the effects of fermented cassava root pulp and protein level on gut development and gut environment in growing Moo Lath and Large White pigs
- To investigate the effect of rice bran inclusion on the chemical properties and nutrient content of ensiled cassava root pulp and soybean pulp
- To investigate the nutritive value of cassava root pulp and soybean pulp ensiled with different inclusion levels of rice bran and fed to growing Large White pigs.

*Hypothesis of the studies:*

- Fermentation of cassava root pulp and soybean pulp results in a low pH product with potential for long-term storage
- Fermentation of cassava root pulp and soybean pulp improves the nutritive value and has positive effects on digestibility, nitrogen retention and nitrogen utilisation when included in the diet of growing Moo Lath and Large White pigs.

# 2 Background

## 2.1 Status and prospects for livestock and pig production in Lao PDR

Livestock productivity has been recognised as an important national goal to foster sustainable growth of the Lao PDR economy and reduce rural poverty and food insecurity (Khounsy & Conlan, 2008; Nampanya *et al.*, 2010). Livestock production in Lao PDR has been estimated to contribute 16% to total gross domestic product (GDP), with most live animals and products being produced in traditional small-scale production systems. It is estimated that around 90% of all households in Lao PDR keep one or more species of livestock (Wilson, 2007). On average, each holding has 0-4 ruminants (buffalo and cattle), 2-5 goats, 2-4 pigs and 20-30 poultry, of which 35% own cattle (with 22% of households owning both species), 55% own pigs (65% in rural areas) and 4% own goats (SCAC, 2000). The major ruminant species in Lao PDR are buffalo (1.9 million head in 2011-2012) and cattle (1.9 million head), followed by goats and sheep (894,255 head). Local and imported (exotic) pigs (3.5 million head) and poultry (40.1 million head) are major contributors to household livelihoods and to the national economy (Theungphachan, 2012).

In urban areas of Lao PDR, animal protein consumption is estimated to be 35 kg per person and year (comprising 10 kg fish, 8 kg pork, 5 kg poultry meat, 3 kg beef, 3 kg buffalo meat and 6 kg eggs), while in rural areas it is estimated to be 22 kg per person and year (comprising 8 kg fish, 5 kg pork, 4 kg poultry meat, 1.2 kg beef, 1.8 kg buffalo meat and 1 kg eggs) (Bouahom, 1999). However, production has grown to match consumption, with an annual increase of about 15% for beef, 14% for poultry and 6% for pork (Quirke *et al.*, 2003). Pig production in Lao PDR has increased by 4.5% due to increased pork consumption, with the bulk coming from local breeds kept by smallholders within low input/low output systems for domestic use and local trade (FAO, 2006).

The long-term goal of the Lao PDR government is to increase livestock production in order to reduce poverty and to increase protein consumption from 22 to 50 kg per person in rural areas and from 33 to 70 kg per person in urban areas (ABD, 2005). To achieve this goal, strategies are needed to engage poor households and minimise potential negative impacts of large-scale livestock intensification on smallholder farmers (Millar & Photakoun, 2008). Moreover, providing effective interventions requires a better understanding of current livestock production, in order to manage health and husbandry constraints that compromise smallholder livestock productivity and market access (Windsor *et al.*, 2008; Nampanya *et al.*, 2010).

## 2.2 Feed resources for pigs in Lao PDR

The type of animal feed used in Lao PDR depends on the farming system, the availability of labour and access to suitable vegetation. In areas where many farmers grow paddy rice for sale, pig feed is based on rice bran as the main feed source (Thorne, 2005). The rice bran is fed together with small amounts of green feed and vegetables, planted crops, agricultural by-products and household scraps (Stür Werner, 2002; Phonvisay Singkham, 2003; Phengsavanh *et al.*, 2010). However, suitable feedstuffs for pigs are in short supply, which results in an imbalance of nutrients, especially amino acids, mineral and vitamins, in the diet. This is a great problem for smallholder pig production (Phengsavanh, 2008) and results in low daily live weight gain of pigs (135 g/day) in rural areas of Lao PDR (Phengsavanh *et al.*, 2010). Other major constraints in smallholder pig production systems in Lao PDR are outbreaks of disease and high mortality of piglets (Phengsavanh *et al.*, 2011).

### 2.2.1 Cassava

Cassava (*Manihot esculenta* Crantz) is widely grown in tropical regions. The cassava root is an excellent source of dietary energy, but is low in protein (Table 1). It is commonly used in pig diets throughout the world (Gomez, 1992). It can be fed fresh, dried or ensiled. A major concern with the use of cassava products as animal feed is that they contain toxic cyanide in the form of the cyanogenic glucoside linamarin (Gomez, 1992; Tewe, 1992). However, appropriate processing of cassava roots (*e.g.* sun-drying, ensiling, grating, soaking and fermentation) can reduce the cyanide content to non-toxic levels (Tewe, 1992).

In general, cassava is a crop traditionally grown for root production. In addition, the leaves are available as a crop residue when the root is harvested (Vongsamphanh *et al.*, 2004). The residue following starch extraction from the cassava root is called cassava root pulp (CRP) and contains 50-70% starch and 20-30% fibre on a dry matter (DM) basis (Pandey *et al.*, 2000; Rattanachomsri *et al.*, 2009). According to the Lao PDR Trade Portal (LTP, 2015), cassava planting in Lao PDR is strongly supported. Production is expanding both in quality and in quantity throughout the country, which has a positive impact on food security, on-farm earnings for rural people and the supply of raw material for agricultural enterprises (Table 2).

Table 1. *Chemical composition (% of dry matter) of cassava root (CR, two different analyses), dehydrated cassava root (CRD), peeled cassava root (CRPe) and cassava root pulp (CRP)*

| Component | CR§ | CRD§§ | CR§§ | CRPe§§ | CRP# |
|---|---|---|---|---|---|
| Dry matter | 37.0 | 87.6 | 37.6 | 28.5 | 13.1 |
| Crude protein | 3.5 | 2.9 | 2.6 | 2.2 | 1.7 |
| Crude fiber | 4.3 | 3.9 | 3.7 | 1.0 | 17.7 |
| NDF | 11.4 | 8.0 | 7.8 | 3.7 | - |
| ADF | 6.3 | 5.4 | 5.3 | 1.6 | - |
| Lignin | - | 1.7 | 1.6 | 0.0 | - |
| Ether extract | 0.9 | 0.7 | 0.8 | 0.6 | 1.3 |
| Ash | 3.0 | 3.9 | 2.8 | 3.8 | 3.7 |
| Starch | 69.2 | 80.4 | 80.8 | - | 52.3 |
| Total sugar | - | 2.4 | - | - | 3.3 |
| Gross energy MJ/kg DM | - | 16.8 | 17.1 | - | 17.7 |

Source: § *McDonald et al. (2011)*; §§ Heuzé *et al. (2016)*, # https://www.feedipedia.org/node/526

Table 2. *Cassava production (1,000 tons) in ASEAN countries 2015-2017*

| Country | 2015 | 2016 | 2017 |
|---|---|---|---|
| Brunei | 0.17 | 0.17 | 0.18 |
| Cambodia | 11,943.20 | 13,222.25 | 13,386.69 |
| Indonesia | 21,801.42 | 20,744.67 | 23,578.97 |
| Lao PDR | 2,382.48 | 2,410.00 | 1,557.75 |
| Malaysia | 67.71 | 74.48 | 81.93 |
| Myanmar | 484.91 | 460.48 | 764.40 |
| Philippines | 2,628.01 | 2,729.14 | 2,800.73 |
| Singapore | - | - | - |
| Thailand | 32,357.74 | 30,557.86 | 31,187.28 |
| Vietnam | 10,452.70 | 10,578.22 | 10,653.47 |
| Total | 82,118.34 | 80,777.28 | 84,011.40 |

Source: *AFSIS (2016)*

### 2.2.2 Maize

Maize (*Zea mays*) is the second most important crop, after rice, for smallholder farmers in Lao PDR (Roder, 2000; LSB, 2011) and is used as food for humans and feed for livestock (Phengsavanh *et al.*, 2010). Maize is cultivated on 196,815 ha, with the main production areas located in Xayaboury (29.2% of total), Oudomxay (18.8%) and Borkeo (10.2%), which together thus account for 58.2% of the total maize production in Lao PDR (1.1 million tons) (MAF, 2007).

Maize is high in starch (730 g/kg) and is an excellent source of energy, but is low in crude protein (90-140 g/kg), fibre, vitamins and minerals. Lysine and tryptophan are the first two limiting amino acids when maize is used for feeding pigs. The fat content of maize ranges from 40 to 60 g/kg DM and is high in linoleic acid, which can produce soft body fat in pigs (McDonald *et al.*, 2011). By-products of maize after the grain has been processed for human use (*e.g.* bran and germ meal) are high in fibre and low in energy, but can be successfully used in pig diets (Holness, 1991).

### 2.2.3 Broken rice and Rice bran

Rice (*Oryza sativa*) is the staple food for humans in Lao PDR (Onphanhdala, 2009). It is grown on 939,011 ha (65% in the rain-fed lowlands and 21% in the rain-fed uplands), which accounts for over 80% of the total agricultural land. Total rice production in 2012 was 3.5 million tons (DOA, 2012).

Rice bran, a by-product of rice milling, is commonly used as animal feed. It is obtained from the outer layer of brown (husked) rice kernels and makes up 12-20% of total kernel weight including pericarp (Moongngarm *et al.*, 2012). Rice bran is the most nutritious part of rice, with a high content of carbohydrates, protein, fat and minerals (Juliano, 1985; Saikia & Deka, 2011; Sharif *et al.*, 2014) (Table 3).

Table 3. *Chemical composition of broken rice (BR) and rice bran (RB) of different quality (% of dry matter)*

| Component | BR | RBI | RBII | RBIII | RBIV |
|---|---|---|---|---|---|
| Dry mater | 87.5 | 90 | 90.1 | 90.2 | 91.7 |
| Crude protein | 9.0 | 14.2 | 14.8 | 12.7 | 8.8 |
| Crude fiber | 1.6 | 4.1 | 8.6 | 16.3 | 28.3 |
| NDF | 6.2 | 12.4 | 25.2 | 34.4 | 48.7 |
| ADF | 2.1 | 3.2 | 11.2 | 19.6 | 32.7 |
| Lignin | 0.5 | 1.2 | 4.1 | 6.8 | 11 |
| Ether extract | 1.9 | 13.2 | 17.2 | 14.4 | 10.3 |
| Ash | 1.4 | 6.9 | 9.4 | 12.4 | 13.6 |
| Starch | 85.5 | 42 | 28.8 | 22.4 | 14.7 |
| Total sugar | 0.7 | 3.8 | 2.8 | 2.8 | 1.1 |
| Gross energy MJ/kg DM | 18.1 | 20.5 | 21.2 | 20.1 | 19.3 |

RBI = rice bran, fibre<4%, RBII = rice bran, fibre 4-11%, RBIII = rice bran, fibre 11-20%, RBIV = rice bran, fibre >20%.
Source: *https://www.feedipedia.org/node/750.*

Rice bran has potential for use as an alternative to grains in pig production (Sharif *et al.*, 2014). However, it also contains anti-nutritional factors such as phytic acid and trypsin inhibitors and has a high fibre content (Ersin *et al.*, 2006). According to Han *et al.* (2015), rice bran protein is a promising protein source with good biological value and digestibility.

Adebiyi *et al.* (2018) report that inclusion of up to 30% extruded rice bran in the diet of weaner pigs improves performance in terms of body weight gain and feed conversion efficiency, and improves intestinal morphology.

### 2.2.4 Soybean

Globally, soybean (*Glycine max*) is the most common and most important oilseed crop used in livestock feeding. It is an excellent source of both energy and protein, and has a balanced amino acid profile to meet the amino acid requirements for monogastric animals (*e.g.* pigs and poultry) (Baker, 1999). The first limiting amino acid in soybean meal is methionine, which makes it an ideal combination with maize (Holness, 1991). Soybean has high protein and fat content, in addition to fibre and non-fibre carbohydrates (FEFAC, 2007) (Table 4). One factor of major concern is the anti-nutritional factors present in soybean (*e.g.* protease inhibitors and lectins). However, both protease inhibitors and lectins are inactivated by moist heat treatment of soy products (Araba, 1990; Liener, 1999).

The residue left after soy milk extraction from soybean (soybean pulp) is a cheap and potentially useful feed ingredient for smallholder farmers.

Table 4. *Chemical composition of soybean meal (g/kg of dry matter)*

| Component | Soybean meal | Soybean, full-fat | Soybean pulp§ |
|---|---|---|---|
| DM (g/kg) | 900 | 898 | - |
| CP | 503 | 415 | 254 - 284 |
| CF | 58 | 48 | 91 - 186 |
| EE | 17 | 222 | - |
| Ash | 62 | 54 | 30 - 37 |
| NDF | 125 | 122 | - |
| ADF | 91 | 82 | - |
| ADIN | 2.2 | - | - |
| Starch and sugar | 124 | 91 | 38-53 |

Source: *McDonald et al. (2011),* § *Li et al. (2012)*

### 2.2.5 Fish meal

Fish meal has long been recognised as a very digestible protein source with a high content of amino acids, vitamins and minerals (Mason & Weidner, 1964). However, the quality of fish meal can vary and depends on factors such as the type and species of fish, the freshness of the fish before processing and the processing system used for the meal (Kjelden *et al.*, 1983). In a study by Kim and Easter (2001) of pigs during week 1 to 4 after weaning, young pigs fed mackerel fish meal dried at 70 °C showed 10.3% greater average daily gain than pigs fed menhaden fish meal. Moreover, the protein content of various fish meals varies over a range of about 500 to 750 g/kg (Table 5). Fish meal is rich in essential amino acids, particularly lysine, cysteine, methionine and tryptophan, and has a high mineral content (100-200 g/kg), in particular of calcium (Ca), phosphorus (P) and trace minerals, including manganese (Mn), iron (Fe) and iodine (I). Fish meal is also a good source of B vitamin complex, particularly choline, $B_{12}$ and riboflavin (Miller *et al*., 1991; McDonald *et al*., 2011).

Table 5. *Chemical composition (g/kg DM) of fish meal*

| Items | Fish meal, UK-produced | Fish meal, herring | Fish meal, South American |
|---|---|---|---|
| CP | 699 | 793 | 733 |
| Ash | 238 | 122 | 197 |
| EE | 69 | 75 | 60 |

Source: *McDonald et al. (2011)*

## 2.3 Breeds of pig in Lao PDR

### 2.3.1 Local breeds

The native pigs in Lao PDR have been characterised and classified into four type of breeds by phenotypes (Table 6). Several breeds are recognised, including Moo Chid (Figure 1), Moo Lath (Figure 2), Moo Nonghad (Figure 3) and Moo Deng (Figure 4). Most native pigs tend to have high fat content and low lean meat content in the carcass (Table 6), and they are black in colour and swaybacked, as are most Asian breeds. In 60 kg live weight Moo Lath pigs, the fat content in the carcass is reported to be 48-59%, with a lean meat content of 34-40% (Keonouchanh *et al*., 2011**).** These breeds reach a mature weight of 60-100 kg and are hardy and able to scavenge at least part of their feed requirements in free-range conditions. Growth rates tend to be low in extensive management systems and animals may take 15 months to reach a weight of 40-50 kg

(Kennard, 1996). Female body weight at puberty (6 months old) is about 21-30 kg and male body weight 30 kg. Piglets weaned at 2-3 months weigh 7-8 kg and sows produce 1.5 litters per year (Bouahom, 1999). However, farmers report that many sows only have one litter per year, with 6-8 piglets per litter.

