Cricket Farming

An Alternative for Producing Food and Feed in Cambodia

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Cover: Native Cambodia field crickets (*Teleogryllus testaceus*) and two-spotted crickets (*Gryllus bimaculatus*), males and females.
(photo: Phalla Miech, 2014)

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Abstract

This thesis examined use of weeds and agricultural by-products as feed for Cambodian field crickets (*Teleogryllus testaceus*) and the value of crickets as feed for monogastric animals. In a 70-day study, survival and growth of cricket nymphs fed weeds and food/agricultural by-products were evaluated. The weeds were *Alternanthera sessilis, Amaranthus spinosus, Commelina benghalensis, Cleome rutidosperma, Cleome viscosa, Boerhavia diffusa* and *Synedrela nodiflora*. The by-products were rice bran, cassava tops, water spinach, spent grain and mungbean sprout residues. Chicken feed was used as the control. Cricket survival did not differ between feeds except for *B. diffusa* and *A. sessilis*, which gave lower survival than the control. Weight of crickets fed cassava tops and *C. rutidosperma* did not differ from the control. Analyses of amino acid and mineral content in dried and frozen samples of field crickets fed the control, cassava tops, *S. nodiflora* or *C. rutidosperma* revealed that methionine content was highest in crickets fed cassava tops. There were no differences in mineral content.

Apparent faecal digestibility and nitrogen retention were evaluated in piglets (n=21, age 30-45 days) fed three iso-nitrogenous diets (18.4% crude protein) containing whole cricket meal (WC), cricket body meal (BC) or fish meal (control) for 25 days. Total collection of faeces and urine was performed during the last 5 days. Dry matter and nutrient intake were higher for WC and BC than for the control. From day 10, piglets fed BC and WC were heavier than piglets fed the control, but there were no differences between WC and BC. Dry matter digestibility was highest for WC. Feed conversion ratio was lower for WC and BC than for the control and nitrogen retention (% of digested) was higher.

Growth was also evaluated in native chickens (n=60, age 30-45 days) fed iso-nitrogenous (21% crude protein) diets containing WC, cricket leg meal (LC) and fish meal (control) for 35 days. Daily weight gain, daily feed intake and feed conversion ratio of chickens fed WC and LC did not differ from the control, but daily weight gain was higher and feed conversion ratio lower in chickens fed WC compared with LC.

These results show that Cambodian field crickets can be reared using simple means, with *e.g.* cassava tops and *C. rutidosperma* (purple cleome) as feed. They also demonstrate that field cricket meal is a nutritious feedstuff for pigs (and probably also humans) and that peeling (removal of legs) does not improve digestibility and nitrogen retention. However, residues from peeled crickets, *i.e.* leg meal, can successfully replace fish meal in the diet of native chickens.

Keywords: Nutrition, food, feed, peeled, wild, cricket farming, weeds, and by-products.

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Thesis at glance

Years	Animal/design	Diets	Measurement	Main results	Conclusion
2014 Paper I: Survival and growth of field crickets	Field crickets/ • 13 treatments, n=80/ treatment, 4 replicates • 50:50, male & female • 70-day trial	13 diets fed *ad libitum:* Chicken feed (control), cassava tops, water spinach, rice bran, spent grain, mungbean sprout residues, *A. sessilis, A. spinosus, B. diffusa, C. benghalensis, C. rutidosperma, C. viscosa, S. nodiflora*	Feed offered and refusals every day. Counting and weighing every 7 days	• Survival did not differ from control, except for *B. diffusa* (at 35-49 days) and *A. sessilis* (lower) • Weight of crickets fed cassava tops and *C. rutidosperma* did not differ from control, *A. sessilis, A. spinosus* and *B. diffusa* gave lower weight • Feed conversion ranged from 1.6 to 3.9. It was ≤1.9 in crickets fed control, cassava tops and *C. rutidosperma*	Cassava tops and *C. rutidosperma* are suitable feeds for field crickets, while *A. sessilis, A. spinosus* and *B. diffusa* are appear to be unsuitable
2017 Paper II: Nutrient composition of field crickets	Field crickets/ Samples of field crickets fed chicken feed, cassava tops, *S. nodiflora* and *C. rutidosperma* (from Paper I)	Wings and legs separated from the body by hand, and analyzed separately for amino acid and mineral content	Weight and length of crickets. Body and wings and legs analyzed separately	• Crickets fed cassava tops were heavier than crickets on the other diets, but wings were heaviest in crickets fed chicken feed • Crickets fed cassava tops had lower DM and higher tyrosine content than crickets fed chicken feed and *S. nodiflora* • Methionine content was higher in crickets fed cassava tops than in crickets fed chicken feed, *S. nodiflora* and *C. rutidosperma*.	Amino acid profile of field crickets can be altered with diet. Cassava tops seem to be a good feed resource with respect to amino acid quality
2016 Paper III: Growth and digestibility of cricket meal in a monogastric animal	Multi-blood strain pigs/ • 3 treatments, n=7 piglets/ treatment • All male pigs • 25-day trial	Three diets (18% CP in DM) fed *ad libitum* and including either: • Fish meal (control) • Whole cricket meal (WC) • Cricket body (legs removed) meal (BC)	Feed offered and refusals every day. Weight every 5 days.	• Feed intake was higher for WC and BC than for control • Piglets fed BC and WC were heavier than piglets fed control (from day 10). No differences between WC and BC • Dry matter digestibility was highest for WC, and ash, crude fiber and crude fat digestibility were higher for BC and WC than for control • Feed conversion ratio was lower for WC and BC than for control, and nitrogen retention was higher.	Field cricket meal is a nutritious feedstuff for monogastric animals, and most likely also for humans. Removal of legs does not improve digestive response and nutritional value.
2017 Paper IV: Growth of native chickens fed cricket meal	Native chickens • 3 treatments, n=60/ treatment, 6 replicates • 50:50, male & female, • 35-day trial	Three diets (21% CP in DM) fed at 9.6% of BW and including either: • Fish meal (control) • Whole cricket meal (WC) • Cricket leg meal (LC)	Feed offered and refusals every day. Weight every 7 days.	• Daily feed intake, feed conversion ratio, body weight and weight gain of chickens fed WC and LC diets did not differ from control • Body weight and daily weight gain was higher with WC compared with LC and feed conversion ratio was lower.	Cricket leg meal is a nutritious feedstuff for native chickens

Preface

Cricket farming as an alternative to produce food and feed is an interesting topic in the current context of Cambodia. The mortality rate of children under 5 years is still high and underweight in women (15-49 years) is common. These conditions are mainly caused by limited food diversification beyond rice. Protein sources of animal origin are urgently needed to alleviate this problem.

