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Use of fresh stylosanthes (*Styosanthes guianensis, CIAT 184*) and cassava foliage (*Manihot esculenta*, Crantz) as a protein source for crossbred pigs

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Appendix

The present thesis is based on the following papers, which will be referred to the text by their Roman numerals I and II:

I. Bounlieng, K., Ogle, B., and Bouahom, B. 2005. Effect of including Stylosanthes and cassava foliages, fed separately or in a mixture on digestibility, intake, and N retention in growing pigs.

II. Bounlieng, K., Ogle, B., and Bouahom, B. 2005. Effect of Stylosanthes or cassava foliages on intake and growth performance of pigs.

1. Introduction

In view of the predicted world shortage of cereal grains because of competing needs of the expanding human population, the availability and supply of grains and protein feedstuffs for livestock is likely to become more limited (Close, 1993). There is thus an urgent need for research to develop alternative feed resources, especially for pigs and poultry.

Cassava, or tapioca, (*Manihot esculenta*, Crantz) is an annual tuber crop grown widely in tropical and sup-tropical areas. Its thrives in sandy-loam soils with low organic matter, and in areas receiving low rainfall and with high temperatures It is therefore a cash crop cultivated by small-holder farmers within the existing farming systems in many countries (Wanapat *et al.*, 2000). Considerable amounts of cassava leaves are readily available as a by-product at the time of harvesting the roots. However, in the rainy season it is difficult to sun-dry the leaves and extending the drying period diminishes the nutritional quality of the product (Phuc, 2001).

Among the potentially valuable unconventional feeds, cassava leaves have attracted the attention of many researchers because their high protein content (170 to 400g /kg CP on a dry matter basis) (Ravindran, 1993). While cassava leaf protein is low in sulphur amino acids (Gomez and Valdivieso, 1984), the content of most other essential amino acids is higher than in soya bean meal (Eggum, 1970). The higher protein content and relatively good profile of essential amino acids are reasons for believing that cassava leaves could be a potentially valuable protein source for monogastric animals.

One plant that has also been given much attention is stylo (*Stylosanthes guianensis*), which is a perennial fodder legume of Latin American and Central American origin, introduced into other areas throughout the world. It is a sub-shrub, semi-erect or erect, with strong tap root and small round root nodules. Its stem has many branches, is herbaceous or lignified at the base, and grows to a height of 1 m. Leaves are trifoliate, leaflets elliptical to lanceoelate. Inflorescence is a loosely capitates spike, terminal or auxiliary, with more than 4 flowers (Mannetje and Jones, 1992).

Stylo, CIAT 184 grows well and is well-adapted to a wide range of environmental conditions. It was initially widely used for ruminants, but in Laos is now more commonly fed to pigs rather than ruminants. Legumes can provide extra protein, as they have much higher levels of protein in their leaves than grasses; legume leaves also provide essential minerals and vitamins for animal growth. Stylo was introduced to small farmers in Laos as a feed for ruminants, but it was soon realized that farmers were feeding it to their pigs (Horne and Sture, 2000).

2. Objectives

The aims of this study were to determine ways of improving the utilization of stylosanthes and cassava foliages as a protein source for pigs by:

- Evaluating the effects of including stylosanthes and cassava foliage, fed separately or in a mixture, on digestibility, intake, and nitrogen (N) retention in growing pigs.
- Studying the effects of stylosanthes and cassava foliages, fed separately or in a mixture, on feed and nutrient intake, growth performance and carcass quality of pigs.

3. General discussion

3.1 Livestock production systems in Laos

The livestock sub-sector plays an important role in the livelihood of farmers in the rural areas of Laos. It accounted for about 18% of GDP in 2003, and generates more than half of farmers' incomes (MAF, 2003). Meat and egg consumption of the people in urban areas in 2000 was about 35 kg/capita/year (including 10kg /capita/year of fish). In rural areas, however, it was estimated to be only about 22 kg/capita/year (Agricultural Census, 2000). The majority of the livestock production, possibly up to 90%, is based on smallholder farms, whose production systems are characterized as traditional, extensive and with low inputs and low outputs (Phonvisay, 2001). There is considerable potential to increase production, both for local consumption and also of value-added products for export, and there are a number research and development areas that could improve animal health, nutrition and livestock breeding, through improving the genetic potential to create animals suitable for the local conditions and environment. It is expected that the livestock sub-sector will continue to be an important source of farm income and for the national economy.