Table 6. *Performance of four types of native pig breeds in Lao PDR*

| Parameter | Moo Chid | Moo Lath | Moo Nonghad | Moo Deng |
|---|---|---|---|---|
| Body length, cm | 92 | 85-100 | 100-105 | 88-120 |
| The girth, cm | 72-85 | 84-102 | 115-130 | 84-116 |
| Height, cm | 46-54 | 51-70 | 55-76 | 60-70 |
| First oestrus, day | 182-197[#] | 189-586[§] | 150-180[*] | - |
| Weight for mature sows, kg | 42-48 | 47-61 | 65-85 | 65-90 |
| Weight for boars, kg | 18-30 | 30-50 | 60-80 | 65-90 |
| First farrowing, day | 360 | 360 | 300-330 | 330-360 |
| Litters per year | 1.5 | 1.5-1.8 | 1.5-1.8 | 1.5-1.8 |
| Number of piglets per litter | 7-8 | 7-8 | 7-10 | 7-10 |
| Weaning age, month | 3 | 2-3 | 2-3 | 2-3 |
| Weight at weaning, kg | 7.8 | 9.5 | 8 | 8.5 |

[#]At body weight 21-31 kg; males have lower body weight than females (20.5 kg at 170-200 days), [§]at body weight 39 kg; male have lower body weight (25 kg) than female pigs.
[*]At body weight 30-40 kg.
Source: *Keonouchanh et al. (2011)*

*Figure 1.* Moo Chid, Moo Markadon, Moo boua

*Figure 2.* Moo Lath

Figure 3. Moo Nonghad or Moo Hmong

*Figure 4.* Moo Deng or Moo Berk

### 2.3.2 Exotic breeds

Several breeds of ‘exotic’ pigs are raised by farmers in Lao PDR, such as Large White, Landrace, Duroc Jersey and Chinese Meishan (Keonouchanh *et al*., 2011).

Large White and Yorkshire are lean meat pigs (55-60% lean meat in the carcass) found world-wide and are the most widespread of modern pigs. Their ability to cross with and improve other breeds has given them a leading role in commercial pig production systems,. They are easily recognised as wholly white and with pricked ears (Figure 5). The female has a mature live weight of above 300 kg (Whittemore, 1993).

The Landrace breed is a general description of a white lop-eared type pig (Figure 6). Its main use world-wide is in crossing with the Large White. It is known for its high litter performance and excellent maternal traits (Christiansen, 2010).

The Duroc pig is coloured, ranging from gold through red to dark brown, and probably originates from the Berkshire breed (Figure 7). Used as a sire line, it offers high daily weight gain and excellent feed conversion ratio (Christiansen, 2010). It may also be used in the female line, where it contributes robustness and disease resistance, particularly in the context of outdoor and extensive pig-keeping systems (Whittemore, 1993).

Meishan is a Chinese pig breed with an adult live weight of no more than 150 kg, a growth rate of about 400 g per day and a carcass with only 45% lean meat. The female reaches puberty at less than 100 days of age and the litter size is around 14 piglets with a birth weight 0.8 kg (Figure 8) (Whittemore, 1993).

A study by Phengvilaysouk *et al.* (2017) found that on large-scale pig farms (more than 30 sows) in Lao PDR, the sows were crosses between Large White and Landrace, which were mated with a Duroc boar. In these systems the sows were on average 7.8 months at first service, weighing 96.6 kg, and they had 2.0 litters per year, with 10.5 born piglets per litter and 9.5 weaned piglets per litter. The piglets were found to be weaned at 1.3 months of age, weighing 11.5 kg. When the same breeds are used in European production systems, the sows are generally are about 7.5 months at first service and weigh about 130-140 kg (Christiansen, 2010). Moreover, the average production values for sows in InterPIG countries in 2016 indicate that a sow has 2.31 litters per year and weans 11.9 piglets per litter (AHDB, 2017). Finisher pigs in InterPIG countries are slaughtered at 120 kg and grow by on average 823 g/day during the finishing period, with a feed conversion ratio of 2.8 (AHDB, 2017).

*Figure 5*. Large White or Yorkshire

*Figure 6*. Landrace

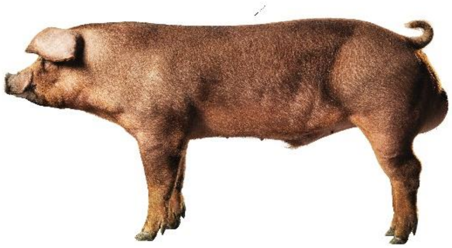

*Figure 7*. Duroc

*Figure 8*. Meishan

Source: *Figure 5-7, Topigs Norsvin, (2018).*

## 2.4 Nutrient requirements of growing pigs

### 2.4.1 Energy

Energy requirements are expressed as digestible energy (DE) or metabolisable energy (ME), and can be transformed into net energy (NE) requirements (Noblet, 2007). In growing pigs, energy is needed for maintenance, synthesis of body tissues and body heat regulation. Energy supplied in excess of maintenance requirements can be used for production, while additional energy is deposited as body tissues (Noblet, 2007; McDonald *et al.*, 2011).

Net energy is the only energy system in which energy requirements and diet energy values are expressed on the same basis (Noblet & Henry, 1993). The net energy value is defined as: ME minus the heat increment associated with metabolic utilisation of ME and with the energy cost of ingestion, digestion and some physical activity. It is generally calculated as the sum of fasting heat production and retained energy (Noblet *et al.*, 1994). The efficiency of utilisation of metabolisable energy for net energy differs between dietary components, with higher values for starch (0.80) than for dietary fibre (0.50-0.60). However, the contribution of dietary fibre to the energy balance of pigs can be affected by climate conditions, as the heat increment of dietary fibre is used for thermoregulation, and by changes in animal behaviour (Noblet & Le Goff, 2001).

Energy and nutrient digestibility depend on chemical characteristics of feed, biotechnological treatments, animal factors (body weight), the role of dietary fibre and interactions between these factors (Noblet & Van Milgen, 2004). Thus, a deeper knowledge of how these factors affect digestibility will lead to improvements in prediction of the energy value of pig feeds.

### 2.4.2 Protein and amino acids

Pigs require a number of essential nutrients to meet their needs for physiological processes, maintenance, growth, reproduction, lactation and other functions (Fashina, 1991). Dietary protein can normally supply the amino acids needed for normal body function. Twelve of 22 amino acids are synthesised by the animal, while the other 10 (essential amino acids) must be provided in the diet in appropriate amounts and proportions needed for a particular level of performance (Adesehinawa & Ogunmodede, 1995). The 10 essential amino acids for pigs are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (NRC, 2012; McDonald *et al*., 2011). If the protein and amino acid requirements are not met, animal performance, health and welfare can be negatively affected (NRC, 1998). The protein and essential amino acid requirements of weaning pigs and growing pigs, as suggested by NRC (2012), are shown in Table 7.

Conventional pig diets are formulated on the basis of crude protein, calculated as the nitrogen (N) content of the feedstuff multiplied by a factor of 6.25) (NRC, 1988). A low protein diet or a diet with insufficient amounts of high quality protein will result in poor growth, impaired feed utilisation, increased fat content in the carcass and/or impaired reproductive performance (Adesehinawa & Ogunmodede, 1995). A protein source with a well-balanced amino acid profile is required in lower amounts to maximise growth and efficiency of gain (Bender, 1975). The amino acid requirement decreases as the pig becomes heavier, which means that the requirement is greatest during the rapidly growing stages of young animals (Conrad, 1984). Thus changes in the rate of growth and body composition are the basis for recommending different dietary protein levels to meet the amino acid requirements during the life of the pig (Fanimo, 1991).

Native Lao pigs, such as the Moo Lath breed, can be assumed to require less dietary crude protein than exotic (*e.g*. European) breeds. Therefore, it is of great interest and practical relevance to establish suitable levels of crude protein in the diet of native Lao pigs. Phengsavanh and Lindberg (2013) suggest that a reasonable target level for crude protein (as a proportion of dietary DM) in feed formulation for growing Moo Lath pigs should be 18% for weaners, 15% for growers and 11% for finishers.

Table 7. *Recommended dietary content of digestible (DE), metabolisable (ME) and net (NE) energy (kcal/kg), minerals (%), essential amino acids (%), standardised ileal digestible crude protein (SID CP) and crude protein (CP) for growing pigs fed ad libitum (90% dry matter) (NRC, 2012)*

| Component | Growing pigs (kg) | | |
|---|---|---|---|
| | 7-11 | 11-25 | 25-50 |
| DE content[§] | 3,542 | 3,490 | 3,402 |
| ME content[§] | 3,400 | 3,350 | 3,300 |
| NE content[§] | 2,448 | 2,412 | 2,475 |
| Body weight gain (g/day) | 335 | 585 | 758 |
| Mineral (%) | | | |
| Total calcium | 0.80 | 0.70 | 0.66 |
| Total phosphorus | 0.65 | 0.60 | 0.56 |
| Sodium | 0.35 | 0.28 | 0.10 |
| Chloride | 0.45 | 0.32 | 0.08 |
| Potassium | 0.28 | 0.26 | 0.23 |
| Essential Amino Acids (%) | | | |
| Arginine | 0.61 | 0.56 | 0.45 |
| Histidine | 0.46 | 0.42 | 0.34 |
| Isoleucine | 0.69 | 0.63 | 0.51 |
| Leucine | 1.35 | 1.23 | 0.99 |
| Lysine | 1.35 | 1.23 | 0.98 |
| Methionine | 0.39 | 0.36 | 0.28 |
| Methionine+cysteine | 0.74 | 0.68 | 0.55 |
| Phenylalanine | 0.79 | 0.72 | 0.59 |
| Phenylalanine+tyrosine | 1.25 | 1.14 | 0.92 |
| Threonine | 0.79 | 0.73 | 0.59 |
| Tryptophan | 0.22 | 0.20 | 0.17 |
| Valine | 0.86 | 0.78 | 0.64 |
| SID CP (%) | 17.5 | 16.0 | 13.2 |
| CP (%) | 23.7 | 20.9 | 18.0 |

[§]Basal diet based on maize and soybean.

### 2.4.3 Minerals

In adult pigs, minerals are required for a variety of biochemical functions (Mahan & Vallet, 1997). In determining the calcium requirements of the pig, development of the skeleton should receive primary consideration because it has a supportive role and serves as a reservoir. Moreover, calcium, phosphorus and vitamin D are critical nutrients for growth and maintenance of the hard and soft tissue of the body (Miller *et al*., 1991). The skeleton contains 98% of total body calcium and 80% of total body phosphorus, in a Ca:P ratio of around 2:1, in the form of hydroxyapatite. Bone contains 45% mineral, 35% protein and 20% fat, with a calcium content in bone ash of 40% and a phosphorus content of 20% (Whittemore, 1993). Moreover, the allowance of calcium and phosphorus can be increased by 30% above requirements without affecting animal performance, and this can be a way to insure strong skeletal development (Nimmo *et al.*, 1981). The calcium and phosphorus requirements of pigs decrease as they grow (Suttle, 2010). Examples of good calcium and phosphorus sources are milk, green leafy plants, especially legumes, and sugar beet pulp. Moreover, animal by-products containing bones, such as fish meal, are excellent sources of calcium and phosphorus (McDonald *et al.*, 2011).

Sodium (Na), chlorine (Cl) and potassium (K) are major controllers of the ionic balance in the body tissues. Sodium and chlorine salts are well digested, normally absorbed in excess of requirements and included at supplementary levels of 1-3 g salt/kg diet. The sodium content of cereals is around 0.3 g Na/kg, while fish meal contains around 6 g Na per kg. The potassium requirement is around 3 g K per kg feed; cereals contain around 4 g K per kg, while soybean meal contains around 20 g K per kg (Whittemore, 1993). The magnesium (Mg) requirement of pigs is 0.4 g per kg feed, which is commonly met from dietary sources. Cereals contain around 1 g Mg per kg. The calcium, phosphorus and potassium requirements in growing and finishing pig diets are given in Table 8.

Table 8. *Recommended calcium (Ca), phosphorus (P) and potassium (K) levels in pig diets*

| Component | Pigs up to 15 kg live weight | | Pigs of 15-150 kg live weight | |
|---|---|---|---|---|
| | g/kg diet | g/MJ DE | g/kg diet | g/MJ DE |
| Ca | 9-15 | 0.80 | 8-10 | 0.65 |
| P | 7-11 | 0.60 | 6-8 | 0.50 |
| K | 1.0-2.5 | 0.14 | 1.0-2.5 | 0.14 |

Source: *Whittemore (1993).*

### 2.4.4 Vitamins

Vitamins are organic compounds that are required in small amounts for maintenance, normal growth, disease resistance, reproductive ability, physiological and metabolic function in the body, and to support health and well-being (Whittemore, 1993; Pond & Mersmann, 2001; McDonald, 2002).

Vitamin D is a companion nutrient to calcium and phosphorus, and signs of vitamin D deficiency are similar to those manifested with calcium and phosphorus deficiency, which makes it difficult to distinguish the cause of observed rickets or osteomalacia (Miller *et al.*, 1991). Vitamin D is found in two forms, ergocalciferol ($D_2$), which is produced in the body, and cholecalciferol ($D_3$), which is a constituent of plants and is abundant only in some fish (McDonald *et al.*, 2011). Vitamin $D_3$ is absorbed in the small intestine from the diet (Miller *et al.*, 1991).

Vitamin E plays an important role in the development and function of the immune system, and is involved in the regulation of cell signalling and gene expression, as is vitamin A (McDonald *et al.*, 2011). The requirement of both selenium (Se) and vitamin E during the later growth and finishing period is lower than for weaner pigs. The selenium requirement declines to approximately 0.1 ppm and the vitamin E requirement to 10-20 IU per kg diet by the time pigs reach market weight (Mahan *et al.*, 1977). Vitamin E and selenium deficiency is manifested in two main diseases, myopathy and cardiac disease (mulberry heart). Myopathy normally affects young, fast-growing pigs, but it can occur at any age (McDonald *et al.*, 2011).

Many vitamins are destroyed by oxidation, a process accelerated by the action of heat, light and certain metals such as iron. This fact is important, since the conditions under which a food is stored will affect the final vitamin potency (McDonald *et al.*, 2011).

## 2.5 Nutrient digestibility in pigs

Energy digestibility is affected by animal-specific factors. In growing pigs, the digestibility coefficient of energy increases with increasing body weight (Noblet & Van Milgen, 2004). The improvement in energy digestibility with body weight is mainly related to improved utilisation of dietary fibre (Le Goff et al., 2002). Moreover, Noblet *et al.* (1994) found that digestible nutrients were used differently for net energy, with the efficiency of ME utilisation (*k*) values varying from approximately 0.60 for digestible crude protein or digestible cell wall fractions to 0.82 for starch and 0.90 for digestible ether extract.

Protein digestion is dependent on both the animal and the feedstuff ingested. Ingested protein that is not excreted in the faeces, and has thus disappeared from the digestive tract, is by definition assumed to have been digested. The average crude protein digestibility for high quality feeds for pigs is within the range 80 to 85% (Whittemore, 1993).

In pig diets, the digestibility coefficient of energy varies between 0.70 and 0.90, but the variation is larger for feed ingredients (0-1.0) (Sauvant *et al.*, 2002). Most of the variation in energy digestibility is related to the presence of dietary fibre (sum of non-starch polysaccharides and lignin), which is less digestible than other nutrients (<50%, compared with 80-100% for starch, sugars, fat and protein). Increasing the fibre content in the diet affects the faecal digestibility of dietary components (Wilfart *et al*., 2007). In general, increased content of dietary fibre causes a decrease in the apparent faecal digestibility of dietary components such as crude protein and fat (Noblet & Perez, 1993; Noblet & Le Goff, 2001). However, there are also benefits from having fibre in the diet, such as increased well-being (Low, 1985).

## 2.6 Methodology

The digestibility of energy and nutrient components in a feedstuff or a diet can be determined *in vivo* by using the total collection technique and the indicator technique. The total collection technique is the 'gold standard' for determination of digestibility (McDonald *et al*., 2011). With the total collection technique, intake of feed and the total output of faeces or ileal digesta have to be measured accurately. Samples of the diet and faeces or ileal digesta are analysed for the content of energy or nutrient components and the apparent digestibility of these is calculated as:

$$D_A = ((A_I - A_D)/A_I) \times 100 \quad \text{(eq. 1)}$$

Where $D_A$ is the apparent faecal of ileal digestibility value of a component in the assay diets (%), $A_I$ is the amount of component consumed (g) and $A_D$ is the amount of component excreted in faeces or ileal digesta (g).

In contrast to the total collection technique, the indicator technique avoids the necessity for total collection of faeces or ileal digesta and measurement of feed consumption. With the indicator technique, an indigestible marker (e.g. $Cr_2O_3$ or $TiO_2$) is included in the assay diet and spot samples of faeces or ileal digesta are collected. The apparent digestibility value is then calculated as:

$$D_D = 100\% - ((I_D \times A_F)/(I_F \times A_D)) \times 100\% \quad \text{(eq. 2)}$$

Where $D_D$ is the apparent digestibility value of a component in the assay diet (%), $I_D$ is the indicator concentration in the assay diet (g/kg), $A_F$ is the component concentration in faeces or ileal digesta (g/kg) and $I_F$ is the indicator concentration in the assay diet (g/kg).