This thesis presents the results of applied research, but also contains useful information on cricket domestication, nursery and simple farming techniques, and concrete information on the composition and nutrient value of whole and peeled crickets as food for smallholders and cricket consumers. The thesis provides basic background information for smallholders on cricket farming. Cricket rearing for food by smallholders could play a vital role in fighting malnutrition among vulnerable groups such as mothers and children under 5 years.

The thesis is based on Papers I-IV, but it also includes data from two other studies performed during the research period and on our practical experiences of rearing crickets.

Dedication

To my Mum, Yi Khieu, and my Dad, Yang Miech, who always encouraged and motivated me keeping to go to school, while other teenagers of similar age were asked to leave school.

"In the sweat of thy face shalt thou eat bread, ~"
Genesis 3:19, King James Version (KJV)

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. Miech, P., J.E. Lindberg, Å. Berggren, T. Chhay, B. Khieu & A. Jansson. (2016). Growth and survival of reared Cambodian field crickets (*Teleogryllus testaceus*) fed weeds and agricultural and food industry by-products. *Journal of Insects as Food and Feed:* 2 (4), 285-292.

II. Jansson, A., P. Miech, G. Håkansson, Å. Berggren & J.E. Lindberg. (2018). Nutrient content of field crickets fed chicken feed, cassava tops and two types of weed. Manuscript.

III. Miech, P., J.E. Lindberg, Å. Berggren, T. Chhay & A. Jansson. (2017). Apparent faecal digestibility and nitrogen retention in piglets fed whole and peeled Cambodian field cricket meal. *Journal of Insects as Food and Feed* 3:4, 279-288.

IV. Miech, P., J.E. Lindberg, Å. Berggren, T. Chhay, B. Khieu & A. Jansson. (2018). Growth of native chicken fed whole meal and leg meal of Cambodian field cricket (*Teleogryllus testaceus*). Manuscript.

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Abbreviations

A. sessilis	*Alternanthera sessilis*
A. spinosus	*Amaranthus spinosus*
B. diffusa	*Boerhavia diffusa*
B. mutica	*Brachiaria mutica*
B. reptans	*Brachiaria reptans*
BC	Body cricket meal
BW	Body weight
C. benghalensis	*Commelina benghalensis*
C. dactylon	*Cynodon dactylon*
C. rutidosperma	*Cleome rutidosperma*
C. viscosa	*Cleome viscosa*
CassaT	Cassava plant tops
CelAgrid	Centre for Livestock and Agriculture Development
CF	Crude fibre
Cfat	Crude fat
CP	Crude protein
D. aegyptium	*Dactyloctenium aegyptium*
DM	Dry matter
E. colona	*Echinochloa colona*
E. indica	*Eleusine indica*
FCR	Feed conversion ratio
Field cricket	Cambodia field cricket (*Teleogryllus testaceus*)
HCN	Hydrogen cyanide
LC	Cricket leg meal
ME	Metabolisable energy
Mung	Mungbean sprout residue
RiceB	Rice bran
S. halepense	*Sorghum halepense*
S. nodiflora	*Synedrela nodiflora*
SpenG	Spent grain
Two-spotted cricket	Cambodia two-spotted cricket *(Gryllus bimaculatus)*
WateS	Water spinach
WC	Whole cricket meal

1 Introduction

In Cambodia, around 25% of the population is food-deprived and eats less than the minimum daily energy requirement (USAID, 2017). Cambodia is a predominantly rice-eating country and its people suffer from several forms of malnutrition, such as protein, iron, iodine and vitamin A deficiency (Unicef, 2016). Limited diversification in daily food items is common for vulnerable groups (mothers and child under 5). According to the report *Overview of the Nutrition Situation in Seven Countries in Southeast Asia*, the proportion of children under two years whose diet meets the minimum standards for dietary diversity is lowest in Cambodia (37%) and highest in Vietnam (72%) (Chaparro *et al.*, 2014). Crickets are an interesting food and feed option (Wang *et al.*, 2004; FAO, 2013) due to their nutritional value. In particular, they contain high contents of protein (58-78%) and trace minerals (Adul Razak *et al.*, 2012; Finke *et al.*, 2002; Barker *et al.*, 1998; Nakagaki *et al.*, 1987). Crickets are well accepted as food and popular in Cambodia (Münke, 2012) and are consumed whole (*i.e.* including body, wings and legs). In Europe, it is suggested that crickets are peeled to improve the eating experience and reduce choking risks (EFSA, 2015). If crickets are peeled, wings and legs can be a valuable by-product. However, studies on the use of cricket products and by-products as food or animal feed are lacking.

The main supply of crickets to the current market is from light trap owners harvesting wild crickets (Münke, 2012). However, this system is not likely to meet future year-round demand for crickets, as Cambodia began experiencing depleted stocks in 2010 and started importing crickets from Thailand to meet domestic demand (Pordes & Khyhay, 2010). However, it has also been reported that a surplus of crickets was exported to Thailand in 2007 (Ratanchan, 2009). Catching wild crickets could be a potential threat to existing wild cricket populations due to the harvesting season partly coinciding with the mating period (reproduction stage) of wild crickets. Therefore, sustainable cricket rearing systems are needed to ensure that crickets can be supplied to meet year-round market demand. Cricket farming may be an attractive option for smallholder families in terms of supplying diversified food items that include animal protein, since it can be conducted with small resources and the crickets can be harvested for family consumption.