In Lao PDR, buffalo and cattle are important economic animals, not only because they are used for draft power and as a source of protein, but because they are becoming increasingly important as an export product and a source of foreign exchange (Phonvisay, 2001). The livestock population in 2004 was as follows: buffalo 1.115 million head, cattle 1.248 million head, and sheep and goats 0.139 million head. There were also 1.728 million pigs and 19.481 million poultry. Fisheries and aquaculture yield about 25,000 tonnes of fish each year (DLF, 2003). Livestock production in shifting cultivation forms part of integrated crop-livestock production systems Pigs and poultry are mainly raised in the backyard, and fed with agricultural by-products such as rice bran, broken rice, cassava, maize, and forages and weeds, which are gathered from upland fields. During the cropping season (June-November) buffalo and cattle are allowed to graze freely in the forests or on fallow land. In some areas, where the cropping areas are not fenced, the animals are tethered or left to graze in a confined space. The owners of the livestock will visit them regularly to tend them during this season. After the harvest, the weather is cool and dry; cattle and buffalo are brought back to the village and grazed on rice straw and browse (Phonepaseuth, 1997). The animals then stay in the fields around the villages until the next cropping season, when they are taken back to the forests. In the dry period, large ruminants suffer from a shortage of feed and at this time they often lose weight, especially the young animals. Rice straw and browse are common roughages eaten during the dry season. The animals regain their weight when feed becomes available in the rainy season (Bouahom, 1993). Small livestock (especially pigs and poultry) have a particular significance in rural livelihoods, security and poverty reduction in Laos. Pig production in particular is very labour intensive, and women and children commonly spend two to four hours each day collecting fresh leaves such as water yam (*Caladium spp*), amaranth (*Amaranthaceae*) and banana stem, or heart of the stem, from the forests and fields to feed their pigs. Supplementary cooked feed (mainly maize, cassava and taro) is provided in most villages (Stur *et al.*, 2002).

The Asian Development Bank (ADB, 2002) reviewed the livestock sector, and noted that livestock are a key feature in creating employment and poverty alleviation. As well as providing a source of cash, livestock products such as milk, meat, fish and eggs can be used by the family for its own consumption, contributing to better family health. Livestock rearing also creates employment opportunities, and absorbs any slack in farm family labour. Although the per capita annual meat and fish consumption in the Lao PDR, estimated at 20.0 and 14.2 kg, respectively, is considerably higher than in many developing countries (10-12 kg) and well above the minimum dietary requirement (7.3 kg) for animal protein, demand still seems not to be saturated. Consequently it is reasonable to assume that increases in livestock production will generate more sales and, as such, increased incomes.

Livestock thus have a vital role in sustaining the upland farming systems of the Lao PDR, and improving living standards. In fact, crop cultivation without any livestock would be almost impossible in the uplands. Livestock are kept in the household with multiple objectives, and rearing livestock is the second most important economic activity in the country after cropping, but it is the main source of the households' cash income (Bouahom, 1993). A survey conducted by the Lao-IRRI, Upland Rice Project in several provinces of the northern part of Laos found that the average cash income for families in the uplands was about USS 150/year. Of this, livestock contributed 44%, crops 26%, outside work 13%, handicrafts 7%, loans 6%, and forest products 1%. It was also found that livestock are often sold for cash when this is needed.

3.2 Cassava leaves as a protein source for pigs

According to Montaldo (1977), cassava plants can withstand defoliation for several years if they receive adequate fertilization and irrigation, and under such management conditions have the potential to produce up to 4 tonnes of protein per hectare, annually. The management of cassava as a forage crop with high levels of fertilization has recently been documented by Preston *et al.,* (2000) and Preston (2001). Annual yield of fresh forage is between 80 and 120 tonnes/ha.