However, the nutritive value of protein in feedstuffs for monogastric animals is determined not only by the amino acid composition, but also by the bioavailability of individual amino acids. Animal growth assays and digestibility assays are the two major evaluation tools available for assessing the bioavailable of amino acids in feedstuffs for pigs (Sauer *et al*., 2000). Several in vivo assay methods have been developed for determining digestible amino acids in feedstuffs for pigs and considerable efforts have been made to compare the validity of different amino acid digestibility assays (Stein *et al.*, 2007). Moreover, the bioavailability of protein and amino acid can be estimated based on nitrogen balance studies in which nitrogen intake and excretion (faeces and urine) are determined (Just *et al.*, 1982; Eggum, 1989). This is a robust and simple in vivo assay that can be applied without requiring access to sophisticated analytical equipment.

## 2.7 Fermentation

Microorganisms can be used for preservation of biomass and for interventions to change the chemical and nutritional properties of the material (Bernard & Barrington, 1962; McDonald *et al.*, 2011). Both aerobic and anaerobic fermentation has been used for preservation of moist plant biomass (Ashbell, 1996; Ubalua, 2007; McDonald *et al.*, 2011) and for nutritional interventions using yeast and bacteria (Hahn-Hägerdal *et al.*, 1994; Driehuis *et al.*, 2000; Aro, 2008; Nasseri *et al.*, 2011; Polyorach *et al.*, 2013; Napasirth *et al.*, 2015).

### 2.7.1 Aerobic fermentation

Aerobic fermentation processes for the production of a high protein product use a primary source of carbon and oxygenated hydrocarbons in aqueous inorganic salt growth medium and oxygen-containing gas, together with microorganisms capable of growth on oxygenated hydrocarbon (Douros, 1970). Aerobic fermentation with yeast is used in the production of antibiotics, steroids and other useful products (Bernard & Barrington, 1962). Oxygen transfer from the gas phase to solution in the proximity of the cell is a major factor in aerobic fermentation, with oxygen transfer being the rate limiting step (Darlington, 1964).

### 2.7.2 Anaerobic fermentation

The process by which forage with high moisture content is preserved by anaerobic fermentation is called ensiling, and the product is called silage. The main objective of ensiling forage is to preserve it for use in those seasons when fresh forage is not available (Driehuis & Elferink, 2000). The container/pit used for ensiling the material is called a silo or bunker, but a plastic wrapping can also be used for smaller portions (bale silage). Almost any crop can be preserved as silage, such as grasses, legumes and whole cereals (McDonald *et al.*, 2011). Biochemical change occurs during fermentation and can result losses of soluble carbohydrates and proteins, as well as dry matter and energy because the activities of lactic acid bacteria are low (McDonald *et al*., 1991). Anaerobic conditions are established within the silage and anaerobic microflora dominates the fermentation process (McDonald *et al*., 2011). The main principles of preservation by ensiling are rapid achievement of low pH by lactic acid fermentation and maintenance of anaerobic conditions (McDonald *et al*., 1991).

# 3 Summary of materials and methods

## 3.1 Location of studies

The experiment reported in Paper I in this thesis was performed at the Department of Molecular Sciences, Swedish University of Agricultural Sciences (SLU) in Uppsala Sweden.

The experiments described in Papers II, III and IV were conducted at the Integrated Farming Demonstration Center of Champasack University, 13 km south of Pakse city, Champasack province, Lao PDR. There are two seasons in this region, a dry season (November-April) and a rainy season (May-October), with mean daily temperature of approximately 29 °C in both seasons (Lao Statistics Bureau, 2014). The experiments were conducted from May to June 2016 (Paper II), in February 2017 (Paper III) and from January to April 2018 (Paper IV).

## 3.2 Experimental design and treatments

In Paper I, the experiments were arranged in a 4 x 3 factorial design with two replicates per treatment where CRP was aerobically fermented with different yeast sources at different nitrogen addition rates. The factors were yeast source (*Schwanniomyces occidentalis*; *Rhodotorula toruloides*; *Saccharomyces cerevisiae*; Lao alcohol yeast) and nitrogen (N) addition rate (0, 1.25 and 2.5 % N on a DM basis). Ammonium-sulphate was used as the nitrogen source.

In Paper II, the experiment was structured according to a 2×4 factorial design with two pig breeds Moo Lath and Large White and four diets based on CRP and SBP ((Unfermented cassava root pulp-low protein (UCLP), Fermented cassava root pulp-medium protein (FCMP), Soybean pulp-medium protein (SPMP) and Soybean pulp-high protein SPHP)). The animals were selected from six litters (three litters of Moo Lath and three litters of Large White), and the experiment was performed in four periods, using four animals per breed, diet and period. Each period lasted for 12 days, with seven days for adaptation and five days for collection of data. Faeces and urine were collected daily and kept frozen until sample preparation. Sulphuric acid was added to urine collection trays to minimise nitrogen losses.

In Paper III, the experiment was randomised with three replicates per treatment and incubation time. CRP and SBP were anaerobically fermented (ensiled) for 7 and 14 days with different inclusion levels of rice bran (0, 5, 10 and 20% on a fresh weight basis). Sugar cane molasses (5% on a fresh weight basis) was added to all treatments.

In Paper IV, three experiments (Exp. 1-3) were performed with growing Large White pigs. In total, 24 Large White pigs with an initial body weight of 20 kg were used. In Exp. 1, eight pigs were used in a change-over design with two diets (rice bran (RB) and rice bran + fish meal (RBFM)) and two periods, while in Exp. 2 and Exp. 3, eight pigs were used in each experiment in a double change-over design with four diets and four periods. The diets in Exp. 2 were: CRP fermented without rice bran + fish meal (CRPF0), CRP fermented with 5% rice bran + fish meal (CRPF5), CRP fermented with 10% rice bran + fish meal (CRPF10), and CRP fermented with 20% rice bran + fish meal (CRPF20), and in Exp. 3: SBP fermented without rice bran (SBP0), SBP fermented with 5% rice bran (SBP5), SBP fermented with 10% rice bran (SBP10) and SBP fermented with 20% rice bran (SBP20). Each period lasted for 14 days, with nine days for adaptation and five days for collection for data. Faeces and urine were collected daily and kept frozen until sample preparation. Sulphuric acid was added to urine collection trays to minimise nitrogen losses.

## 3.3 Experimental animals, management and feeding

In Paper II, 32 pigs (16 Moo Lath and 16 Large White), were used in the experiment. The Moo Lath pigs were bought from farmers in Champasack Province and the Large White pigs were bought from a private farm in the city. They were 60 days old, and the initial body weight of Moo Lath pigs was 7.5 kg and that of Large White pigs was 13.7 kg.

In Paper IV, 24 Large White pigs with an initial body weight of 20 kg were used. The pigs were bought from a private farm in the city at 75 days old and with an initial body weight of 20 kg. Pigs were selected from three litters per experiment.

Before the experiments started in Papers II and IV, all animals were vaccinated against hog cholera and de-wormed with Ivermectin (0.05 mL/kg body weight). The pigs were kept in individual metabolism cages (0.66 m × 0.63 m × 0.60 m) that were elevated 0.6 m above the ground, allowing separate collection of faeces and urine. The cages were made of bamboo and wood, and provided with feeders and automatic water drinkers. The animals had free access to water. The feed was given in equal amounts twice per day, at 8:00 and 16:00 hours. Uneaten feed was removed and weighed before fresh feed was added.

## 3.4 Fermentation studies

In Paper I, dry CRP was reconstituted to 20% DM with tap water, steamed for 25 minutes and then cooled to room temperature before being mixed with yeast and nitrogen. This was done in a plastic bag by mixing the yeast suspension vigorously with CRP. The prepared treatments (2 g DM CRP per replicate or approximately 10 g wet CRP per replicate) were placed in Falcon tubes (50 mL) with a lid loosely attached (or with a hole punched in the lid of the tubes with a nail) to allow air exchange (aerobic conditions) during fermentation. The Falcon tubes were kept at 30 °C in a temperature-controlled incubator. Before inoculation, the yeasts were cultivated into yeast peptone dextrose broth (YPD) (yeast extract 10 g/L, peptone 20 g/L, dextrose 20 g/L) in 1000 mL shake flasks at 30 °C for 24 hours and then washed. The pellet was re-suspended in sterile sodium chloride solution (NaCl 9 g/L) to reach a concentration of $10^5$ cells per g CRP.

The CRP used in Paper III was collected from a starch factory situated about 11 km from the Integrated Farming Demonstration Center of Champasack University. The SBP used was bought at the local market in Pakse city. Fresh CRP and fresh SBP were mixed with different levels of rice bran (0, 5, 10 and 20% on a fresh weight basis in 2 kg mixture) and with 5% of sugar cane molasses (50 g/kg mixture on a fresh weight basis). After mixing, the material was placed in plastic bags, which were compressed to remove air and closed carefully to achieve anaerobic conditions. The samples were left to ferment at room temperature (23-30 °C) for a total of 14 days. Two experiments were performed in parallel, one with CRP and one with SBP.

## 3.5 Sample collection and analyses

In Paper I, samples were taken at the start and after 2, 4, 6, 8, 10 and 14 days of fermentation. Each replicate was carefully mixed and sampled, and were subjected to yeast culturing (serial dilutions and plating) and analysis of fermentation characteristics (pH and temperature) and DM. Samples were immediately transferred to plastic tubes, frozen at -20 °C and kept frozen until preparation for analysis.

In Papers II and IV, the total amount of feeds offered and residues, faeces and urine were recorded daily during the last five days of each period. In addition, samples of faeces were collected twice daily, 3-4 hours after each meal, from plastic sheeting placed under the metabolism cage. The faeces samples collected from each pig per treatment and period were pooled and stored at -18 °C until analysis. Urine was collected in a plastic bucket to which sulphuric acid was added to maintain the pH below 4.0 (10 mL of 10% concentrated $H_2SO_4$). The volume of urine was measured every day and 10% of the total volume was collected and stored for analysis. The pigs were weighed in the morning before feeding at the beginning and end of each period.

In Paper II, on day 13 of the experiment, two to three hours after the morning feeding, the pigs were weighed and then killed by injection of Thiopental (20 mg/kg body weight), for assessment of gut environment and morphometric measurement of visceral organs. The gastrointestinal tract was removed and segmented into stomach, small intestine, caecum and colon plus rectum. The removal of gastrointestinal tract started from the anus, using scissors to remove each segment in turn (rectum, colon, caecum, small intestine and stomach). Segments of full and empty digestive tract were weighed, and the length of each segment section of intestine was recorded. Digesta were collected from the ileum (about 60 cm of small intestine before the ileo-caecal ostium) and colon (20 cm from the caecum) and immediately transferred to plastic tubes, frozen at -20 °C and kept frozen until preparation for analysis.

In Paper III, samples were taken at the start and after 0, 7 and 14 days of ensiling. Each replicate was carefully mixed and sampled. Samples were immediately transferred to plastic tubes, frozen at -20 °C and kept frozen until preparation for analysis. to plastic tube, frozen at -20°C and kept frozen until preparation for analysis.

## 3.6 Statistical analyses

In Paper I, the data were analysed as a 3 x 4 factorial design with nitrogen level, yeast type and their interaction (Yeast x N level) as factors, using the general linear model (GLM) procedure of Minitab Software, version 16 (Minitab, 2010).

In Paper II, the data were analysed as 2×4 factorial design with diet, breed and interaction (diet x breed) as factors, using the GLM procedure of Minitab Software, version 16 (Minitab, 2010). The following model was used to analyse treatment effects on digestibility:

$$Y_{ijk} = \mu + D_i + B_j + (D_i \times B_j) + e_{ijk} \qquad \text{(eq. 3)}$$

Where $Y_{ijk}$ is nutrient digestibility, μ is the overall mean, $D_i$ is the effect of diet *I*, $B_j$ is the effect of breed *j*, ($D_i \times B_j$) is the interaction between diet *i* and breed *j* and $e_{ijk}$ is the random error.

In Paper III, the data from each experiment were analysed by ANOVA using the GLM procedure of Minitab Software, version 16 (Minitab, 2010).

In Paper IV, the data were analysed using the GLM procedure of Minitab Software, version 16 (Minitab, 2010).

For all experiments, treatment means which showed significant differences at $p<0.05$ were compared using Tukey's pair-wise comparison procedure.

# 4 Summary of results

## 4.1 Effect of aerobic and anaerobic fermentation on the nutrient content of cassava root pulp and soybean pulp (Papers I, III and IV)

In CRP fermented with different types of yeast and different levels of N, the pH differed between yeast sources and N levels at day 0 and day 14 (Table 9). Moreover, there was an interaction between yeast source and N level at 0 and 14 days of fermentation. The pH at start (day 0) was around 7.1 in most cases. For all yeast sources and N levels, the pH at day 14 was between 3.2 and 3.9 in Paper I. In Paper III, the pH of CRP and SBP ensiled with different levels of rice bran decreased with increasing days of ensiling (0, 7 and 14 days). The pH at day 14 ranged from 4.0 to 4.8 for CRP and from 3.9 to 5.2 for SBP, with the highest values observed for the highest inclusion of rice bran. In Paper IV, the pH in fermented CRP and SBP mixed with different levels of rice bran (0, 5, 10 and 20%) ranged from 3.8 to 4.6 for CRP and from 3.9 to 4.3 for SBP after 21 days of fermentation.

In Paper I, the DM content at day 0 increased with N addition level for all yeasts (Table 9). The DM content increased with the yeast *Rhodotorula toruloides*, while there was a reduction in DM content for other yeasts after 14 days of fermentation. Lao alcohol yeast reduced the DM of CRP by 7.0, 8.2 and 9.6% for an N level of 0, 1.25 and 2.5 %, respectively. This was the greatest DM reduction among the yeast sources tested.

In Paper III, higher DM content was observed with increasing time of ensiling for both CRP and SBP, and DM content also increased with increasing inclusion rate of rice bran. When 20% rice bran was included, the DM content increased from 23.7% at day 0 to 37.6% at day 14 for CRP and from 15.7% at day 0 to 26.7% at day 14 for SBP. Similar values for the DM content of ensiled

CRP and SBP with 20% inclusion of rice bran (37.0% and 28.4%, respectively) were obtained in Paper IV.

In Paper I, the crude protein and ash content increased and the starch content decreased with days of fermentation for all yeast sources and at all levels of N addition (Table 9). The greatest increase in crude protein and ash content with duration of fermentation was observed for the Lao alcohol yeast, and corresponded to 42.8, 27.2 and 5.7 % for CRP when N was added at a level of 2.5, 1.25 and 0 %, respectively. The greatest decrease in starch content was found for Lao alcohol yeast, followed by *Schwanniomyces occidentalis, Saccharomyces cerevisiae* and *Rhodotorula toruloides*, respectively.