Cricket species like the field cricket (*Teleogryllus testaceus*) and two-spotted cricket (*Gryllus bimaculatus*) are native species that occur naturally in paddy fields and fallow land in Cambodia. They can also be reared artificially (Megido *et al.,* 2016). Crickets are omnivores and have the potential to eat a wide range of food sources from animals, plants, residues or by-products. Low-cost feeds such as weeds and agricultural and food industry by-products are therefore among the top priority feeds for small-scale cricket farming. Today, cricket farming in Cambodia is based on poultry feed and thus vulnerable to feed price rises (Hanboonsong *et al.,* 2013). Cricket farmers also have limited technical knowledge of farming (Hanboonsong *et al.*, 2013), which is one of the underlying causes of many farming failures. A technical handbook for small-scale cricket farming has been developed based on existing experiences, in order to assist small cricket farmers (CelAgrid, 2016).

According to Münke (2012), trap owners consume some crickets themselves (2-3 handfuls per catch) and sell the rest. Also cricket farmers may consume some of the crickets themselves. This is in contrast to many farmers who keep large livestock *(e.g. cattle, pigs, and poultry)*. These animals are not commonly slaughtered and consumed by households' members but sold for cash. With respect to counteracting malnutrition, cricket rearing could therefore serve rural households better than large livestock.

2 Aims and hypothesis

Objectives of the study

The overall aims of this thesis were: i) to study the feasibility of rearing native crickets and ii) to investigate potential feeds for crickets and potential use of cricket products as an alternative food and feed.

Specific objectives were:

- To evaluate survival and growth of Cambodian field crickets fed different local weed species and agricultural and food industry by-products
- To investigate the amino acid and mineral content of Cambodian field crickets fed selected weeds and agricultural and food industry by-products
- To investigate apparent faecal digestibility and nitrogen retention in piglets fed whole and peeled Cambodian field cricket meal
- To study growth performance of native chickens fed diets including either whole meal or leg meal of wild Cambodian field crickets.

Hypotheses of the study

- Wild field crickets from natural habitats in Cambodia can be reared in captivity in a mass rearing system
- Weeds, and agricultural and food industry by-products can be used to feed crickets reared in captivity
- Feed source affects the content of amino acids and minerals in crickets
- Whole cricket meal and body (legs removed) cricket meal have different effects on apparent faecal digestibility and nitrogen retention in piglets
- Whole cricket meal and cricket leg meal affect growth performance of native chickens differently

3 Scope of the PhD project

Wild field crickets were caught live by hand using light traps at night in the provinces of Kampong Thom and Kampong Chhnang, Cambodia (Figure 1). They were nursed in concrete block pens at the research farm of the Centre of Livestock and Agriculture Development (CelAgrid). Nymphs were produced at CelAgrid from wild-caught field crickets for the studies and used as a model of simple cricket rearing practice.

Breeding was successful and the second generation that hatched in captivity was used for the study on potential feed resources (Paper I). The chemical composition of the field crickets was analyzed and the effect of peeling was evaluated (Paper II). Finally, whole field cricket meal and field cricket body (legs removed) meal were used as a protein source in diets for piglets (as an animal model for humans) (Paper III), while whole cricket meal and field cricket leg meal were used as a protein source in diets for native chickens (Paper IV).

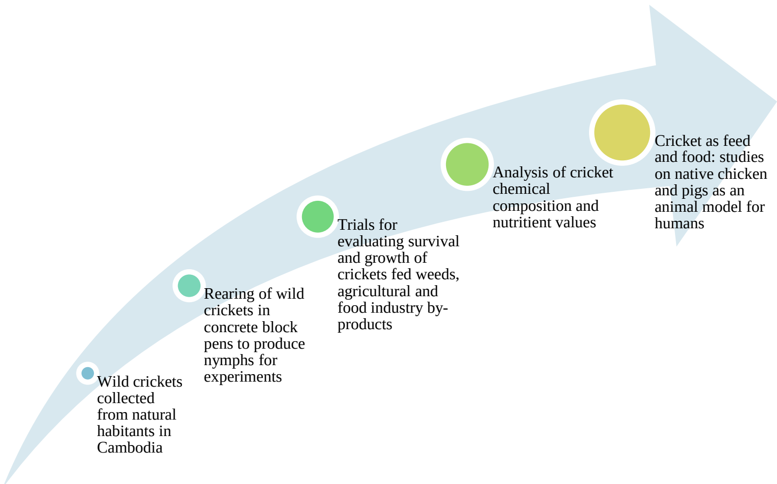

Figure 1. Scope of the work presented in this thesis.

4 Background

Geography and climate

Cambodia is located in the Indochina peninsula of Southeast Asia, between 10°-15°N and 102°-108°E and covers 181 035 km^2. The country borders Thailand, Lao PDR and Vietnam. It is roughly square in shape and is divided into four main agro-ecological zones: the Coastal zone, the Mekong floodplain, the Tonle Sap floodplain and the Upland zone. The Mekong and Tonle Sap floodplains traverse the country from north to south. There are two seasons, wet and dry, and a tropical monsoon climate with sunshine throughout almost the whole year. The wet season runs from May to September, with the heaviest rainfall in August and September (up to 80% of the annual, 1000-2500 mm). The dry season runs from October to April. Average temperature ranges from 24 °C in the cooler months of October, November and December to 35 °C in the hottest period of the year, from March to June.

Cambodian landless/land poor and its causes

Landless people are those who have involuntarily lost land, possess no skills other than in farming and face an uncertain livelihood because of the loss of land (Sophal *et al.*, 2001). Agricultural landless households were estimated to comprise 5% of the Cambodian population in 1990 (Williams, 1999), 12% in 1997 and 16% in 1999 (Sophal & Acharya, 2002). By 2004, up to 45% of families were landless or near landless (World Bank, 2004). About 91% of the poor live in the countryside, of which small farmers who own less than 0.5 hectares and the landless poor comprise an estimated 46% of the rural population (World Bank, 2007). Low productivity and the high price of land are the main causes of landlessness or near-landlessness (Sophal *et al.*, 2001). Other causes include land grabbing by rich and powerful individuals and forced evictions through lack of legal land ownership rights and tenure (LICADHO, 2009). Under the economic land concession and development (ELC) plan, approximately 133,000 residents, or 11% of the total population (1.2 million) in Phnom Penh, have been evicted since 1990 (COHRE, 2009). Large-scale ELCs were granted to private investors in 1995, allowing the private sector to obtain large amounts of land (up to 10,000 ha) for agricultural production and agro-industry development (Sub-decree on ELCs, 2005). By November 2011, the government had granted ELCs for 1,980,888 ha, equivalent to 54.9% of Cambodia's arable land, to private companies (LICADHO, 2011).