Cassava leaf is a rich source of protein, minerals and vitamins, with an average of 21% crude protein (ranging from 16.7 to 39.9%) according to Chinh *et al.*, (1994). In fresh cassava leaf there is a high content of glycosides, linamarin and lotaustralin, which are hydrolysed by the linamarase enzyme, resulting in the release of hydrocyanic acid (HCN), which is toxic to the animal. HCN is colourless, volatile and extremely poisonous. The content of HCN in cassava leaf differs among varieties and generally ranges from 200 to 800 mg/kg DM in the fresh leaf, but values as low as 80 mg/kg DM (Wood, 1965) and as high as over 4000 mg/kg DM (Ravindran, 1988) have been reported. The HCN content also depends on the nutritional status of the plant, and is increased by nitrogen fertilization. The glycoside concentration in cassava leaves decreases with age (Lutaladio, 1984; Ravindran and Ravindran, 1988). The elimination of cyanogens by heating will depend on the temperature, the stage of development of the plant, and the type of heat. Simple sun drying reduces the cyanogens content of cassava leaf more effectively than ensiling because of the stability of linamarase at low pH values (Oke, 1994). Despite its high content of HCN, documented cases of poisoning due to the ingestion of cassava leaf are rare (Ravindran, 1993).

Ravindran *et al.*, (1987) evaluated cassava leaf meal (CLM) as a substitute for coconut meal. The results showed that CLM could replace up to 66 percent of the coconut meal (26 percent of the total diet) in growing pig diets without adverse effects on performance. The most efficient gains were obtained at 33 percent replacement (13 percent of the total diet). Attempts to utilize CLM as a replacement for other protein supplements in pig diets have been less encouraging. Alhassan and Odoi (1982) reported depressions in live weight gains and feed efficiency when CLM was included at 20 and 30% levels to replace part of the peanut meal, fish meal and maize in the basal ration for growing-finishing pigs. Ravindran (1990) substituted 10, 20 and 30% CLM in a maize-soybean meal basal diet and reported that live weight gain and feed efficiency of growing pigs were lowered linearly with increasing levels of leaf meal. Processed cassava leaves and roots, however, have been used successfully as feed for pigs. Phuc *et al.* (1996) and Ngoan *et al.*(1998). It was demonstrated by Phuc *et al.* (2000) that an ADG of 550g/head/day in fattening pigs could be attained when cassava root or leaf meal made up 30-40% on DM basis of the diet.

3.3 Stylosanthes guianensis as a protein source for pigs

Stylo CIAT-184 is a short-lived perennial legume (2 to 3 years) that grows into a small shrub with some woody stems. It is adapted to a wide range of soils and climates but is one of the few herbaceous legumes which will grow well on infertile, acid soils. It will not grow on very alkaline soils (pH > 8). Unlike earlier varieties of *S. guianensis* (e.g. cv. Schofield, Cook and Graham) Stylo 184 has shown good resistance to the fungal disease anthracnose in Southeast Asia. It is usually grown as a cover crop, which is cut every 2 to 3 months. It effectively suppresses weeds and is a good feed supplement for most animals, including chickens, pigs and fish. Stylo 184 can be fed fresh or dried for hay and leaf meal. It does not tolerate being cut close to the ground since there are few buds on the lower stem for regrowth, which can be improved by making the first cut at 10 to 20 cm to encourage branching close to the ground. Subsequent cuts must be made higher (>25 cm) to ensure good regrowth (Horne and Stür, 1999). N concentrations of *Stylosanthes guianensis* range from 1.5 to 3 % in DM. DM digestibility of young plant material lies between 60 to 70%, but with increasing age and lignification this may be reduced to below 40% (Mannetje and Jones, 1992). In a study on the effects of cutting height and time on DM yield and chemical composition of Stylo 184, increasing cutting height from 20 to 30 cm reduced DM yield from 4.7 to 4.3 t/ha (Kryothong, 2002). Cutting at 60 to 75 cm and 90 days reduced yield from 4.2 to 4.4 and 0.9 t/ha respectively. Increasing the cutting height from 20 to 39 cm affected CP content, which tended to increase from 17.8 to 18.8%, whereas NDF and ADF values tended to decrease, from 55.7 to 53.8 and from 41.0 to 36,4%, respectively. Cutting at 60, 70 and 90 days affected CP content, which increased from 15.4 to 17.9 and 21.9%, respectively, while NDF and ADF values decreased from 63.3 to 55.8 and 45.2 and from 47 to 38 and 31%, respectively. Ca and P showed no difference, and were in the range of 0.12 and 0.39 to 0.50%, respectively (Kryothong, 2002).