Table 9. *pH, dry matter (DM) and crude protein content of fermented cassava root pulp (CRP) and soybean pulp (SBP) in Papers I, III and IV*

| Experimental factor | Level, (%) | Paper | pH | DM, (%) | CP (% in DM) |
|---|---|---|---|---|---|
| Lao alcohol yeast* | 0 | I | 3.8 | 7.0 | 5.7 |
| | 1.25 | I | 3.4 | 8.2 | 27.2 |
| | 2.5 | I | 3.4 | 9.6 | 42.8 |
| R. totuloides* | 0 | I | 3.6 | 17.4 | 2.1 |
| | 1.25 | I | 3.9 | 18.0 | 12.8 |
| | 2.5 | I | 3.2 | 18.1 | 17.1 |
| S. cerevisiae* | 0 | I | 3.5 | 16.4 | 3.5 |
| | 1.25 | I | 3.3 | 17.7 | 13.9 |
| | 2.5 | I | 3.4 | 14.9 | 28.7 |
| S. occidentalis* | 0 | I | 3.8 | 11.3 | 3.4 |
| | 1.25 | I | 3.4 | 13.2 | 14.6 |
| | 2.5 | I | 3.3 | 16.4 | 23.7 |
| CRP0* | 0 | III | 4.0 | 27.6 | 2.7 |
| CRP5* | 5 | III | 4.4 | 30.7 | 4.3 |
| CRP10* | 10 | III | 4.7 | 33.3 | 5.4 |
| CRP20* | 20 | III | 4.8 | 37.6 | 7.2 |
| SBP0* | 0 | III | 3.9 | 17.8 | 23.2 |
| SBP5* | 5 | III | 4.3 | 22.9 | 22.7 |
| SBP10* | 10 | III | 4.8 | 23.8 | 21.4 |
| SBP20* | 20 | III | 5.2 | 26.7 | 19.3 |
| CRP0# | 0 | IV | 3.8 | 28.9 | 2.7 |
| CRP5# | 5 | IV | 4.0 | 31.5 | 4.0 |
| CRP10# | 10 | IV | 4.4 | 33.6 | 5.1 |
| CRP20# | 20 | IV | 4.6 | 37.0 | 6.7 |
| SBP0# | 0 | IV | 3.9 | 18.5 | 22.6 |
| SBP5# | 5 | IV | 4.0 | 24.6 | 21.3 |
| SBP10# | 10 | IV | 4.1 | 25.3 | 19.8 |
| SBP20# | 20 | IV | 4.3 | 28.4 | 17.4 |

*After 14 days of fermentation; #after 21 days of fermentation, 0, 5, 10 and 20 % level of rice bran inclusion). *R. toruloides = Rhodotorula toruloides; S. cerevisiae = Saccharomyces cerevisiae; Sch. occidentalis = Schwanniomyces occidentalis*.

In Paper III, the crude protein content in CRP increased with days of ensiling within level of rice bran inclusion. The crude protein content in CRP was highest

(7.2%) when the CRP was mixed with 20% rice bran. For SBP, the crude protein content decreased with days of ensiling, and was 23.2% for diet SBP0, 22.7% for diet SBP5, 21.4% for diet SBP10 and 19.3% for diet SBP20 after 14 days of ensiling (Paper III). These values were higher than the crude protein content in the fermented CRP and SBP with 20% rice bran inclusion in Paper IV, which was 6.7% and 17.4%, respectively.

The ammonia-nitrogen ($NH_3$-N) content (% of DM) in both CRP and SBP increased with days of ensiling and within rice bran inclusion. The cyanide (HCN) content (% of DM) in CRP decreased with increasing rice bran inclusion and with days of ensiling. The range of HCN was between 39.3 to 48.2 mg/kg DM (Paper III).

## 4.2 Effect of fermented CRP and SBP on dry matter intake, apparent total tract digestibility, nitrogen metabolism, organ size and gut environment in growing Moo Lath and Large White pigs (Papers II and IV)

In Paper II, where Moo Lath and Large White pigs were fed unfermented and fermented CRP and SBP, higher dry matter intake (DMI) was found in Large White pigs than Moo Lath pigs (Table 10). The highest DMI was found in pigs fed diet SPHP (593 g/day) and diet SPMP (481 g/day) compared with diet FCMP (224 g/day) and diet UCLP (224 g/day). Moreover, in Paper IV, differences in DMI were found for the fermented CRP diets, with pigs fed diets CRPF10 and CRPF20 having the highest DMI and pigs fed diet CRPF0 the lowest. No differences were found in DMI between pigs fed rice bran (diet RB) and pigs fed rice bran and fish meal (diet RBFM), or between pigs fed the different SBP diets. In general, the highest DMI was observed for the SBP diets and the lowest for the CRP diets.

In Paper II, the apparent total tract digestibility (ATTD) of organic matter (OM) and crude protein differed between diets, with the highest value for both OM and crude protein in diet SPHP, followed by diets SPMP, FCMP and UCLP (Table 10). However, there were no differences between breeds and no interaction between breed and diets. In Paper IV, the ATTD of DM and OM in the CRP diets was highest for diet CRPF10 (82 and 84 %, respectively), followed in descending order by diets CRPF20 (78 and 81%, respectively), CRPF5 (76 and 79 %, respectively) and CRPF0 (73 and 76 %, respectively), while there was no difference between diets in the ATTD of crude protein. Moreover, the ATTD of DM and OM in SBP diets was lower in diet SBP0 (66 and 70 %, respectively) than in diets SBP5 (71 and 74 %, respectively), SBP10 (74 and 77 %, respectively) and SBP20 (74 and 77 %, respectively). The ATTAD of crude protein was higher in diet SBP20 (85%) than in diets SBP10, SBP5 and SBP0 (82, 74 and 77 %, respectively) (Table 10).

Table 10. *Dry matter intake (DMI, g/day), apparent total tract digestibility (%) of organic matter (dOM) and crude protein (dCP), N intake ($N_{in}$, g/day) and N retention ($N_{re}$, g/day; $N_{reT}$, % of total N intake; $N_{reD}$, % of digested N) in growing pigs fed fermented cassava root pulp (CRP) and soybean pulp (SBP)*

| Items | Paper | DMI | dOM | dCP | $N_{in}$ | $N_{re}$ | $N_{reT}$ | $N_{reD}$ |
|---|---|---|---|---|---|---|---|---|
| UCLP | I | 239 | 63 | 64 | 3.7 | 1.2 | 32 | 52 |
| FCMP | I | 224 | 65 | 75 | 4.5 | 2.4 | 53 | 71 |
| SPMP | I | 481 | 83 | 76 | 10.4 | 5.4 | 52 | 68 |
| SPHP | I | 593 | 88 | 87 | 11.9 | 6.9 | 58 | 68 |
| RB | II | 651 | 77 | 81 | 9.3 | 6.1 | 66 | 81 |
| RBM | II | 613 | 82 | 86 | 18.2 | 12.9 | 71 | 83 |
| CRPF0 | II | 439 | 76 | 79 | 7.1 | 3.8 | 54 | 59 |
| CRPF5 | II | 568 | 79 | 82 | 9.5 | 5.6 | 60 | 72 |
| CRPF10 | II | 646 | 84 | 85 | 11.4 | 7.8 | 68 | 80 |
| CRPF20 | II | 673 | 81 | 80 | 12.7 | 7.5 | 59 | 74 |
| SBP0 | II | 835 | 70 | 77 | 18.6 | 8.9 | 52 | 61 |
| SBP5 | II | 848 | 75 | 74 | 18.4 | 9.3 | 50 | 67 |
| SBP10 | II | 884 | 77 | 82 | 18.6 | 11.9 | 64 | 78 |
| SBP20 | II | 910 | 78 | 85 | 17.1 | 11.9 | 70 | 82 |

UCLP = unfermented cassava root pulp-low protein, FCRP = fermented cassava root pulp-medium protein, SPMP = soybean pulp-medium protein, SPHP = soybean pulp-high protein, CRPF0 = cassava root pulp fermented without rice bran; CRPF5 = cassava root pulp fermented with 5% rice bran; CRPF10 = cassava root pulp fermented with 10% rice bran; CRPF20 = cassava root pulp fermented with 20% rice bran; SBP0 = soybean pulp fermented without rice bran; SBP5 = soybean pulp fermented with 5% rice bran; SBP10 = soybean pulp fermented with 10% rice bran; SBP20 = soybean pulp fermented with 20% rice bran.

In Paper II, total N intake was highest for diet SPHP (11.9 g/day) followed in descending order by diets SPMP (10.4 g/day), FCMP (4.5 g/day) and UCLP (3.7 g/day). Higher values were obtained in Large White pigs than Moo Lath pigs in terms of N intake (10.4 compared with 5.9 g/day), N loss in urine (2.1 compared with 1.2 g/day) and N retention (68 compared with 46 % of N intake). Nitrogen retention (expressed in g/day and as a percentage of N intake) differed between breeds, while N retention expressed as a proportion of N digested was similar between breeds. Nitrogen retention (g/day and % of N intake) was highest for diet SPHP, followed in descending order by diets SPMP, FCMP and UCLP. In contrast, N retention expressed as a proportion of N digested was highest for diet FCMP, followed in descending order by diets SPHP, SPMP and UCLP.

In Paper IV, N intake was higher in pigs fed diet CRPF20 than in pigs fed diets CRPF5 and CRPF0, while pigs fed diet CRPF10 had higher N intake than pigs fed diet CRPF0. Pigs fed diet CRPF20 also had the highest N excretion in faeces and, together with pigs fed CRPF5, the highest N excretion in urine.

Nitrogen retention in g/day was higher in pigs fed diets CRPF20 and CRPF10 than in pigs fed diet CRPF0, while N retention as a percentage of total N was higher in pigs fed diet CRPF10 than in pigs fed diet CRPF0. Nitrogen retention (% of digested N) was highest for pigs fed diet CRPF10 and lowest for pigs fed diet CRPF0, with diets CRPF20 and CRPF5 being intermediate. In addition, N intake was lower in pigs fed diet SBP20 than in pigs fed diets SBP0 and SBP10, while N excretion in faeces was lower in pigs fed diet SBP20 than in pigs fed diets SBP0 and SBP5. Nitrogen excretion in urine decreased linearly with increasing rice bran inclusion and the value (in g/day, % of total N and % of digested N) was higher in pigs fed diets SBP10 and SBP20 than in pigs fed diets SBP0 and SBP5.

Organ measurements performed in Paper II showed that the length of small intestine, caecum and colon + rectum and total intestine length (cm) were greater for Large White than for Moo Lath pigs. No effects of diet or interactions between diet and breed were observed. Organic acids were also measured in Paper II and the results showed that pigs fed diet FCMP had the highest proportion of propionic acid in ileal digesta, followed by pigs fed diets UCLP, SPMP and SPHP. Propionic acid concentration was affected by diet and was 17.8 mol-% for diet FCMP, 15.0 mol-% for diet UCLP, 11.9 mol-% for diet SPMP and 11.5 mol-% for diet SPHP.

# 5 General discussion

## 5.1 Small-holder pig farms

Around 85% of pigs in Lao PDR are kept in traditional smallholder farming systems, mainly in mountainous regions of the country (Thorne, 2005). Moreover, farmers keep native breeds, as local people prefer pork from native breeds to pork from imported (exotic) breeds.

There are a number of constraints affecting smallholder pig production systems in Lao PDR. One of the most important factors is animal diseases, which can cause mortality at rates ranging from 50 to 100 % depending on the severity of disease. One example is classical swine fever (CSF) (Osbjer, 2006; Conlan *et al.*, 2008). Moreover, animal performance is limited by nutritional factors, which vary depending on species (Devendra *et al.*, 1997; Phengsavanh & Phimphachanh, 2007) and the severity of feed shortage.

## 5.2 Feed sources

The focus in this thesis was on evaluating the nutritive value of fermented cassava root and soybean pulps for pigs in Lao PDR, as a potential strategy to improve feed availability for smallholder farmers. However, there are a range of potential feed sources in Lao PDR, comprising planted crops (banana pseudo-stem, taro, yams, maize, mulberry, sweet potato leaves and cassava), agricultural by-products (rice bran, broken rice, distiller's waste and brewer's grain) and vegetables (Stür Werner, 2002; Phonvisay Singkham, 2003; Phengsavanh *et al.*, 2010). In addition, the use of household scraps and leftovers (swill) is common practice on smallholder pig farms (Manivanh & Preston, 2016). Nevertheless, in general feed availability is a limiting factor in many areas (Phengsavanh, 2006; Conlan *et al.*, 2008), which results in high prices of feed ingredients and high feeding costs. One consequence of this is poor nutrition of pigs due to underfeeding and resulting health problems (Conlan *et al*., 2008; Phengsavanh *et al*., 2011). Some smallholder farmers overcome the prohibitive cost of feeds by adding supplement to feed concentrates (Huynh *et al.*, 2007).

It is common practice to feed crosses between native and exotic pig breeds a mixture of commercial feed and cheap locally available by-products. Intensive and semi-intensive pig production systems make up a small proportion of pigs produced in Lao PDR (Hoffmann, 1999). Semi-intensive pig farms commonly use commercial feed mixed with rice bran, cassava root meal or maize meal, and fish meal.

### 5.2.1 Cultivated crops

*Rice*: Rice is the most important cultivated crop in Lao PDR (CIAT, 2001) as a staple for human consumption, which includes both paddy rice and sticky rice. It is only the by-products from rice production (*e.g*. broken rice & rice bran) that are used as feed for livestock in Lao PDR (Phengsavanh & Stür, 2006).

*Maize:* The second most important cultivated staple crop for human consumption is maize (yellow). However, pig farms in maize cultivation areas raising imported pig breeds commonly use maize as animal feed (LSB, 2011).

*Cassava:* Cassava is the third most important crop in Lao PDR, after rice and maize (CIAT, 2001). Cassava can produce very high yields (Preston, 2001) and is a crop traditionally grown for root production, as the root is an excellent energy source for people and the leaves are available as a crop residue when the root is harvested (Vongsamphanh *et al.*, 2004). In addition to being an important food crop, cassava (root and leaves) is also a very important animal feed source for pigs, poultry and cattle (Borin *et al*., 2005). In traditional cultivation, there are two common varieties of cassava; bitter and sweet. The cyanide content is higher in the bitter than in the sweet varieties, which has an impact on the risk of cyanide toxicity in both humans and livestock (Tewe, 1992; Hong *et al.*, 2017). However, several other varieties of cassava are also found in Lao PDR, including ordinary cassava, yolk cassava, Japanese cassava, mottled cassava and animal feed cassava (Biodiversity Country Report, 2003). Cassava is mostly grown on small farms, usually intercropped with other plants, such as sweet potatoes, melon and maize. It is widely grown throughout the country by upland farmers, but in small areas using local varieties and with very low inputs. According to the Lao PDR Trade Portal (LTP, 2015) cassava planting in Lao PDR is strongly supported (LTP, 2015) and production is expanding both in quality and in quantity throughout the country. This has a positive impact on food security, on-farm earnings for rural people and supply of raw material for agriculture.

*Sweet potato* (root & leaves): Sweet potato (*Ipomoea batatas* Lam) is commonly used as a human food in Lao PDR when there is rice insufficiency and it is also used as a feed for pigs (Sipaseuth, 2007). Sweet potato leaves are a good source of protein and sweet potato root is a source of energy (Apata & Babalola, 2012). Sweet potato leaves are high in crude protein and have high digestibility (An & Lindberg, 2004) and can replace fish meal and groundnut cake in the traditional Vietnamese diets for growing pigs (An *et al.*, 2005).

*Taro* (root & leaves): Taro (*Colocasia esculenta* (L.) Schott) is a green plant used as a feed for pigs in Lao PDR. The crude protein content in taro leaves ranges from 160 to 260 g/kg DM, with a content of 40-45 g essential amino acids per kg DM. It has been shown that taro leaves are a good protein source for growing pigs (Phengsavanh *et al*., 2010; Kaensombath & Lindberg, 2013). Taro leaf silage is a good source of digestible crude protein (Table 11), *e.g*. Kaensombath & Lindberg (2013) found that taro leaf silage could replace up to 50% of soybean meal in diets for both Large White and Moo Lath pigs.

*Banana pseudo-stem*: Banana trees are widely planted in Lao PDR, especially in upland areas. They are easy to grow without fertiliser and pesticides (Evrard & Goudineau, 2004; Phengsavanh *et al.*, 2010). Banana is a crop that can be harvested at any time of the year (Friis, 2015) and is used as food for humans (banana fruits, flowers and root) and as feed for pigs, poultry and cattle (banana wastes including small-sized, damaged bananas, banana peel, leaves, young stalks and banana pseudo-stem) (Wadhwa & Bakshi, 2013). The banana pseudo-stem is traditionally used as feed for pigs. It is chopped into small pieces and cooked, and then mixed with other feed ingredients such as rice bran, vegetables and kitchen waste (Manivanh & Preston, 2016; Sivilai *et al*., 2017). Ripe banana peel contains up to 8% crude protein, 6.2% ether extract, 13.8% soluble sugars and 4.8% total phenolics, and is rich in minerals (iron, copper and zinc). Banana leaves contain 10-17% crude protein, while banana pseudo-stem contains 3-5% crude protein and has a high fibre content (Tuan & Van Hai, 2004; Wadhwa & Bakshi, 2013).