In 2014, the Cambodian poverty rate was 13.5% and around 4.5 million people (approximately 30%) remain near-poor, *i.e.* vulnerable to falling back into poverty when exposed to economic and other external shocks (World Bank, 2017). Landlessness can be one of the underlying causes of poverty, food insecurity and migration, while food insecurity is an underlying cause of malnutrition (Chaparro *et al.*, 2014), especially for mothers and children under 5 years.

Malnutrition in mothers and children under 5 years

In 2014, the proportion of underweight, stunted and wasted children under 5 years was 29%, 40% and 11%, respectively, and the mortality rate was 54%, with child deaths mostly caused by factors that could be prevented, such as lack of food (Chaparro *et al.*, 2014). In 2010, the prevalence of underweight women (15-49 years) was 19% and the proportion of women with short stature was 6.3% (Chaparro *et al.*, 2014). In the same year, the proportion of children under 5 years and women (15-49 years) suffering with anaemia associated with malnutrition was 55% and 44% (of which 52.7% were pregnant), respectively (DHS, 2010). Iron and vitamin A deficiency can contribute to anaemia (George *et al.*, 2012). *The review of the nutrition situation in seven countries in Southeast Asia* by Chaparro *et al.* (2014) showed that the proportion of children under 2 years whose diet meets the minimum standards for dietary diversity is lowest in Cambodia (37%) and highest in Vietnam (72%).

The livelihoods of smallholders and the landless poor depend mainly on agricultural activities using natural common property resources (CPR), harvesting informal food items from the wild and off-farm work. Food and nutrition security issues and impending threats to the existing small-scale farms of smallholders with low productivity and limited diversified agricultural activities strongly affect malnutrition, especially for women and children. As an alternative approach to produce food items in order to diversify diets and to provide adequate nutrients and food energy sources from animal protein, smallholders should consider producing food commodities in intensive systems with less negative effect on environment and year-round production.

Crickets as a human food

Previous studies have shown that the crude protein (CP) content of house cricket (*Acheta domesticus*), field cricket and Mormon cricket (*Anabrus simplex*) is up to 78% (dry matter (DM) basis), that the fat content is up to 22.8% and that these cricket species are rich in trace minerals (Moreki *et al.,* 2012; Wang *et al.,* 2005; Finke, 2002; Barker *et al.,* 1998; Finke *et al.,* 1989; Nakagaki *et al.,* 1987; DeFoliart *et al.,* 1982). The field cricket is a common native species in Cambodia and a popular food, and preliminary data from our laboratory indicate that it may contain 58-65% CP (DM basis). The species is therefore of interest as human food. Crickets are well accepted as food in Cambodia and they are mainly consumed whole, *i.e*. including body, wings and legs, as a deep-fried snack. In work on introducing crickets as food in Europe, it has been suggested that they should be peeled, *i.e.* legs and wings removed, before consumption (EFSA, 2015) to improve the eating experience and reduce choking risks. Wings and legs can be expected to have a high chitin and chitosan content, which may be difficult to chew. However, data are lacking on specific problems caused by cricket consumption, either whole or any parts. Chitinases have been found in several human tissues and their role has primarily been associated with defense against parasite infections, while chitin digestion by humans has been questioned. However, an acidic mammalian chitinase (AMCase) is found in the human gastric mucosa (Boot *et al.,* 2001) and gastric juice (Paoletti *et al.,* 2007). It appears reasonable to assume the AMCase is involved in the digestion of chitin in mammals, including humans, although the extent of digestion of chitin of different origin is at present unknown. Studies are lacking on the feed or food value of field crickets and the impact of peeling on the feed value in monogastric species.

Due to the high contents of protein, unsaturated fatty acids, amino acids and minerals, entomophagy could be one option to improve nutrition, by providing high-quality animal protein. Crickets have been suggested as an excellent food source, resolving the scarcity of food, and have also been suggested as a health supplement in the future (Ryu1 *et al.,* 2016).

Cricket as feed option

Previous studies have examined replacing fish meal or soybean meal in the diet of broiler chickens with cricket meal made from whole Mormon crickets, house crickets and field crickets (Abdul Razak *et al.,* 2012; Wang *et al.,* 2005; Nakagaki *et al.,* 1987; Finke *et al.,* 1985; Defoliar *et al.,* 1982). These studies indicate that cricket (whole) meal has great potential to replace fish meal and soybean meal in poultry production. Besides being fed as meal, crickets can be fed fresh directly to lizards, poultry and fish. In Cambodia, it is very rare to use fresh crickets as animal feed. However, if cricket production were to increase above the human food demand in the future, then crickets may be used in animal feed. In addition, if Cambodia follows the food safety procedure as suggested by EFSA (2015) that crickets should be peeled (legs and wings removed), this will produce residues that may contain valuable muscle protein with potential to be used as animal feed.

To obtain cricket products or by-products for food and feed there are two possible alternatives: either harvest them from the wild or rear them in farming systems. Both options have advantages and disadvantages.

Wild cricket harvesting

Wild crickets are harvested using light traps at night. The light trap is a very simple construction consisting of a piece of white plastic sheeting, a bamboo frame and poles and a light, powered by battery, generator or electricity, for attracting insects (Figure 2). Light trap owners operate mainly in the areas around Tonle Sap Lake in Battam Bong, Pursat, Kampong Chhnang, Kampong Thom and Siem Reab provinces. The season for trapping is the early wet season, with peak trapping season appearing to be in May-July (Münke, 2012). However, trap owners in Kampong Thom province report year-round harvesting (Münke, 2012). The traps are placed on common property resources, on the banks of water channel, in rice paddy fields, on roadsides and around the house of trap owners.

The amount (kg/trap) of crickets harvested per night depends mainly on quantity of rainfall, wind speed and degree of moonlight. The exact volume of wild crickets harvested annually is not known, since there are no records. However, the volume of wild crickets exported from Cambodia to Thailand during November-October 2007 was 225 tons of field cricket, 33 tons of mole cricket (*Gryllotalpa africana*), 5 tons of two-spotted cricket and 2.5 tons of short-tail cricket (*Brachytrupes portensosus*) (Ratanchan, 2009). A major concern with wild harvesting is that, as more farmers become involved in this harvesting practice (and some farmers even harvest during the breeding season), there will be an imbalance in the wild cricket population, which may have a great impact on the ecosystem. For example, overharvesting may result in a lack of feed for lizards and weed plants may grow faster because wild crickets no longer forage on them.