Growing and processing (chopping) Stylo 184 foliage is a simple technique, and the product is cheap and suited to the conditions of small-holder farmers in the upland areas of Laos. Intake of a maize / rice bran diet was increased and rate of live weight gain almost tripled with increasing levels of Stylo 184 foliage in the diet (Chanphone, 2003), Laotian indigenous pigs given a maize / rice bran basal diet over the live weight range of 10 to 40 kg were shown to consume Stylo 184 foliage at up to 6.4% of the diet DM without any negative effects on health and with superior growth performance compared with the traditional diet, and feeding Stylo also resulted in a higher profit for the farmers.

3.4 Requirement of protein and amino acid for growing pigs

According to Church (1979) the usefulness of a protein source depends on its amino acid composition, because the real need of the pig is for amino acids and not protein as such. Protein quality is a term used to describe the amino acid balance of a protein.

A good quality protein contains all of the essential amino acids in the amounts and proportions necessary for the particular need of the animal, i.e., growth, fetal development, lactation etc. A poor quality protein is one that is deficient in either content or balance of the essential amino acids. It is possible for a 12% protein diet that is well balanced in the essential amino acids to support better growth of weanling pigs than a 16% protein diet that has a poor balance of these amino acids. However, combinations of most common feed ingredients do not provide this superior balance of amino acids.

Pigs require about twenty amino acids and some of these, which cannot be synthesized by the animal or are synthesized at an insufficient rate to meet its requirement, are called essential (or indispensable) amino acids. For optimum performance, a diet must provide adequate amounts of essential amino acids, energy and other indispensable nutrients. Protein requirement may be stated in terms of "Ideal protein" (McDonald *et al.*, 1995). The pig's requirements for total proteins are usually determined in feeding trials in which growth rate is the main criterion of adequacy, and are stated as the concentration of protein in the diet. The pig has specific requirements for ten essential amino acids, and the ratio of amino acids to lysine is particularly important. NRC (1988) and NIAH (1998) recommend a ratio of methionine + cystine to lysine of 50%, and of the threonine to lysine of 60%. In pig diets, protein quality is often limited by a deficiency of one or two of the indispensable amino acids. If total protein standards are accompanied by standards for these amino acids they become more meaningful. The concept of "limiting" amino acids refers to the most deficient amino acids, and for the pig it is likely to be lysine (McDonald *et al.,* 1995). Protein requirements of growing pigs depend on many factors, such as breed, age and growth rate.

3.5 Digestion of protein in the pig

In industrial systems the aim of animal production is to obtain the maximum biological performance of animals. However, this should not be the main objective of small-scale pig production systems in the tropics, as diets for animals should if possible be based on local resources. These feeds usually have low protein and high fibre contents. In order to improve performance, the farmers have to know about the requirements of their pigs, the characteristics of the feed resources and feed digestibility (Tran Thi Thu Hong, 2003).

In monogastric animals, the initial protein digestion occurs in the stomach, followed by a rapid protein digestion in the duodenum. The absorption from the products of protein hydrolysis takes place throughout the small intestine where the jejunum is the main absorptive site (McDonald *et al.,* 1995). In a normal diet, there is always a certain amount of protein, which is resistant to the action of enzymes secreted into the alimentary canal. The undegraded fractions, endogenous N as well as mucus from intestinal desquamation, reach the large intestine and are exposed to microbial degradation. There is no secretion of proteases by the large intestinal mucosa and pancreatic enzymes are inactivated by the resident micro-organisms (Mason, *et al.*, 1986). The slow rate of passage and availability of different nutrient sources encourages the growth of bacteria. The hindgut micro flora ferment undigested nitrogen residues, mucoproteins and urea and release amines and ammonia, of little or no nutritional value to the animal (Zebrowska, 1973; Mason, 1986). Most nitrogenous components are either absorbed into the blood or are degraded and assimilated by the micro-organisms and used for cell synthesis (Payne, 1975; Mosenthin *et al*, 1992).