### 5.2.2 Wild plants

Forage legume leaf meal, which includes stylo (S*tylosanthes guianensis* Composite) and porcupine joint vetch (*Aeschynomene histrix* BRA 9690), can be used to replace some of the soybean meal in the diet of growing pigs (Phengsavanh & Lindberg, 2013). Moreover, Kaensombath *et al*. (2013) concluded that ensiled stylo could replace up to 25% of soybean meal in the diet of growing Large White and Moo Lath pigs without any negative effects on the performance and carcass traits.

Cocoyam is a green plant that smallholder farms can collect from the forest and use as a feed for pigs. It has a crude protein content of 170-240 g/kg DM with an NDF content of 218-398 g/kg DM. Cocoyam leaves are a good source of essential amino acids (lysine range is 43-57 g/kg protein) and minerals (calcium up to 69 g/kg DM, potassium, iron and manganese) (Leterme *et al.*, 2005).

Chittavong *et al.* (2012) report that elephant yam (*Amorphophallus paeoniifolius*) and taro leaves are used in cooked form, comprising up to 32-35% of the diet, in smallholder pig production systems in central Lao PDR. Moreover, Phuc *et al.* (2001) report that tropical green plants such as water spinach, duckweed, mungbean and groundnut foliage and cassava leaves have nutritional properties to justify inclusion in the diet of animals.

Based on chemical composition and *in vitro* digestibility, Kambashi *et al.* (2014) concluded that several forage plants (*i.e*. smooth pigweed, sweet potato, African winged bean, cowpea, leaves of cassava and moringa) have potential for use as low cost feed ingredients that can improve pig feeding in the tropics.

### 5.2.3 By-products

*Broken rice & rice bran:* In areas where farmers grow paddy rice for sale, pig feed based on rice bran is the main feed source, fed together with a small amount of green feed. Farmers in some Lao Loum villages also feed brewery waste from making rice wine and spirits, and in some case also broken rice. In all situations, the main feed ingredient is rice bran of varying quality. The different qualities mainly have an effect on the amount of digestible crude protein, with the high quality rice bran used in Papers III and IV having around twice the values reported for coarse rice bran (Table 11). Rice bran tends to be available for most of the year except for a short period (July-September) before the new rice is harvested (Phengsavanh & Stür, 2006). The rice bran used in Paper I was comparable to that used in Papers III and IV.

*Cassava root pulp (CRP):* CRP is a by-product obtained after starch extraction from cassava roots in a starch factory. It is a cheap and potentially useful feed ingredient for livestock on smallholder farms in the tropics (Balagopalan *et al.,* 1991). However, the cassava root is low in crude protein (0.7-1.8%; Ngiki et al., 2014), which was reflected in CRP in this thesis (1.8% CP; Papers I and III). Ensiling CRP, as in Paper IV, decreases the amount of digestible OM (Table 11), but could be a way to make the product storable.

*Soy bean pulp (SBP):* SBP is the residue after soy milk extraction from the soy bean (*Glycine max*). SBP is a cheap and potentially useful feed ingredient for pigs in smallholder farming systems. The crude protein content of SBP in this thesis (Papers II, III & IV) was higher (29.5%) than the 25.4-28.4% reported by Li *et al*. (2012). This could be due to differences in the amount of hull present and the soy milk extraction process used. Ensiling SBP, as in Paper IV, decreased the crude protein content to 22.6% but, as for CRP, ensiling appeared to be a good strategy to make the product more storable. The ensiled SBP had a similar content of digestible crude protein to another soy bean by-product, namely tofu residue, although the amount of digestible OM is higher in tofu residue (Table 11).

*Cassava leaves:* Phuc *et al.* (2000) reported that cassava leaf meal has potential as a feed for pigs in the tropics. In contrast to cassava root pulp, the leaves are rich in digestible crude (Table 11) and represent an unconventional feed resource that could be developed into a feed with all the impact of alfalfa meal in temperate countries.

*Sweet potato leaves:* Sweet potato leaves have potential to improve dietary protein and amino acid supply in low fibre diets for pigs (Hong & Lindberg, 2004).

Table 11. *Organic matter (OM) and crude protein (CP) content (g/kg DM), apparent total tract digestibility (ATTD) and amount of digestible OM and CP in diets CRP0 and SBP0 and in commonly used tropical feed ingredients*

| Component | OM, g | ATTD OM% | Digestible OM, g | CP, g | ATTD CP% | Digestible CP, g | Reference |
|---|---|---|---|---|---|---|---|
| CRP0 | 966 | 73 | 705 | 27 | 45 | 12 | Paper IV |
| Cassava residues | 985 | 90 | 887 | 20 | 85 | 17 | Dung et al. (2002) |
| Cassava leaf meal | 928 | 59 | 548 | 324 | 61 | 198 | Phuc et al. (2001) |
| Ensiled Taro leaf silage | 917 | 86 | 789 | 259 | 72 | 186 | Buntha et al. (2008) |
| Rice bran | 848 | 77 | 653 | 141 | 81 | 115 | Paper IV |
| Coarse rice bran | 806 | 78 | 629 | 64 | 75 | 48 | Dung et al. (2002) |
| SBP0 | 888 | 70 | 622 | 226 | 77 | 174 | Paper IV |
| Tofu residue | 970 | 83 | 805 | 237 | 73 | 173 | Dung et al. (2002) |
| Fish meal | 775 | 94 | 729 | 435 | 89 | 387 | Paper IV |
| Fish meal | 731 | 80 | 585 | 462 | 90 | 416 | Ngoan & Lindberg (2001) |

### 5.2.4 Household scraps

It is very common to feed pigs and poultry with household scraps and food leftovers on smallholder farms in Lao PDR (Manivanh & Preston, 2016; Sivilai *et al.*, 2017). Feeding food surplus (swill) to pigs is a 9000-year-old practice which ensures a minimum of food waste by circulating nutrients between humans and pigs. However, this practice was banned in the EU in 2001 following an outbreak of foot-and-mouth disease in the UK (Luyckx, 2018). In addition to foot-and-mouth, feeding swill to pigs constitutes an increased risk of spreading diseases such as swine vesicular disease, classical swine fever, porcine reproductive and respiratory disease, and African swine fever (Schembri *et al.*, 2010). Primary outbreaks of classical swine fever in Germany were concluded to be caused by illegal feeding of swill to domestic pigs (Fritzemeier *et al.*, 2000). However, heating swill prior to feeding reduces the risk of spreading diseases and also eliminates harmful pathogens (*e.g.* salmonella and campylobacter) (Meyer *et al.*, 1999; Fritzemeier *et al.*, 2000; Luyckx, 2018). It was concluded by an EU expert panel (Luyckx, 2018) that feeding swill to pigs is technically viable, provided that certain safety measures are used (*i.e.* a combination of heat treatment and acidification). Catering and retail food surplus is fed to pigs in Japan, New Zealand and the United States after treatment to ensure its safety (Luyckx, 2018).

### 5.2.5 Imported feedstuffs

Fish meal and soybean meal are the two most common protein feedstuffs used in pig production in Lao PDR. Fish meal is mainly imported from Thailand, while soybean meal is imported from Vietnam. However, in both cases the price depends on world market prices, which in general are too high to be affordable for smallholder pig farmers. Moreover, the nutritional properties of both fish meal and soybean meal vary from batch to batch, depending on the origin and the processing conditions.

The crude protein content of the fish meal in this thesis (Paper IV) was low (43.5%) compared with the 50-70% reported by Miller *et al.*, (1991), while it was similar to the 46.2% recorded by Ngoan and Lindberg (2001) in a study in the same region. The quality of fish meal varies with processing factors such as partial decomposition before processing, overheating, mould and excess oil. Moreover, the nutritive value decreases when there is a higher amount of bone and head present than meat of the fish (Miller *et al.*, 1991).

## 5.3 Protein enrichment

The crude protein content in CRP is very low, which will result in a reduced protein supply if CRP is used to replace other protein-rich feed ingredients. This can be solved by supplementation with protein-rich feedstuffs. However, an alternative approach is to ferment the CRP with suitable microorganisms prior to adding it to the diet. Protein enrichment of CRP would markedly increase the potential nutritive value as a feed ingredient.

Microbial fermentation has been identified as one of the less expensive means of increasing the protein content and quality of cassava (Balagopalan *et al.,* 1991). Recently, Boonnop *et al.* (2009) showed that yeast (*Saccharomyces cerevisiae*) fermentation of fresh cassava root pulp and cassava chips increased the content of crude protein, true protein and lysine, and reduced the content of cyanide.

The crude protein content in yeast-fermented CRP increased with nitrogen addition rate in Paper I and reached values comparable to those reported by Phoneyaphon *et al.* (2016) and Hong *et al.* (2017). Earlier studies on yeast- and fungus-fermented cassava products have shown that the crude protein content increases with days of ensiling (Oboh, 2006; Boonnop *et al.*, 2009; Huu & Khammeng, 2014; Bayitse *et al*., 2015; Hong *et al.*, 2017). In Paper I, the crude protein content in fermented CRP without nitrogen addition increased 3-fold for Lao alcohol yeast, 1.2-fold for *R. toruloides*, 1.3-fold for *S. cerevisiae* and 1.9-fold for *Sch. occidentalis*. However, the increased crude protein content in the yeast-fermented CRP without nitrogen addition was mainly at the expense of starch, creating a protein-enriched product. This was also reflected in an increase in the total protein content in fermented CRP without nitrogen addition. The increase in total protein content in the fermented CRP showed a similar pattern to the crude protein content for the different yeast sources. However, the relative proportion of total protein (as % of total CP content) decreased from 48-70% for 0 N addition to 16-22% for 1.25% N addition and 10-12% for 2.5% N addition. Thus, a major part of the nitrogen added to the CRP was not built into protein of the yeast sources during fermentation. In contrast, Hong *et al.* (2017) observed a marked increase in total protein content in CRP fermented for seven days with the yeast *Pichia kudriavzevii* (with addition of 0.5% urea and 1% di-ammonium phosphate). After 7 days of fermentation, the total protein content in CRP in that study (11.6% in DM) made up around 48% of the crude protein content.

## 5.4 Feed preservation

The low dry matter content of both CRP and SBP makes it impossible to store them for any length of time and maintain acceptable hygienic properties, without some form of preservation. Fermentation is one of the oldest technologies used for food preservation and can be applied at household level, in small-scale food industries and in large enterprises (Motarjemi, 2002; Bhalla & Savitri, 2017).

The loss of sugar is crucial for fermentation, as sugars are the principal substrate for yeasts and lactic acid bacteria to produce the acid needed for preserving the feed or food (Bhalla & Savitri, 2017). During ensiling, the main aerobic phase losses occur during exposure to air before a given layer of material is covered by a sufficient quantity of additional material to isolate it from the atmosphere or before an impermeable cover is applied.

Anaerobic microbial lactic acid fermentation is an efficient, well-established and cheap process that can be used for safe preservation of moist feed sources (McDonald *et al*., 1991; Rahmi *et al*., 2008). Silage lactic acid bacteria prefer a temperature between 20-40 °C, with an optimum around 30 °C. Depending on dominant species and type of material, these bacteria can decrease the silage pH to 4-5. Lactic acid bacteria can grow below pH 4 and some can grow at pH as low as 3.5 (McDonald *et al*., 1991; Driehuis & Elferink, 2000). In addition, lactic acid bacteria contribute antifungal activity or biological agents for food safety (Ström, 2005; Dalié *et al*., 2010).

In Paper III, the maximum fermentation period of CRP and SBP was 14 days, while in Paper IV the period was at least 21 days. The data obtained in Paper IV showed that pH in the ensiled material continued to decrease up to 21 days of ensiling (Table 8). This indicates that a longer fermentation period of CRP and SBP may be beneficial to reach a pH that will allow long-term storage with reduced risk of deterioration (aerobic spoilage).

In Paper I, all yeast sources showed a marked increase in growth during the first two days of fermentation, after which the growth pattern differed between yeast sources. However, there was a decline in growth after 8-10 days of fermentation. The aerobic yeast fermentation of CRP was successful, with pH values of 3.2 to 3.9 after 14 days of fermentation. The pH values of the yeast-fermented CRP were in the same range as recorded in Paper III for anaerobically fermented (ensiled) CRP. Moreover, the pH values obtained at day 14 in yeast-fermented CRP clearly met the target value of approximately 4.2 for good quality cassava root silage (Lounglawan *et al*., 2011). Thus, it appears reasonable to assume that aerobic yeast fermentation of CRP should allow long-term storage with reduced risk of growth of spoilage bacteria and subsequent deterioration.

## 5.5 Impact of fermentation on protein quality

During fermentation of plant biomass two enzymatic processes take place, namely respiration and proteolysis. Respiration is the complete breakdown of plant sugars to carbon dioxide and water, using oxygen and releasing heat (McDonald *et al*., 1991). Proteolysis is an undesirable protein decomposition process resulting in inhibition of acidification, which may have an impact on silage quality (Slottner, 2004). Breakdown of amino acids occurs because of plant enzyme action and most destruction of amino acids in silage is brought about by microbial activity rather than plant enzymes (McDonald *et al*., 2011).

In this thesis, the initial relative proportion of ammonia-nitrogen ($NH_3$-$N_{prop}$, % of N) was markedly higher in CRP than in SBP (Paper III), which could be due to differences in the processing procedures used for extracting the starch from cassava (and storing the pulp) and extracting the milk from soybean. In the process of extracting soybean milk, soybean is heated prior to pressing the pulp, which will inactivate the endogenous proteases. In contrast, no heating is applied in the cassava starch extraction process. However, in this thesis there was an increase in both the $NH_3$-N content and $NH_3$-$N_{prop}$ with days of ensiling in both CRP and SBP. This has also been reported for ensiling of other feedstock (McDonald *et al*., 1991) and is caused by microbial proteases during the ensiling process.

## 5.6 Aerobic spoilage

The silage microflora consists of beneficial microorganisms, *i.e.* lactic acid bacteria responsible for the silage fermentation processes. As the lactic acid bacteria grow, they produce increasing amounts of lactic acid, and thereby dominate the remaining microflora in the forage mass. This process continues until the pH drops so low that even growth of lactic acid bacteria is inhibited. This phase should generate rapid acidification and sufficiently high concentrations of lactic acid so that silage pH decreases to a stable level (Knicky, 2005).

Aerobic spoilage of silage is associated with penetration of oxygen into the silage during storage and feeding. Pettersson (1988) reported that the main factors affecting growth of microorganisms during aerobic deterioration are the concentration of oxygen, increasing competition for nutrients, temperature, water activity, pH, concentration of organic acids and use of additives. Lactate-oxidising yeasts are generally responsible for the initiation of aerobic spoilage. The secondary aerobic spoilage flora consists of moulds, bacilli, listeria and enterobacteria. Mycotoxcin-producing mould, *Bacillus cereus* and *Listeria* *monocytogenes* in aerobically deteriorated silage pose a serious risk to the quality and safety of milk and to animal health (Driehuis & Elferink, 2000).

Increasing the dry matter content of moist material has shown to be an efficient way of facilitating initiation of the ensiling process of forage and stabilising the resulting silage after completion of ensiling (McDonald *et al*., 1991). Inclusion of rice bran increases the low dry matter content of both CRP and SBP, and could thereby be expected to facilitate the ensiling process. However, this could not be verified in this thesis (Paper III), as ensiling of CRP and SBP with inclusion of more than 5% rice bran (fresh weight basis) resulted in pH >4.7, which is too high to inhibit the growth of spoilage bacteria and cannot be recommended. In contrast, ensiling CRP and SBP without inclusion of rice bran resulted in pH 3.9-4.0, which should allow long-term storage with maintained silage quality.

## 5.7 Nutritional properties of available feed sources

The change in chemical composition observed during fermentation of CRP and SBP (Papers I & III) has implications for the potential nutritive value of these materials for livestock. This applies to both the energy and protein value of the fermented material.

Hong *et al*. (2017) reported that replacement of 25% fish meal protein with yeast-enriched CRP (4% yeast-enriched CRP in diet DM) in the diet to growing-fattening pigs reduced feed intake, while live weight gain was unaffected and feed conversion tended to be improved. At higher proportions of yeast-enriched CRP in the diet, feed intake and live weight gain decreased linearly (Hong *et al*., 2017). Those authors suggest that this may be related to toxic effects of non-protein nitrogen compounds in the yeast-enriched CRP due to incomplete conversion of these compounds to yeast protein. In Paper II, nitrogen intake, nitrogen in faeces and nitrogen in urine were higher for diets with SBP than for diets with CRP. Moreover, nitrogen retention expressed as g/day and % of N intake was highest in the SBP diets. However, nitrogen retention expressed as % of N digested was highest for the diet with fermented CRP (diet FCMP), followed by the SBP diets. The lowest values were found for the diet with unfermented CRP (diet UCLP). This indicates a positive effect of fermentation of CRP on the protein value of the fermented feed.