Figure 2. Two night-traps for catching wild crickets at night. They are made using a bamboo frame, wooden poles and plastic sheets, with an electric light to attract the crickets.

Local media have reported a decline in the number of crickets caught and claim that Cambodia's insect industry is struggling to meet the demand for one of "the Kingdom's favorite snacks" (Pordes & Khyhay, 2010). Trap owners have also observed that the amount of crickets harvested from the wild is decreasing year on year (Pordes & Khyhay, 2010). Government officials have recognized dwindling cricket reserves and suggest overharvesting as a possible cause (Pordes & Khyhay, 2010). Food safety may also be at risk with the dwindling harvest, because wild crickets may be contaminated with an infectious zoonotic or pesticides/herbicides from agricultural land (Hanboonsong *et al.,* 2013).

One option to make sure that the supply of crickets is adequate all year round, that harvesting does not affect the ecosystem and that crickets are safe for consumers is to produce farm-reared crickets rather than harvesting wild crickets (Hanboonsong *et al.*, 2013). Thus, farmed crickets may play a vital role in replacing the loss of wild crickets in nature. In addition, more valuable species can be produced.

Cricket farming - an option

Advantages of cricket farming

Crickets have a short production cycle across three development stages, egg, nymph and adult. The length of the production cycle depends on the cricket species, temperature and rearing purposes. The most common cricket species farmed include field cricket, two-spotted cricket and house cricket (FAO, 2013). In current cricket farming systems for food, the production cycle from hatching to harvest is 35, 45, and 55 days for two-spotted cricket, field cricket and house cricket, respectively (CelAgrid, 2016).

Crickets seem to convert feed very efficiently into weight gain. To gain 1 kg of body weight, the feed requirement for chickens is 1.8 kg (PoultryHub, 2018), for pork 5 kg and for beef 10 kg (Smil, 2002), while crickets may require as little as 1.7 kg (Collavo *et al*., 2005). The edible proportion of crickets is estimated to be 80% (100% in Cambodia), compared with 55% for poultry and pigs and 40% for cattle (van Huis, 2013; Nakagaki & DeFoliart, 1991). In addition, cricket production has been suggested to require less water consumption than conventional livestock production (van Huis *et al.,* 2013). However, water consumption by crickets needs further investigation.

Cricket production requires a small area and crickets can be reared at high density, with an approximate average density range (depending on cricket species) of 6000-12,000 crickets/m^2 and with total fresh yield of 7.3 kg/m^2 (recalculated from Hanboonsong *et al.*, 2013). A study on crowding has shown that a crawl space of 2.5 cm^2 per cricket is needed to minimize mortality and maximize growth rate from hatch (Patton, 1978). That is equivalent to approximately 4000 crickets/m^2 (calculation based on Patton, 1978). Cricket species produce less greenhouse gas emissions (g/kg mass gain) than conventional livestock such as pigs and cattle (Oonincx *et al.*, 2010). In addition, crickets are reported to carry less risk of transmitting infections to humans, livestock and wildlife, compared with mammals and birds (van Huis *et al.*, 2013).

Furthermore, cricket production may be cost- and area-effective compared with production of other animals, which is very important for poor and landless people (Table 1). The output in terms of both edible (kg) and money per m^2 is higher or comparable to that in conventional livestock production.

Table 1. *Calculated productivity and earnings (weight (W), number (No.), production cycle (Cy), edible proportion (E), meat price (M) and facility earnings (F)) of some livestock systems*

Livestock/Species	**W[1 a]**	**W[2]**	**No.[3 b]**	**Cy[4 c]**	**E[5 d]**	**W[6]**	**M[7 e]**	**F[8]**
Meat chicken	1.5	25.0	16.7	5	55	33	4.5	149
Meat duck	1.3	14.4	11.1	5	55	19	3.3	62
Fattening pig, 85 kg	85	77.3	0.9	6	55	85	3.5	298
Beef cattle, 100 kg*	100	66.7	0.7	18	40	18	8.8	156
House cricket [#]	0.0006	15.4	25592	1.8	100	101	2.0	201
Field cricket [#]	0.0009	15.4	17062	1.5	100	123	3.0	369
Two-spotted cricket [#]	0.0013	15.4	11812	1.2	100	158	4.0	632

[1]kg/head, [2]kg/m^2, [3]head/m^2, [4]months per production cycle, [5] %, [6]kg/year, [7]\$/kg, [8]\$/m^2 per year.

[#] Calculation in cubic meter (m^3).

[a, b] Based on data obtained in current observations in Cambodia (2017) and own data for chicken and crickets in practical trial (2017), adapted data from Animal Welfare Approved Standards (2014) for minimum indoor bedded lying area of meat chicken/duck, fattening pig and beef cattle, data on mass rearing cricket farming from Hanboonsong *et al*., (2013) for cricket.

[c] Own data from practical trial for chicken and cricket and current observation for meat duck, fattening pig and beef cattle (2017).

[d] Based on data from van Huis (2013) for meat chicken/duck, pig and beef cattle, data from current observation (2017) in Cambodia for crickets.

[e] Meat price obtained in shop investigation on 18/1/2018, Preho market, Takhmao city, Kandal province, Cambodia ($US1=4000 Riels).

*Beef cattle refers to local red cattle from the uplands of Cambodia, 100 kg is a common slaughter weight for this breed.

Availability of native cricket species

When new cricket farms are started there are always questions about where to get crickets, how to get crickets and which cricket species is best for the farm. Cricket farming in Cambodia was introduced around early 2010 and scaled up rapidly both to small and medium-sized farms. The trend seems to have peaked in 2012, based on local media and internet sources. There are no data available on the actual cricket species first used in farming in Cambodia. However, local media reports that house cricket, two-spotted cricket and field cricket were imported from Thailand and Vietnam in the form of eggs in plastic bowls. Local cricket farmers sold these eggs to other farmers who were interested in cricket farming and shared information about rearing techniques. Currently cricket farming is in decline due to some challenges, including lack of supply of quality cricket stocks and the high costs of feed. Today, most cricket farms feed chicken feed concentrate, which is costly.