3.6 Digestion of fibre in the pig

The utilization of fibre in monogastric animal species such as pigs is very important, due to the fact that digestion of fibre may highly influence performance traits of economic importance (Siers, 1975; Frank *et al*., 1983). In this connection, structural carbohydrates, or fibre, utilization in growing pigs largely depends on the level of fibre fed, source of fibre, stage of forage maturity, and level of other nutrients in the diet (Farrell and Johnson, 1973; Close, 1993). Feeding diets with high fibre content will increase the time needed to consume the daily allowances (Morz *et al.*, 1986). The addition of fibre might also be involved in inducing satiety through increasing gut distension. According to Fernandez and Jorgensen (1986), 94 to 99% of all carbohydrates are digested by the time they reach the terminal ileum in pigs. However, digestion of hemicelluloses and cellulose up to the terminal ileum is very limited (Key and DeBarthe, 1974), and the amount of carbohydrates and other nutrients transferred from the small intestine into the large intestine is highly dependent on diet composition. Digestibility of lignin by the large intestinal microbes is very limited, and lignin is not degraded in noticeable amounts (Dierick *et al*, 1989). Fibre is not digested by endogenous processes, but only by microbial flora. The digestibility of fibre is more variable than other components (Noblet and Le Goff, 2001). Microbial fibre degradation in the caecum and colon generates volatile fatty acids (VFA), which include mainly acetate, propionate and butyrate, and the gases H_2, CO_2 and CH_4 (Varel, 1984). However, only small amounts of hydrogen and methane are absorbed (Graeve and Demeyer, 1988). Acetic acid tends to predominate, with lower proportions of propionic and butyric acids. The proportions vary according to type of dietary fibre (Low, 1985). As shown by Kass *et al.,* (1980) the acid acetic proportion increases as the content of forage fibre in the diet increases. The VFAs are normally absorbed into the bloodstream directly across the colon wall in the form of free acids. Thereby acidity is relieved and pH of the colon is maintained above 6, which normal fibre-digesting bacteria require (Van Soest., 1994). The VFA may provide between 5 to 30% of the energy requirement of growing pigs

(Farrell and Johnson, 1972; Kass *et al.,* 1980; Ehie, 1982; Varel, 1984; Dierick, 1989), and the VFA supply from fermentation may be considerably higher in adult than in young pigs (Noblet and Back-Knudsen, 1991). Prolonged feeding of high fibre diets can increase the number of cellulolytic bacteria in the pig large intestine and these organisms may represent 10% of the cultivable flora (Varel and Pond, 1984). Two of the predominant cellulitic species found in the rumen, *Bacteriodes succinogenes* and *Ruminococcus flavefaciens*, are the predominant organisms in the large intestine of growing pigs (Varel *et al*., 1984). This provides a partial explanation as to how significant quantities of fibre can be degraded in the large intestine. The source of fibre plays a significant role in digestion and absorption (Wenk and Wenk *et al.,* 2001) and for example Noblet and Back-Knudsen (1991) showed that digestibility in sows was higher for maize fibre and soybean pulp than for wheat bran.

4. Conclusions

The purpose of theses studies was to maximize the utilisation of locally available low protein feed resources for pigs. It was demonstrated that: On a basal diet of about 93.5 g/ kg DM of crude protein supplemented with cassava foliages and fresh stylo leaves fed to pigs of 26 to 30 kg the foliage DM intake was higher when cassava foliages were mixed with fresh stylo in the proportion 50:50 on DM basis as compared with cassava foliages and stylo CIAT-184 offered alone. There was a positive curvilinear relationship between the proportion of foliage supplement in the diet and nitrogen retention. The addition of a mixture of cassava foliages and fresh stylo 184 fed together *ad libitum* improved the quality of the diet, which resulted in higher intake and better growth rate and feed conversion, which led to improved economical efficiency, as well as making use of locally available, low cost resources..

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