The starch content in CRP decreased with days of fermentation for all yeast sources tested (Paper I). The greatest decrease in starch content was found for Lao alcohol yeast, followed by *Schwanniomyces occidentalis, Saccharomyces cerevisiae* and *Rhodotorula toruloides.*

Simultaneously, the neutral detergent fibre content increased with ongoing fermentation for all yeast sources. The greatest increase in NDF content was found for the Lao alcohol yeast, followed by *Sch. occidentalis*, *S. cerevisiae* and *R. toruloides*. The changes in starch and fibre content in the yeast-fermented CRP had implications for the energy value. Thus, the estimated net energy value (www.evapig.com) for CRP fermented with Lao alcohol yeast, *R. toruloides*, *S. cerevisiae* and *Sch. occidentalis* was 3.4, 8.1, 7.5 and 5.9 MJ/kg DM, respectively, compared with 8.8 MJ/kg DM for unfermented CRP. The corresponding values for metabolisable energy were 5.4, 11.3, 10.6 and 8.6 MJ/kg DM for Lao alcohol yeast, *R. toruloides*, *S. cerevisiae* and *Sch. occidentalis*, respectively, and 12.1 MJ/kg DM for unfermented CRP.

The potential nutritive value of a feed is determined not only by the energy and protein values, but also by the palatability. High palatability results in high dry matter intake, which will have positive impacts on overall performance. It has been reported that ensiling increases the palatability of cassava roots for pigs (Loc *et al*., 1997; Ly *et al*., 2011). However, in this thesis the dry matter intake of growing pigs was lower for CRP-based diets than for SBP-based diets (Paper II), and for diets based on ensiled CRP than for ensiled SBP (Paper IV). Thus, despite a possible positive effect of ensiling on dry matter intake, the palatability of CRP seems to be low. This may have negative effects on energy intake and subsequent performance.

## 5.8 Pig breeds

Pig rearing continues to be an important livelihood activity in Lao PDR. More than 80% of pigs in the country (3.2 million head in total) are native breeds kept by smallholder farmers (DLF, 2014). These native breeds have many positive attributes, such as being hardy and able to survive on relatively poor quality feed sources (Phengsavanh & Stür, 2006), whereas imported lean-meat breeds require more feed and high-quality feed sources.

The high labour demand in collecting and preparing feed is a challenge in smallholder pig production systems (Stür *et al.*, 2002). Women and children commonly provide significant labour inputs as the primary caretakers of household pigs and poultry (Phengsavanh *et al.*, 2011; Chittavong *et al.*, 2013). Thus, using native breeds can be way to reduce the labour demand for collection and preparation of feed for the pigs. Moreover, being able to store the feed for some time could be a way to reduce the labour needed for feed collection.

Imported breeds, such as Landrace and Large White and their crosses, are used by a small number of farmers, particularly in semi-commercial pig farms near population centres (Vongthilath & Blacksell, 1999). In this thesis, Large

White pigs showed higher daily dry matter intake (g/day) and greater nitrogen retention than native Moo Lath pigs (Paper II). However, there were no differences in apparent total tract digestibility of organic matter and crude protein between breeds. Kaensombath and Lindberg (2013) found that Large White growing pigs had higher dry matter intake and average daily gain than Moo Lath growing pigs. In particular, Large White pigs fed soybean meal and ensiled taro leaves were found to have better feed conversion ratio than Moo Lath pigs (Kaensombath & Lindberg, 2013), while Large White growing pigs fed soybean meal and ensiled stylo had poorer feed conversion ratio than Moo Lath pigs (Kaensombath *et al*., 2013). Interestingly, Len *et al*. (2007) found that digestibility of energy and nutrients was highest for Mong Cai pigs and lowest for Landrace-Yorkshire crosses, with intermediate values for Mong Cai-Yorkshire crosses. However, nitrogen retention and nitrogen utilisation were higher for Landrace-Yorkshire crosses than for Mong Cai and for Mong Cai-Yorkshire crosses in that study (Len *et al*., 2007).

# 6 General conclusions

- Cassava root pulp can be successfully fermented aerobically with yeast, reaching pH values of 3.2 to 3.9 after 14 days of fermentation. The growth of spoilage bacteria is inhibited at these pH levels, which will allow longer storage time with maintained quality.
- The nutrient content in cassava root pulp changes during aerobic yeast fermentation, resulting in an increase in crude protein, total protein, neutral detergent fibre and ash content, and a decrease in starch content. As a consequence, the protein value and the energy value of fermented cassava root pulp in the diet of pigs differs from that of the unfermented material. Moreover, the yeast source used for fermentation influences the protein and energy value of the fermented product.
- Fermenting cassava root pulp with rice bran and urea increases the crude protein content and improves the nitrogen digestibility and nitrogen retention in both Moo Lath and Large White pigs. Nitrogen utilisation (N retention as a percentage of N digested) for diets with fermented cassava root pulp is comparable to that for diets supplemented with soybean pulp. This suggests improved protein quality in fermented compared with unfermented cassava root pulp. Dry matter intake is markedly higher in pigs fed soybean pulp, which indicates that both unfermented and fermented cassava root pulp have poor palatability.
- Ensiling cassava root pulp and soybean pulp for 14 days without inclusion of rice bran results in pH 3.9-4.0, which should allow long-term storage with maintained silage quality. The pH achieved can be even lower if cassava root pulp and soybean pulp are ensiled for 21 days or more (pH 3.8-3.9).
- Ensiling cassava root pulp and soybean pulp for 14 days with inclusion of more than 5% rice bran (fresh weight basis) results in pH (>4.7) that is too high to inhibit the growth of spoilage bacteria and cannot be

recommended. However, ensiling cassava root pulp and soybean pulp for 21 days or more with inclusion of up to 10% rice bran results in lower pH (4.1-4.4) that should allow medium-term storage.

- There is no empirical support for the general hypothesis that inclusion of rice bran to increase the dry matter content facilitates ensiling of cassava root pulp and soybean pulp.
- Ensiling cassava root pulp and soybean pulp with rice bran results in a feed product that is well digested and utilised in growing pigs. However, dry matter intake appears to be impaired with ensiled cassava root pulp in comparison with ensiled soybean pulp. Moreover, ensiling cassava root pulp and soybean pulp with inclusion of rice bran (10% for cassava root pulp and 20% for soybean pulp) improves the digestibility of organic matter and crude protein, suggesting synergistic effects in the porcine gastrointestinal tract.

# References


ADB (2005) Participatory Livestock Development Project. Final Report. Project Preparation Technical Assistance. ADB PPTT No. 4287-Lao. Vientiane Capital, Lao PDR. Manila: Asian Development Bank. Available at: http://www.mekonginfo.org/assets/midocs/0002027-farming-participatory-livestock-development-project-working-paper-no-6-extension-approaches-for-livestock-development.pdf [2018-10-13]

Adebiyi, O. A., Sodeke, M., Adeleye, O. O. & Adejumo, I. O. (2018). Effects of extruded rice bran based diets on the performance, intestinal microbiota and morphology of weaned pigs. *Agricultura Tropica et Subtropica*, *51*(1), pp. 13-19.

Adesehinwa, A. O. K., Ogunmodede, B. K. (1995). Swine feeds and practical feed composition techniques, In: N.A.E.R.L.S. Workshop training Manual, Moor Plantation, Ibadan, April 3-7, (1995) Nat. Pig Prod, pp. 27-56.

AFSIS. (2016). ASEAN Agricultural Commodity outlook, ASEAN Food Security Information System, Cassava production in ASEAN country, page 71. Available at: http://www.aptfsis.org/uploads/normal/ACO%20Report%2017/aco17.pdf [2018-10-10]

AHDB. (2017). 2016 Pig Cost of Production in Selected Countries, pp 12-15. Available at: https://pork.ahdb.org.uk/media/274535/2016-pig-cost-of-production-in-selected-countries.pdf [2018-10-25]

An, L. V. & Lindberg, J. E., 2004. Ensiling of sweet potato leaves (Ipomoea batats (L.) Lam) and the nutritive value of sweet potato leaf silage for growing pigs. *Asian-Australasian Journal of Animal Science* 17 (4), pp. 497-503.

An, L. V, Tran Thi Thu Hong & Lindberg, J. E., 2004. Ileal and total tract digestibility in growing pigs fed cassava root meal with inclusion of fresh, dry and ensiled sweet potato (Ipomoea batats (L.) Lam) leaves. *Animal Feed Science and Technology* 114, pp. 127-139.

An, L. V, Hong, T.T.T., Ogle, B. and Lindberg, J.E. 2005. Utilisation of ensiled sweet potato leaves as a protein supplement in diets for growing pigs. *Tropical Animal Health and Production* 37, pp. 77-88.

Apata, D. F., & Babalola, T. O. (2012). The use of cassava, sweet potato and cocoyam, and their by-products by non–ruminants. *International Journal of Food Science and Nutrition Engineering*, *2*(4), pp. 54-62.

Araba, M., & Dale, N. M. (1990). Evaluation of protein solubility as an indicator of under processing of soybean meal. *Poultry Science*, *69*(10), pp. 1749-1752.

Aro, S. O. (2008). Improvement in the nutritive quality of cassava and its by-products through microbial fermentation. *African Journal of Biotechnology*, *7*(25), pp. 4789-4797.

Ashbell, G. (1996). Silage fermentation and silage additives. *Asian-Australasian Journal of Animal Sciences*.

Baker, D. H. (1999). Nutritional constraints to use soy products by animals. In: Soy in Animal Nutrition, J. K. Drackley (ed). *Federation of Animal Science Societies*, Savoy, Illinois, USA, pp. 1-12.

Balagopalan, C., Padmaja, G., & George, M. (1991). Improving the nutritional value of cassava products using microbial techniques. *Machin, David; Nyvold, Solveig (eds.). FAO Expert Consultation on Roots, Tubers, Plantains and Bananas in Animal Feeding (1991, Cali, Colombia). Roots, Tubers, Plantains and Bananas in Animal Feeding: Proceedings.*

Bayitse, R., Hou, X., Laryea, G., & Bjerre, A. B. (2015). Protein enrichment of cassava residue using Trichoderma pseudokoningii (ATCC 26801). *AMB Express*, *5*(1), pp. 80.

Bender, A. E. (1975). Nutritional aspects of amino acids. *Amino Acid Metabolism. John Wiley and Sons, London*, *8*, pp. 192-202.

Bernard, W., & Barrington, L. F. (1962). "Aerobic fermentation process." U.S. Patent 3,041,250, issued June 26, 1962.

Bhalla, T. C. & Savitri, 2017. Yeasts and traditional fermented foods and beverages. In: Yeast diversity in human welfare, Eds. Satyanarayana, T. & Kunze, G., Springer Nature, Singapore. pp. 385-416.

Biodiversity Country Report. (2003). Ministry for Agriculture and forestry, Vientiane Lao PDR, Science Technology and Environment Agency, Vientiane, Lao PDR.

Bolsen, K. K., Ashbell, G., & Weinberg, Z. G. (1996). Silage fermentation and silage additives-Review. *Asian-Australasian Journal of Animal Sciences*, *9*(5), 483-494.

Boonnop, K., Wanapat, M., Nontaso, N., & Wanapat, S. (2009). Enriching nutritive value of cassava root by yeast fermentation. *Scientia Agricola*, *66*(5), pp. 629-633.

Borin, K., Lindberg, J. E., & Ogle, R. B. (2005). Effect of variety and preservation method of cassava leaves on diet digestibility by indigenous and improved pigs. *Animal Science*, *80*(3), 319-324.

Bouahom, B. (1999, July). Animal genetic resources in the Lao PDR: current status and production systems. In *Proceedings of the Workshop on the Implications of the Asian Economic Crisis for the Livestock Industry, (RAP Publication 1999/29*.

Chittavong, M., Lindberg, J. E., & Jansson, A. (2012a). Feeding regime and management of local Lao pigs in Central Lao PDR. *Tropical Animal Health and Production*, *45*(1), pp. 149-155.

Chittavong, M. (2012b). Nutritional status of local pigs in Central Lao PDR. Diss. Uppsala: Swedish University of Agricultural Science. (Vol. 2012, No. 87).

Chittavong, M., Lindberg, J. E., & Jansson, A. (2013). A field study on feed supplementation, body weight and selected blood parameters in local pigs in Laos. *Tropical Animal Health and Production*, *45*(2), pp. 505-510.

Christiansen, J. P. (2010). The Basic of Pig Production. 2$^{nd}$ edition. Aarhus, Denmark: Knowledge Center for Agriculture, Agricultural Publishing.

CIAT. (2001). Project sustainable system for smallholders: integrating improved germplasm and resource management for enhanced crop and livestock production systems. Annual Reported. Centro Internacional de Agricultura Tropiccal, Cali, Colombia, pp. 136.

Conlan, J. V., Khounsy, S., Phithakhep, L., Phruaravanh, M., Soukvilai, V., Colling, A., & Gleeson, L. (2008). Pig production and health in Bolikhamxay province, Lao PDR. ACIAR. Available at: http://researchrepository.murdoch.edu.au/id/eprint/15984/1/pig_production_and_health.pdf [2018-10-07]

Conrad, J. H. (1984). Feed formulation with soybean meal for efficient swine production. *Animal Nutrition Resource Highlight (Special edition). Quarterly Publication of the American Soybean Association*.

Dalié, D. K. D., Deschamps, A. M., & Richard-Forget, F. (2010). Lactic acid bacteria–Potential for control of mould growth and mycotoxins: A review. *Food control*, *21*(4), pp. 370-380.

Darlington, W. A. (1964). Aerobic hydrocarbon fermentation-a practical evaluation. *Biotechnology and Bioengineering*, *6*(2), pp. 241-242.

Devendra, C. (1997). Improvement of livestock production in crop-animal systems in rainfed agro-ecological zones of South-East Asia. ILRI (aka ILCA and ILRAD).

DLF. (2014). Agricultural Statistics for 2014, Ministry of Agriculture and Forestry, Department of Livestock and forestry, Vientiane Lao PDR.

DOA. (2012). Department of Agriculture. Crop Statistic in Rainy Season Year 2012. Vientiane: Lao PDR, pp.126. Available at: http://www.fao.org/fileadmin/user_upload/tci/docs/1_Laos%20document%20low%20resolution.pdf [2018-10-25]

Douros Jr, J. D., Naslund, L. A., & Lahl, W. J. (1970). *U.S. Patent No. 3,546,071*. Washington, DC: U.S. Patent and Trademark Office.

Driehuis, F., & Elferink, S. O. (2000). The impact of the quality of silage on animal health and food safety: a review. *Veterinary Quarterly*, *22*(4), pp. 212-216.

Dung, N. N. X., Manh, L. H., & Udén, P. (2002). Tropical fibre sources for pigs—digestibility, digesta retention and estimation of fibre digestibility in vitro. *Animal Feed Science and Technology*, *102*(1-4), 109-124.

Eggum, B. O., 1989. Biochemical and methodological principles. In Protein Metabolism in Farm Animals-Evaluation, digestion, absorption and metabolism, Eds. Bock, H. D., Eggum, B. O., Low, A. G., Simon, O. and Zebrowska, T. VEB Deutscher Landwirtschaftsverlag Berlin. pp. 1-52.

Ersin Samli, H., SENKOYLU, N., AKYUREK, H., & AGMA, A. (2006). Using rice bran in laying hen diets. *Journal of Central European Agriculture*, *7*(1), pp. 135-140.

Evrard, O., & Goudineau, Y. (2004). Planned resettlement, unexpected migrations and cultural trauma in Laos. *Development and change*, *35*(5), pp. 937-962.

Fanimo, A. O. (1991). Substitution of soybean and animal by-products for fish meal in pig rations. Ph.D. Thesis, University of Ibadan, Ibadan.

FAO. (2006). The Importance of Agricultural Biodiversity for Food Security, Nutrition and Sustainable Livelihoods in Lao PDR and relevant Policies. Workshop background document prepared as a contribution to future the implementation of the National Agricultural Biodiversity Program.