The most common cricket species found in natural habitats in Cambodia and consumed by local people are shown in Table 2. These species can all be harvested by light traps at night. The species that can be kept in captivity and intensive farming systems are house cricket, two-spotted cricket and field cricket, while attempts to rear the mole cricket and short-tail cricket in captivity have generally failed. There is currently limited knowledge on how to rear the latter two species, both of which exhibit behavior that differs from that of the other species mentioned above, *i.e.* they live belowground. Such specific demands might be costly to overcome in a domestication process aiming for intensive livestock production.

Table 2. *Common cricket species harvested from the wild in Cambodia*

Khmer	**English**	**Scientific Name**	**Order/Family**
ចង្រិតខ្មៅ/ដែក	Two-spotted cricket	*Gryllus bimaculatus*	Orthopetra/Grylliadae
ចង្រិតស/ទឹកឃ្មុំ	House cricket	*Acheta domesticus?*	Orthopetra/Grylliadae
ចង្រិតក្រហម	Field cricket	*Teleogryllus testaceus*	Orthopetra/Grylliadae
មេមុល	Mole cricket	*Gryllotalpa africana*	Orthopetra/Gryllotalpidae
ចង្រិតដូង	Short-tail cricket	*Brachytrupes portensosus*	Orthopetra/Grylliadae

Source: Münke (2012) and scientific names by Khon Khaen University (2012).

Availability of local feed sources

Crickets (omnivores) can eat a wide range of food sources from animals and plants. Crickets can be fed seeds, nuts, alfalfa and wheat bran, as well as vegetables, tubers and fruits (http://cricket-breeding.com/what-to-feed-crickets). It is known that the house cricket (*Acheta domesticus*) can survive and grow well on a variety of organic materials (Makkar *et al.*, 2014) including forage diets (Tyree *et al.*, 1974). A study by Megido *et al.* (2016) indicates that *T. testaceus* might have similar capacity. Accordingly, agricultural and food industry by-products and plants considered weeds are potential cheap and sustainable feed sources. In Cambodia, agricultural by-products such as rice bran and cassava tops or leaves are potential feed resources. By-products from the food industry, such as spent grain and residues from mungbean sprout production, may also have potential.

Some weeds are already harvested and used as feed for livestock. For example, *Commelina benghalensis* and *Boerhavia diffusa* can be used to feed pigs (Chikwanha *et al.*, 2007) and *Synedrela nodiflora* and *Amaranthus spinosus* have been used as feed and food (Adjibode *et al.*, 2015). Moreover, *Cleome* *viscosa* has been suggested to be useful as a feed for ruminants (Akinfemi & Mako, 2012). All the plant species discussed above are present in Cambodia and can be trapped and/or acquired free of charge.

Agricultural and food industry by-products like rice bran from rice mills, spent grain from breweries, residues from mungbean sprout production in small and medium food enterprises, cassava leaves/tops from cassava plantations and water spinach floating on urban wastewater treatment ponds in Phnom Penh are great potential local available sources of animal feeds that can contribute to a low-cost diet for crickets.

Rice bran

Rice (*Oryza sativa*) bran is a by-product from the rice milling process and about 1 million tons may be produced annually in Cambodia (calculated from the amount of rice paddy (MAFF, 2013) and assuming 12% rice bran content in rice (Kahlon, 2009). The CP content of rice bran is 12-13% (fresh matter (FM) basis) (Rosniyana *et al*., 2007). Rice bran therefore has huge potential to become an important feed for animals like crickets.

Brewery spent grain

There are four large commercial breweries in Cambodia: Cambodia Brewery, Khmer Brewery, Cambrew Brewery and Kingdom Brewery. Based on data from Mussatto *et al.* (2006), the estimated amount of spent grain produced in Cambodia is approximately 177,800 tons per year. Spent grain may be used as an alternative feed for poultry, pigs and fish (Mussatto *et al.*, 2006b), and for dairy cattle (Belibasakis & Tsirgogianni, 1996). Spent grain consists of 23.6% CP, 10.6% fat, 14.5% crude fiber (CF), 50.9% neutral detergent fiber (NDF), 17.5% acid detergent fiber (ADF), 0.29% calcium (Ca), 0.48% phosphorus (P) and 7.3 MJ/kg metabolizable energy (ME) (Dong & Ogle, 2003).

Mungbean sprout residues

Anecdotal evidence indicates that 100 kg of dry mungbean (*Vigna radiata*) grain (14% DM) can generate 100-200 kg fresh by-products from mungbean sprout production. In Cambodia, the volume of mungbean sprout residue produced is approximately 59,220 tons annually (calculated from mungbean grain production data in MAFF, 2016). The chemical composition of mungbean sprout residues shows large variation and depends on the processing procedure and mechanism. The residues consist of 45.4% seed coat (hull), 51.2% sprout and 3.4% small hard grains that did not germinate during sprouting (own data).

Cassava leaves

There are two common varieties of cassava, bitter cassava (*Manihot esculenta*) and sweet cassava (*Manihot dulcis*). In Cambodia, farmers plant sweet cassava along the fence around their houses and on small-scale farms for food purposes (roots and plant tops), while bitter cassava is cultivated on large-scale farms and the root is harvested and sold as food and feed. According to

MAFF (2017), cassava plantations in Cambodia cover more than 500,000 ha. Farmers harvest only the root for sale, the stems are collected for replanting and the leaves and tops of the plants are left on the farms as a residue. Unlike other plants, cassava is of limited use as animal feed due to its content of hydrogen cyanide (HCN) and tannins. However, fresh cassava leaves can be fed at 38% of DM intake to fattening pigs (equivalent to 6.28 mg HCN intake per kg live weight) without toxicity (Chhay Ty, 2012). Fresh cassava leaves contain 23.9-34.7% CP, 1.3-15.6% crude fat, 5.0-8.1 % ash, 9.7-16.5% CF and 32-33.5 NDF (Bui Huy, 2000), and have a HCN content in the range 475-507 mg/kg (Chhay Ty *et al.*, 2009). Cassava leaves and cassava tops are different fractions. Cassava tops comprise young leaves, petioles, soft stems and shoots. Thus, tops and leaves may have different properties with respect to nutritive values.