Fashina, H. A. (1991). Utilization of soybean based rations and finisher pigs in hot humid tropics. Ph.D. Thesis, University of Ibadan, Ibadan, Nigeria.

FEFAC. (2007). European Feed Manufacturers Federation. Industrial compound feed production, FEFAC Secretariat General, Brussels, May 2007. Available at: https://slideplayer.com/slide/10797917/ [2018-10-12]

Friis, C. (2015, May). Small-scale land acquisitions, large-scale implications: The case of Chinese banana investments in Northern Laos. In *Conference Paper* (No. 36). Available at: https://www.researchgate.net/profile/Cecilie_Friis/publication/282847756_Small-scale_land_acquisitions_largescale_implications_The_case_of_Chinese_banana_investments_in_Northern_Laos/links/561e23d708ae50795afd8ce4.pdf [2018-10-14]

Fritzemeier, J., Teuffert, J., Greiser-Wilke, I., Staubach, C., Schilter, H. & Moennig, V., 2000. Epidemiology of classical swine fever in Germany in the nineties. *Veterinary Microbiology* 77, pp. 29-41.

Gomez, G. G. (1992). Use of cassava products in pig feeding. In: Roots, tubers, plantains and bananas in animal feeding, D. Machin & S. Nyvold (eds). FAO Animal Production and Health Paper 95, FAO, Rome, Italy, pp. 157-167.

Hahn-Hägerdal, B., Jeppsson, H., Skoog, K., & Prior, B. A. (1994). Biochemistry and physiology of xylose fermentation by yeasts. *Enzyme and Microbial Technology*, *16*(11), pp. 933-943.

Han, S. W., Chee, K. M., & Cho, S. J. 2015. Nutritional quality of rice bran protein in comparison to animal and vegetable protein. *Food Chemistry*, *172*, 766-769.

Hoffmann, D. (1999). Asian livestock to the year 2000 and beyond. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok. Thailand. Available at: http://cdn.aphca.org/dmdocuments/APHCA%20Publications/1999_25_normal.pdf [2018-10-06]

Holness, D. H. (1991). The tropical agriculturist. Pigs. Macmillan Education Ltd, pp. 63-68

Hong, T. T. T., & Lindberg, J. E. (2004). Ileal and total tract digestibility in growing pigs fed cassava root meal diets with inclusion of fresh, dry and ensiled sweet potato (Ipomoea batatas L. (Lam.)) leaves. *Animal Feed Science and Technology*, *114*(1-4), pp. 127-139.

Hong, T. T. T., Passoth, V., & Lindberg, J. E. (2011). Bacterial diversity at different sites of the digestive tract of weaned piglets fed liquid diets. *Asian-Australasian Journal of Animal Sciences*, *24*(6), pp. 834-843.

Hong, T. T. T., Lien, P. T. B., Hai, D. T., Hang, P. T., & Quan, N. H. (2017). Protein-enriched cassava root pulp as partial replacement for fish meal in diets for growing pigs. *Chemical Analysis*, *30*(3), pp. 97.

Huu, H., & Khammeng, T. (2014). Effect of yeast fermented cassava pulp (FCP) on nutrient digestibility and nitrogen balance of post-weaning pigs. *Livestock Research for Rural Development*, *26*(8).

Huynh, T. T. T., Aarnink, A. J. A., Drucker, A., & Verstegen, M. W. A. (2007). Pig production in Cambodia, Laos, Philippines, and Vietnam: a review. *Asian Journal of Agriculture and Development*, *4*(1), pp. 69-90.

Juliano, B. O. (1985). Rice Chemistry and Technology. American Association of Cereal Chemists, pp. 757.

Just, A., Fernandez, J. A. & Jørgensen, H., 1982. Nitrogen balance studies and nitrogen retention. INRA publication No. 12 (Les Colloques de l'INRA), pp. 111-122.

Kaensombath, L., & Lindberg, J. E. (2013a). Effect of additives on ensiling of taro (Colocasia esculenta (L.) Schott) leaves and Stylo CIAT 184 (Stylosanthes guianensis (Aubl.) Sw. var. guianensis) forage. *Livestock Research for Rural Development*, *25*(4).

Kaensombath, L., Neil, M., & Lindberg, J. E. (2013b). Effect of replacing soybean protein with protein from ensiled stylo (Stylosanthes guianensis (Aubl.) Sw. var. guianensis) on growth performance, carcass traits and organ weights of exotic (Landrace× Yorkshire) and native (Moo Lath) Lao pigs. *Tropical Animal Health and Production*, *45*(3), pp. 865-871.

Kambashi, B., Picron, P., Boudry, C., Thewis, A., Kiatoko, H., & Bindelle, J. (2014). Nutritive value of tropical forage plants fed to pigs in the Western provinces of the Democratic Republic of the Congo. *Animal Feed Science and Technology*, *191*, pp. 47-56.

Kennard. (1996). Country report for animal genetic resource management in the Lao PDR, Available at: ftp://ftp.fao.org/docrep/fao/010/a1250e/annexes/CountryReports/LaoPDR.pdf [2017-09-10]

Keo. (2000). Water spinach or stylosanthes as protein supplements in a diet of ensiled cassava roots for local pigs, Available at: http://www.mekarn.org/Research/keolao.htm [2017-09-20]

Keonouchanh, S., Egerszegi, I., Ratky, J., Bounthong, B., Manabe, N., &Brüssow, K. P. (2011). Native pig (Moo Lat) breeds in LaoPDR. *Archives Animal Breeding*, *54*(6), 600-606.

Khounsy, S. & Conlan, J. (2008). Classical swine fever and foot-and-mouth disease in Lao PDR. ACIAR Monograph no. 128. In: Colan, J. V., Blacksell, S. D., Morrissy, C. J. & Colling, A., eds., Management of classical swine fever and foot and mouth disease in Lao PDR, ACIAR proceedings, Vientiane Capital, Lao PDR. pp. 39-42.

Kim, S. W., & Easter, R. A. (2001). Nutritional value of fish meals in the diet for young pigs. *Journal of animal science*, *79*(7), 1829-1839.

Knicky, M. (2005). Possibilities to improve silage conservation. Diss. Uppsala: Swedish University of Agricultural Science (Vol. 2005, No. 62).

Kjeldsen, N. J. V., Danielsen, A., Just, H. E., Neilsen, and B. O. Eggum. (1983) Forskellige kvaliteter fishkemel til Smaagrise. The National Institute of Animals Science, Foulum, Denmark. 449:1-4.

Le Goff, G., Van Milgen, J., & Noblet, J. (2002). Influence of dietary fibre on digestive utilization and rate of passage in growing pigs, finishing pigs and adult sows. *Animal Science*, *74*(3), 503-515.

Len, N. T., J. E. Lindberg and B. Ogle. 2007. Digestibility and nitrogen retention of diets containing different levels of fiber in local (Mong Cai), F1 (Mong Cai x Yorkshire) and exotic (Landrace x Yorkshire) growing pigs in Vietnam. *Journal of Animal Physiology and Animal Nutrition* 91, pp. 297-303.

Le, T. T. H., R. R., Lemke, U., & Zárate, A. V. (2005). *Impact Ot the Use of Exotic Compared to Local Pig Breeds on Socio-economic Development and Biodiversity in Vietnam*. Grauer.

Leterme, P., Londono, A. M., Estrada, F., Souffrant, W. B., & Buldgen, A. (2005). Chemical composition, nutritive value and voluntary intake of tropical tree foliage and cocoyam in pigs. *Journal of the Science of Food and Agriculture*, *85*(10), pp. 1725-1732.

Li, B., Qiao, M., & Lu, F. (2012). Composition, nutrition, and utilization of okara (soybean residue). *Food Reviews International*, *28*(3), pp. 231-252.

Liener, I. E. (1999). Non-nutritive factors and bioactive compounds in soy. In: Soy in Animal Nutrition, J. K. Drackley (ed). Federation of Animal Science Societies, Savoy, Illinois, USA, pp. 13-45.

Loc, N.T., Preston, T.R. and Ogle, B., (1997). Cassava root silage for crossbred pigs under village conditions in Central Vietnam. *Livestock Research for Rural Development*, *9*(2), pp.12-19.

Lounglawan, P., Khungaew, M., & Suksombat, W. (2011). Silage production from cassava peel and cassava pulp as energy source in cattle diets. *Journal of Animal and Veterinary Advances*, *10*, pp. 1007-1011.

Low, A. G. (1985). The role of dietary fibre in pig diet. In: W. Harresign (Ed.) Recent Advance in Animal Nutrition. p 87. Butter worth, London.

LSB. (2011). Year book 2011. Lao Statistic Bareau. Vietiane, Lao People's Democratic Republic. http://www.nsc.gov.la. [2017-10-25]

LTP. (2015). Lao PDR Trade portal. Annual Reported of Agricultural statistic, Vientiane, Lao PDR.

Luyckx, K., 2018. REFRESH Task 6.3.3 Expert panel on the risk management of using treated surplus food in pig feed, Report of the EU project REFRESH. Available at: https://eu-refresh.org/sites/default/files/REFRESH%20animal%20feed%20expert%20seminar%20report%20final%2012.04.18.pdf [2018-10-21]

Ly, N. T., Ngoan, L. D., Verstegen, M. W., & Hendriks, W. H. (2011). Ensiled and dry cassava leaves and sweet potato vines as a protein source in diets for growing Vietnamese Large White× Mong Cai pigs. *Asian-Australasian Journal of Animal Sciences*, *23*(9), pp. 1205-1212.

MAF. (2007). Participative analysis of coffee supply chain in Lao PDR (prepared by the coffee Working Group), Ministry of Agriculture and Forestry, Programme of Capitalisation in Support of Rural Development Policy (PCADR), November, pp. 101.

Mahan, D. C., & Vallet, J. L. (1997). Vitamin and mineral transfer during fetal development and the early postnatal period in pigs. *Journal of Animal Science*, *75*(10), pp. 2731-2738.

Manivanh, N., & Preston, T. R. (2016). Replacing taro (Colocasia esculenta) silage with protein-enriched cassava root improved the nutritive value of a banana stem (Musa spp) based diet and supported better growth in local pigs (Moo Laat breed). *Livestock Research for Rural Development*, *28*.

Mason, V. C., & Weidner, K. (1964). The effect of heat on the amino acid, fatty acid and B-vitamin composition of fish meal. *Acta Agriculturae Scandinavica*, *14*(1), 87-95.

McDonald, P., A, R. Henderson, and S. J. E. 1991**.** The Biochemistry of Silage (2nd ed). Chalcombe Publications., Church Lane, Kingston, Canterbury, Kent, UK.

McDonald, P., Edwards. R. A., Greenhalgh, J. F. D. & Morgan, C. A. (2002). Animal nutrition. (Six Edition). Pearson education.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A. & Wilkinson, R. G. (2011). Animal Nutrition (Seven Edition). *Printed by Ashford Colour Press Ltd, Gosport, United Kingdom.*

Millar, J., & Photakoun, V. (2008). Livestock development and poverty alleviation: Revolution or evolution for upland livelihoods in Lao PDR. *International Journal of Agricultural Sustainability, 6*(1), pp. 89-102.

Miller, E. R., Ullrey, D. E & Lewis, A. J. (1991). Swine Nutrition. *Butterworth-Heinemann. Stoneham, Mass,* pp. 183-281.

Minitab. (2010). Statistical Software Version 16.0. User's Guide to Statistics, Minitab, PA, USA.

Moongngarm, A., Daomukda, N., & Khumpika, S. (2012). Chemical compositions, phytochemicals, and antioxidant capacity of rice bran, rice bran layer, and rice germ. *Apcbee Procedia*, *2*, pp. 73-79.

Motarjemi, Y. (2002). Impact of small scale fermentation technology on food safety in developing countries. *International Journal of Food Microbiology, 75*(3), pp. 213-229.

Myer, R. O., Brendemuhl, J. H. & Johnson, D. D. (1999). Evaluation of dehydrated restaurant food waste products as feedstuffs for finishing pigs. *Journal of Animal Science*, 77, pp. 685–692.

Nampanya, S., Rast, L., Khounsy, S., & Windsor, P. A. (2010). Assessment of farmer knowledge of large ruminant health and production in developing village-level biosecurity in northern Lao PDR. *Transboundary and Emerging Diseases*, *57*(6), pp. 420-429.

Napasirth, V., Napasirth, P., Sulinthone, T., Phommachanh, K., & Cai, Y. (2015). Microbial population, chemical composition and silage fermentation of cassava residues. *Animal Science Journal*, *86*(9), pp. 842-848.

Nasseri, A. T., Rasoul-Amini, S., Morowvat, M. H., & Ghasemi, Y. (2011). Single cell protein: production and process. *American Journal of Food Technology*, *6*(2), pp. 103-116.

Ngiki, Y. U., Igwebuike, J. U., & Moruppa, S. M. (2014). Utilisation of cassava products for poultry feeding: A review. *The International Journal of Science and Technoledge*, *2*(6), pp. 48.

Ngoan, L. D., & Lindberg, J. E. (2001). Ileal and total tract digestibility in growing pigs fed cassava root meal and rice bran diets with inclusion of fish meal and fresh or ensiled shrimp by-products. *Asian-Australasian Journal of Animal Sciences*, *14*(2), 216-223.

Nimmo, R. D., Peo Jr, E. R., Crenshaw, J. D., Moser, B. D., & Lewis, A. J. (1981). Effect of Level of Dietary Calcium-Phosphorus during Growth and Gestation on Calcium-Phosphorus Balance and Reproductive Performance of First Litter Sows. *Journal of Animal Science*, *52*(6), pp. 1343-1349.

Noblet, J., & Henry, Y. (1993). Energy evaluation systems for pig diets: a review. *Livestock Production Science*, *36*(2), 121-141.

Noblet, J., Fortune, H., Shi, X. S., & Dubois, S. (1994). Prediction of net energy value of feeds for growing pigs. *Journal of Animal Science*, *72*(2), pp. 344-354.

Noblet, J., & Le Goff, G. (2001). Effect of dietary fiber on the energy value of feeds for pigs. *Animal Feed Science and Technology*, *90*(1-2), pp. 35-52.

Noblet, J., & Perez, J. M. (1993). Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. *Journal of Animal Science*, *71*(12), pp. 3389-3398.

Noblet, J., & Van Milgen, J. (2004). Energy value of pig feeds: Effect of pig body weight and energy evaluation system. *Journal of Animal Science*, *82*(suppl_13), E229-E238.

Noblet, J. (2007). Recent developments in net energy research for swine. *Advances in Pork Production*, *18*(18), pp. 149-156.

NRC. (1988). Nutrient Requirements of Swine, National Research Council, 9th revised edition. National Academic Press, Washington DC.

NRC. (1998). Nutrient Requirements of Swine, *National Research Council, 10th revised edition, National Academic Press, Washington DC,* pp. 116-117.

NRC. (2012). Nutrient requirements of swine. *National Research Council. National Academies Press, Washington DC.*

Oboh, G. (2006). Nutrient enrichment of cassava peels using a mixed culture of Saccharomyces cerevisae and Lactobacillus spp solid media fermentation techniques. *Electronic Journal of Biotechnology*, *9*(1), 0-0.

Onphanhdala, P. (2009). Farmer education and rice production in Lao PDR. *Journal of International Cooperation Studies*, *16*(3), pp. 105-121.

Osbjer, K. (2006). *Methodology in Recording Data on Pig Health and Production in the Lao People's Democratic Republic: Experiences from a Surveillance Programme on Classical Swine Fever in Lowland Villages in Bolikhamxay Province, Lao PDR* (Doctoral dissertation, Sveriges lantbruksuniversitet).

Pandey, A., Soccol, C. R., Nigam, P., Soccol, V. T., Vandenberghe, L. P., & Mohan, R. (2000). Biotechnological potential of agro-industrial residues. II: cassava bagasse. *Bioresource technology*, *74*(1), pp. 81-87.

Pettersson, K. (1988). Ensiling of forages. *Factors affecting silage fermentation and quality*. Diss. Uppsala: Swedish University of Agricultural Science.

Phengsavanh, P., & Stür, W. (2006, August). The use and potential of supplementing village pigs with Stylosanthes guianensis in Lao PDR. In *Workshop-seminar" Forages for Pigs and Rabbits" MEKARN-CelAgrid, Phnom Penh, Cambodia,* pp. 22-24.

Phengsavanh, P., & Phimphachanhvongsod, V. (2007, March). Forage Development in Lao PDR–Emerging impacts from planting forages in the Upland of Lao PDR. In *International Forage Symposium on "Forages: A pathway to Prosperity for Small-holder Farmers". Ubonrachathani, Thailand*.

Phengsavanh, P., Ogle, B., Stür, W., Frankow-Lindberg, B. E., & Lindberg, J. E. (2010). Feeding and performance of pigs in smallholder production systems in Northern Lao PDR. *Tropical Animal Health and Production*, *42*(8), pp. 1627-1633.

Phengsavanh, P., Ogle, B., Stür, W., Frankow-Lindberg, B. E., & Lindberg, J. E. (2011). Smallholder pig rearing systems in Northern Lao PDR. *Asian-Australasian Journal of Animal Sciences*, *24*(6), pp. 867-874.

Phengsavanh, P., & Lindberg, J. E. (2013a). Effects of Dietary Protein Levels on Growth Performance and Feed Intake in Native Moo Lath Lao Pigs. *Journal of Animal and Veterinary Advances*, *12*(3), pp. 406-411.

Phengsavanh, P. (2013b). Forage legumes as feed for pigs in Small-holder production systems in the North of Lao PDR. Diss. Uppsala: Swedish University of Agricultural Science. (Vol. 2013, No. 12).

Phengsavanh, P., & Lindberg, J. E. (2013). Effect of replacing soybean protein with protein from porcupine joint vetch (Aeschynomene histrix BRA 9690) and stylo (Stylosanthes guianensis Composite) leaf meal on growth performance of native (Moo Lath) Lao pigs. *Tropical Animal Health and Production*, *45*(8), pp. 1795-1802.

Phengvilaysouk, A. and Wanapat, M., (2008). Study on the effect of harvesting frequency on cassava foliage for cassava hay production and its nutritive value. *Livestock Research for Rural Development*, *20*(9).

Phengvilaysouk, A., Jansson, A., Phengsavanh, P., Tiemann, T., Phengvichith, V. & Lindberg, J. E. (2017). Sow and piglet management in small-holder and larger-scale pig farms in Northern part of Laos. *Livestock Research for Rural Development*. Volume 29, Article #201. Retrieved October 10, 2017, Available at: http://www.lrrd.org/lrrd29/10/jan29201.html

Phoneyaphon, V., & Preston, T. R. (2016). Feed intake, digestibility and N retention in Mong Cai pigs fed ensiled cassava (Manihot esculenta Cranz) root and protein-rich foliages. *Livestock Research for Rural Development*, *28*.

Phonvisay Singkham. (2003). Country report For Animal Genetic Resources Management in the Lao Department of Livestock and Fisheries, Ministry of Agriculture and Fisheries, PDR, pp 7, Available at: ftp://ftp.fao.org/docrep/fao/010/a1250e/annexes/CountryReports/LaoPDR.pdf [2017-09-01]

Phuc, B. H. N., Lindberg, J. E., Ogle, B., & Thomke, S. (2001). Determination of the nutritive value of tropical biomass products as dietary ingredients for monogastrics using rats: 1. Comparison of eight forage species at two levels of inclusion in relation to a casein diet. *Asian Australasian Journal of Animal Sciences*, *14*(7), pp. 986-993.

Polyorach, S., Wanapat, M., & Wanapat, S. (2013). Enrichment of protein content in cassava (Manihot esculenta Crantz) bysupplementing with yeast for use as animal feed. *Emirates Journal of Food and Agriculture*, pp. 142-149.

Pond, W. G., & Mersmann, H. J. (2001). Biology of the Domestic Pig. *Comstock Publishing Associates, a division of Cornell University Press, Ithaca and London*, pp. 309-389.

Preston, T.R., (2001), July. Potential of cassava in integrated farming systems. In *International Workshop on Current Research and Development on Use of Cassava as Animal Feed. Khon Kaen University, Thailand*, pp. 5-12.

Quirke, D., Harding, M., Vincent, D. P., & Garrett, D. (2003). *Effects of globalisation and economic development on the Asian livestock sector*. Australian Centre for International Agricultural Research. Available at: http://ageconsearch.umn.edu/record/114079/files/mn97e.pdf [2018-10-06]

Rahmi, B., Yanti, Y., Mizumachi, S., Achmadi, J., Kawamoto, Y., & Purnomoadi, A. (2008). Effects of drying and ensiling methods on cyanides contents and chemical components of cassava roots and stems. *Journal of the Indonesian Tropical Animal Agriculture*, *33*(4), pp. 247-254.

Rattanachomsri, U., Tanapongpipat, S., Eurwilaichitr, L., & Champreda, V. (2009). Simultaneous non-thermal saccharification of cassava pulp by multi-enzyme activity and ethanol fermentation by Candida tropicalis. *Journal of Bioscience and Bioengineering*, *107*(5), pp. 488-493.

Roder, W. (2000). *Slash-and-Burn Rice Systems IN the Hills of Northern Lao PDR*: Description, Challenges, AND Opportunities. IRRI, pp. 20-24.

Rodriguez, L., & Preston, T. R. (1997). Local feed resources and indigenous breeds: fundamental issues in integrated farming systems. *Livestock Research for Rural Development, 9*(2), pp. 92-99.

Saikia, D., & Deka, S. C. (2011). Cereals: from staple food to nutraceuticals. *International Food Research Journal*, *18*(1).

Sansoucy, R., Jabbar, M. A., Ehui, S., & Fitzhugh, H. (1995). Keynote paper: The contribution of livestock to food security and sustainable development. *Livestock Development Strategies for Low Income Countries, FAO/ILRI, Rome/Nairobi*.

Sauer, W. C., Fan, M. Z., Mosenthin, R., & Drochner, W. (2000). Methods for measuring ileal amino acid digestibility in pigs. *Farm Animal Metabolism and Nutrition*, pp. 279-306.

Sauvant, D., J. M. Perez, and G. Tran. (2002). Tables de composition et de valeur nutritive des matie`res premie`res destine´es aux animaux d’e´levage: porcs, volailles, bovins, ovins, caprins, lapins, chevaux, poissons. D. Sauvant, J.M. Perez, and G. Tran, ed. INRA Editions, Versailles.

SCAC. (2000). Lao Agricultural Census, 1998/99. Steering Committee for the Agricultural Census, Agricultural Census Office: Vientiane. Lao PDR. Available at: https://www.lsb.gov.la/pdf/updateAgriculture_Census_98_99.pdf [2018-08-29]

Schembri, N., Hernandez-Jover, M., Toribio, J. A. & Holyoake, P. K., 2010. Feeding of prohibited substances (swill) to pigs in Australia. *Australian Veterinary Journal* 88(8), pp. 294-300.

Slottner, D. (2004). Protein degradation during ensilage. Diss. (sammanfattning/summary) Uppsala : Sveriges lantbruksuniv., Acta Universitatis agriculturae Sueciae. Agraria, pp. 1401-6249.

Sharif, M. K., Butt, M. S., Anjum, F. M., & Khan, S. H. (2014). Rice bran: A novel functional ingredient. *Critical Reviews in Food Science and Nutrition*, *54*(6), pp. 807-816.

Sipaseuth. B. T. (2007). Plant Breeding and Biotechnology Capacity. GIPB-Global Partnership Initiative for Plant Breeding Capacity Building and Global Crop Diversity Trust, Vientiane, September, pp. 10-14. Available at: http://www.fao.org/pgrfa-gpa-archive/lao/LaosFullReport.pdf [2018-07-05]

Sivilai, B., Preston, T. R., & Kaensombath, L. (2016). Feed intake, nutrient digestibility and nitrogen retention by Moo Lath pigs fed ensiled banana pseudo-stem (Musa spp) and ensiled taro foliage (Colocasia esculenta). *Livestock Research for Rural Development*, *28*.

Sivilai, B., & Preston, T. R. (2017). Effect of level of dietary protein on growth and feed conversion of Moo Lath pigs fed a mixture of ensiled taro foliage (Colocasia esculenta) and of ensiled banana pseudo-stem (Musa spp). *Livestock Research for Rural Development*, *29*.

Stein, Hans H., M. F. Fuller, P. J. Moughan, B. Sève, R. Mosenthin, A. J. M. Jansman, J. A. Fernández, and C. F. M. De Lange. "Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs." *Livestock Science* 109, no. 1-3 (2007): pp. 282-285.

Ström, K. (2005). Fungal inhibitory lactic acid bacteria. Diss. Uppsala: Swedish University of Agricultural Science. (Vol. 2005, No. 37).

Stür, W., Gray, D., & Bastin, G. (2002). Review of the Livestock Sector in the Lao People's Democratic Republic. The ASIAN Development Bank. International Livestock Research Institute. Available at: https://cgspace.cgiar.org/bitstream/handle/10568/21136/adb_livestock_review.pdf?sequence=2 [2018-10-01]

Suttle, N. F. (2010). Mineral Nutrition of Livestock. 4th edition. *British library, UK*, pp. 182-200.

Tewe, O. O. (1992). Detoxification of cassava products and effects of residual toxins on consuming animals. *Roots, tubers, plantains and bananas in animal feeding (Editors: D Machin and AW Speedy). FAO Animal Production and Health. Paper*, (95), pp. 85-91.

Theungphachan, T. (2012, October). Country Report: Lao PDR. In *Proceedings of the International Workshop on the Use of Antimicrobials in Livestock Production and Antimicrobial Resistance in the Asia-Pacific Region*, pp. 22-23.

Thorne, P. (2005). Pig raising in Northern Lao PDR. Working paper. No 4 Participatory Livestock Development Project (ADB PPTA No 4287-Lao). CAIT/ILRI: Vientiane, Lao People's Democratic Republic.

Tuan, B. Q., & Van Hai, N. (2004). Research on using banana stem as feed for crossbred milking calves. *Journal of Agricultural Science*, *2*(1), 2004.

Ubalua, A. O. (2007). Cassava wastes: treatment options and value addition alternatives. *African Journal of Biotechnology*, *6*(18), pp. 2065-2073.

Vongthilath, S., & Blacksell, S. (2000) Classical swine fever in Lao PDR. In: Blacksell, S. (ed) *Classical Swine Fever and Emerging Disease In Southeast Asia*. ACIAR proceeding No. 94. ACIAR, Canberra, Australia, pp. 122-125.

Vongsamphanh, P. and Wanapat, M., (2004). Comparison of cassava hay yield and chemical composition of local and introduced varieties and effects of levels of cassava hay supplementation in native beef cattle fed on rice straw. *Livestock Research for Rural Development*, *16*(8), pp.1-11.

Wadhwa, M., & Bakshi, M. P. S. (2013). Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products. *Rap Publication*, *4*, 30.

Whittemore, C. T. (1993). The Science and Practice of Pig Production. First published. Longman Scientific and technical. United kingdom.

Wilfart, A., Montagne, L., Simmins, P. H., Van Milgen, J., & Noblet, J. (2007). Sites of nutrient digestion in growing pigs: Effect of dietary fiber 1. *Journal of Animal Science*, *85*(4), pp. 976-983.

Wilson, R. T. (2007). Status and prospects for livestock production in the Lao People's Democratic Republic. *Tropical Animal Health and Production*, *39*(6), pp. 443-452.

Windsor, P. A. (2008). Final report SRA AH2006/077, 'Identifying Research Priorities for the Development for the Beef Industry in Cambodia and Lao PDR with Special Reference to Animal Health Interventions'. ACIAR, Canberra. Available at: file://storage.slu.se/Home$/lotg0001/Downloads/Final%20Report%20AH-2006-077.pdf [2018-09-20]

# Popular science summary

The tropics present great opportunities for sustainable development, thanks to the cultural and biological riches of these regions. Rational exploitation of local feed sources and local breeds of livestock will support sustainable production systems in the medium and long-term perspective. However, this has received insufficient attention in the past and has not been considered seriously because of the introduction of 'exotic' systems based on high inputs, high technology and breeds of 'high genetic merit'. As result, local breeds of pigs and cattle in many tropical countries have disappeared or their population is decreasing drastically. Local breeds perform well in low-input systems, fulfilling multiple functions for smallholder households. They display poorer performance than exotic breeds, but have lower production input requirements.

In Lao PDR, more than 80% of pig herds are native breeds and belong to smallholders with combined rearing systems. Local pigs are the main livestock raised and their feeds are made from vegetables collected from the forest, cultivated crops and crop residues, and household leftovers. The main constraint in this low-input, low-output system is protein supply. Protein and mineral supply to local pig breeds reared in Central Lao PDR currently appears to be below requirements. This is partly due to low nutrient allowance, but also reflects the fact that obtaining animal feed is becoming an increasing challenge for smallholder farmers in developing countries. The main reason for this is that traditional feed ingredients for these animals (*i.e.* cereals, roots, tubers, soybeans and fish) are also important staple foods for humans. As a result, these feed ingredients have become expensive and scarce. Thus, there is an urgent need to find alternative feed ingredients that can complement or replace the traditional feed ingredients

The high labour demand in collecting and preparing feed is a challenge in smallholder pig production systems. Women and children commonly provide significant labour inputs as the primary caretakers of household pigs and poultry. Thus, using native breeds can be way to reduce the labour demand for collection

and preparation of feed for the pigs. Moreover, being able to store the feed for some time could be a way to reduce the labour needed for feed collection.

This thesis evaluated the impact of fermentation on the nutritive value of cassava root pulp (CRP) and soybean pulp (SBP) as feed for growing pigs. The evaluation was based on four studies (two fermentation and two animal studies). In these, the chemical composition of CRP aerobically fermented with four different yeast sources was evaluated; the digestibility, nitrogen balance, gut environment and visceral organ size were studied in growing Moo Lath and Large White pigs fed unfermented and fermented CRP and SBP; CRP and SBP were anaerobically fermented (ensiled) with or without rice bran; and the digestibility and nitrogen balance of the ensiled products was studied in growing Large White pigs.

The results from this thesis showed that cassava root pulp can be successfully fermented aerobically with yeast, reaching pH values of 3.2 to 3.9 after 14 days of fermentation. The growth of spoilage bacteria is inhibited at these pH levels, which will allow longer storage time with maintained quality. The nutrient content in cassava root pulp changes during aerobic yeast fermentation, resulting in an increase in crude protein, total protein, neutral detergent fibre and ash content, and a decrease in starch content. As a consequence, the protein value and the energy value of fermented cassava root pulp in the diet of pigs differs from that of the unfermented material. Moreover, the yeast source used for fermentation influences the protein and energy value of the fermented product.

Fermenting cassava root pulp with rice bran and urea increases the crude protein content and improves the nitrogen digestibility and nitrogen retention in both Moo Lath and Large White pigs. Nitrogen utilisation (N retention as a percentage of N digested) for diets with fermented cassava root pulp is comparable to that for diets supplemented with soybean pulp. This suggests improved protein quality in fermented compared with unfermented cassava root pulp. Dry matter intake is markedly higher in pigs fed soybean pulp, which indicates that both unfermented and fermented cassava root pulp have poor palatability.

Ensiling cassava root pulp and soybean pulp for 14 days without inclusion of rice bran results in pH 3.9-4.0, which should allow long-term storage with maintained silage quality. The pH achieved can be even lower if cassava root pulp and soybean pulp are ensiled for 21 days or more (pH 3.8-3.9). In contrast, ensiling cassava root pulp and soybean pulp for 14 days with inclusion of more than 5% rice bran (fresh weight basis) results in pH (>4.7) that is too high to inhibit the growth of spoilage bacteria and cannot be recommended. However, ensiling cassava root pulp and soybean pulp for 21 days or more with inclusion of up to 10% rice bran results in lower pH (4.1-4.4) that should allow medium-term storage. There is no empirical support for the general hypothesis that inclusion of rice bran to increase the dry matter content facilitates ensiling of cassava root pulp and soybean pulp.

Ensiling cassava root pulp and soybean pulp with rice bran results in a feed product that is well digested and utilised in growing pigs. However, dry matter intake appears to be impaired with ensiled cassava root pulp in comparison with ensiled soybean pulp. Moreover, ensiling cassava root pulp and soybean pulp with inclusion of rice bran (10% for cassava root pulp and 20% for soybean pulp) improves the digestibility of organic matter and crude protein, suggesting synergistic effects in the porcine gastrointestinal tract.

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