Water spinach

Water spinach (*Ipomoea aquatica*) originates from India, Southeast Asia and South China. It is an aquatic plant which grows either floating on water or in marshy soil (FAO, 2009). In Cambodia, water spinach is cultivated and also grows naturally in ponds, lakes and channels. It can be a weed in rice fields in the wet season and is fast-growing. Water spinach can be harvested within 3-4 weeks after plantation (Khov *et al,* 2007), with an annual yield of 5 tons/ha (Khov *et al.*, 2006). In water spinach cultivated on the ground with proper irrigation, the fresh yield can be high, up to 15 tons/ha within a month (Bunyet, 2003). Water spinach is eaten as a vegetable (Edwards, 1980), and around 40% of all vegetable consumption in Phnom Penh is of water spinach (Khov *et al.*, 2007). It is also used as a feed for conventional livestock such as poultry and pigs. Previous studies have shown that water spinach has potential as a basal diet for rabbits (Samkol *et al.*, 2006; Honthong *et al.*, 2004; Vo Thi, 2004). Water spinach contains about 24-34% CP, 2.7-3.9% lipid, ~13% ash and 10.2-12.7% CF (FAO, 2009).

Broad-leaved and grass/sedge weeds

According to Martin and Chanthy, (2007), "weeds can be a problem in the production of upland crops because they reduce yield by competing for resources essential for growth, such as water, nutrients and light. Weeds can also make it difficult to harvest the crop, and seeds of some species can contaminate the grain and reduce its quality and price". The weeds in Cambodia can be classified into two groups, broadleaved and grass/sedge weeds (Martin & Chanthy, 2007). The main broadleaved weeds are *Cleome viscosa*, *Cleome rutidosperma*, *Commelina benghalensis*, *Synedrela nodiflora, Alternanthera sessilis*, *Amaranthus spinosus* and *Boerhavia diffusa.* The main grass/sedge weeds are *Cynodon dactylon, Sorghum halepense, Brachiaria reptans, Echinochloa colona, Brachiaria mutica*, *Eleusine indica* and *Dactyloctenium aegyptium*. Weeds may be of use as animal feed. Grass/sedge can be grazed or cut and carried to feed to livestock (Martin & Chanthy, 2007), while *C. benghalensis* and *B. diffusa* can be fed to pigs (Chikwanha *et al.*, 2007). *Synedrela nodiflora* and *A. spinosus* can be used as feed and food (Adjibode *et al.*, 2015) and *C. viscosa* has been suggested as a useful feed for ruminants (Akinfemi & Mako, 2012). The chemical composition and nutrient content of both broadleaved and grass/sedge weeds show huge variation, depending on botanical stage. Thus, time of harvest is crucial for the chemical composition.

Cricket rearing techniques for small-scale farming

Cricket species

The house cricket (white cricket or bee cricket, in Khmer) is small, weighing approximately 0.6 g/cricket at harvest (unpublished data by P. Miech). This species seems to be tolerant to infectious disease except for one densovirus (van Huis *et al.*, 2013). It also seems able to cope with polluted living conditions and can tolerate the daily temperature and humidity fluctuations in rearing pens (high survival and low mortality rate) (own data, 2016). House crickets can be reared in high-density conditions in massive rearing and intensive farming and have a long period of egg laying. However, they are sold at lower price ($2/kg fresh weight) than other farmed cricket species, and sometimes there is no demand at all on the local market. Field cricket (red cricket or monkey cricket, in Khmer) is of medium size (larger than house cricket but smaller than two-spotted cricket), approximately 0.9 g/cricket at harvest. This species seems moderately tolerant to infectious disease and polluted living conditions, with high survival and low mortality rate (unpublished data by P. Miech). The price is higher than for house cricket ($3/kg fresh weight) and many cricket farmers report successful rearing attempts (unpublished data by P. Miech). Two-spotted cricket (black cricket or iron cricket in Khmer) is large at harvest (1.3 g/cricket). This cricket species is most valued by consumers and the price is high ($4/kg fresh weight). However, this species is not tolerant to disease and captive living conditions, with high mortality (up to 100%), the causes of which remain unknown. Most of the farmers who have attempted to rear two-spotted cricket have reported failure and have given up after 1-2 years. For short-tail cricket, failure in rearing is also common, but there is one report on successful domestication (Meas, 2012). Thus, the best option for smallholder farmers in Cambodia who want to sell crickets is currently to rear field crickets.

Feed, feeding and watering

Crickets are omnivores and can eat a wide range of food sources from animal and plant materials. The feed sources used by Cambodian cricket farmers vary depending on availability and cost. The feed used is often from the conventional livestock feed industry, *e.g.* chicken feed, duck feed, pig feed, fish/frog feed or sometimes also a combination of these (unpublished data by P. Miech). The techniques used for feeding are also different; some farmers grind the feed, some feed it wet and some feed it dry. There are no data on the effects of these feed and feeding choices. Some famers may add fresh forage or plants as extra feed. Drinking water can be put in a tray with small pebbles or a sponge for crickets

<20 days. For older crickets there is no need to use stones or sponges as long as the water in the trays is less than 1 cm deep.

Crude protein and amino acid requirements

In cricket farming in Thailand, farmers use chicken feed with 14 or 21% protein content, with the higher value for crickets < 20 days old and a mix between 14 and 21% for crickets >20 days old (Hanboonsong *et al.,* 2013). Smallholder farmers in Cambodia use duck feed (18.5% CP, the product '7770' for laying ducks from De Heus Co., Phnom Penh) at the first day of hatching to harvest (45 days). When fed a diet with low protein (14%), low ME (9.1 MJ/kg) and high fiber content (20%), house crickets are reported to perform remarkably well (Nakagaki & DeFoliart, 1991).

Pens and equipment

Pens for field crickets can be of any size and shape and made from any materials (concrete, wood, plastic). A common form used in commercial rearing of both field cricket and two-spotted cricket is a concrete block pen measuring 1.2 m x 2.4 m x 0.6 m. In these pens, yield of 20-35 kg fresh crickets per generation (*i.e*. 45-day cycle) can be expected (Hanboonsong *et al.,* 2013). However, movable pens made of plywood or plastic containers can be a smart design and suitable for small farms. To increase the surface area and to enable the crickets' natural hiding behavior, pens are often equipped with cardboard egg trays. However, egg trays are costly and sugar palm leaves and old rice bags can be used instead. This reduces the cost by one-third compared with using egg trays. The lids of round (30 cm diameter, 1.5 cm height) 20-L paint cans are commonly used as feed and water trays in small-scale cricket farming in Cambodia. Nylon netting (1 mm mesh) is used to cover the pens, to stop crickets from escaping and to protect them from gnats, parasite wasps and other predators. Around the base of the pens, a water-filled channel is generally constructed, to protect the crickets from ants.

Hygiene and prevention

Cricket pens are not easy to clean due to the crowded conditions and cleaning can also cause a disturbance if the pens are cleaned very often. Once weekly is common practice, to remove some residues and waste from the floor. There are usually no feed residues on the floor of the pens when a proper amount of feed is used. However, open water trays generally need to be cleaned every other day, because of contamination with feed and faeces resulting in microbial growth. Cleaning outside the pens is also important, to keep away animals that can enter the pens and eat crickets (lizards, spiders and frogs). A major cleaning event is generally done after each harvest. All wastes and residues are removed from the pens and the pen structures are washed with water and allowed to dry before new stocks are introduced. Egg trays are cleaned by strong shaking or striking to remove wastes and residues attached to the surfaces and then sun-dried before re-use.

Egg collection and incubation

The best time to collect eggs is when female crickets are observed to have a large abdomen and male crickets sing a loud and uniform song. Bowls filled to two-thirds with wet biochar (80% moisture) as a substrate for laying the eggs are often used. The appropriate number of bowls placed in each pen depends on the cricket density. In general, 48 hours of egg laying are allowed to achieve high hatching rate in a short time. The egg bowls are then removed from the pens, put in plastic bags and the bags are tied to maintain a high moisture content in the bowls during the hatching process. Incubation period depends on the cricket species and incubator temperature. At 30 ºC and 70% relative humidity outside and around the incubation egg bowls, two-spotted crickets hatch after 6-10 days, while field cricket and house cricket hatch after 10-14 days. During the hatching period, the farmer needs to check for movements of very tiny nymphs and then remove the bowls from the plastic bags and place them in pens for the development process. The bowls should be placed on their side for 3-5 days to help the nymphs leave the bowl.

Harvest and marketing

Reared crickets can be harvested at any time depending on the purpose. If the purpose is to sell them as food, crickets reared on chicken feed have to be transferred to a diet of vegetables, fruit or tubers for the last a couple of days in the rearing period. The general opinion is that this improves the taste of the crickets (Hanboonsong *et al.,* 2013). It is also common practice to prevent egg laying within the 12-24 hours before harvest, in order to obtain a better flavor. Harvesting is generally performed by shaking the egg trays gently in pairs so that the crickets drop into a collection container. Live crickets are then separated from dead crickets and feed and faecal residues and placed in a fresh container, where they are soaked and killed in fresh water. Clean, dead crickets are placed in baskets with 3 mm holes to allow water to drain for 1-2 hours, before packaging and transport to the market.

Challenges of current cricket farming

Despite the possible huge benefits and advantages of cricket rearing, there are challenges that are currently causing a decline in production. The greatest challenge is the economic conditions for cricket farming. High production costs limit the possibilities to scale up farming for poor rural households. Establishment of large-scale cricket farms requires considerable capital as well as manpower skills, whereas crickets harvested from the wild require low investments (traps), are easy to trap and give a quick return (Münke, 2012). The existing cricket farming system is susceptible to feed price increases, since it uses commercial high-protein chicken feed. Purchased feed costs generally represent 68-77% of the total costs of cricket production (Hanboonsong *et al.,* 2013). In addition, unstable prices for crickets on the market can cause failure of cricket farms, especially small farms. Cricket farming in Cambodia is currently competing with wild crickets harvested from common property resources. Fresh farmed crickets can reduce the price of wild-harvested crickets from $4 to $2 per kg during the peak season.

At present, cricket farming is considered an informal food system industry in Cambodia. There is no legislation or regulation. Therefore, the sector is poorly monitored and supported in terms of technical matters, prevention of diseases, unstable marketing and prices, and lack of policy on insects as food and feed. Most cricket farmers in Cambodia receive basic information on cricket farming techniques through egg suppliers and there is a lack of more science-based knowledge among cricket producers. Due to lack of knowledge on *e.g.* management, nutrition, pest/disease control and farm hygiene, outbreaks of infectious diseases are common in high-density populations (Hanboonsong *et al.*, 2013). Replacement of the brood stock is also a challenge. The quality of eggs imported from Thailand and/or Vietnam may be low, high mortality appears common and inbreeding is suspected.

Cricket farming is still less attractive than other livestock systems for large investors in Cambodia but is much more relevant for smallholders and land-poor farmers in rural areas who farm small plots of land and have limited financial capital. In addition, although cricket farming is an alternative for producing food and feed with great potential, the sector has still not attracted wide interest from researchers in Cambodia. Therefore, this thesis sought to increase knowledge on cheap resources that can be used as feed for (mainly) field crickets and on the nutrient value of field crickets in pigs, as a model of humans, and in poultry.

5 Materials and Methods

Location, experimental seasons and temperature/humidity

All studies were carried out on the organic research farm at the Centre for Livestock and Agriculture Development (CelAgrid), about 25 km south of Phnom Penh City in Prashtheat Village, Sangkat Rolous, Dangkor District, Phnom Penh, Cambodia. The location has a tropical monsoon climate with two seasons, dry season (October-April) and wet season (May-September). The study reported in Paper I, on weeds and agricultural and food industry by-products as feed for crickets, was conducted in the middle of the wet season (October-December 2014), when annual weeds were growing and available to collect. The work in Paper II, on the amino acid and mineral profiles of field crickets, was performed in Sweden during autumn 2017. The study on pigs (Paper III) was conducted in May-October 2016, in the peak harvesting season of wild crickets (May-July), since wild crickets were used to produce the meals for the study. The study on chickens (Paper IV) was conducted in March-April 2017 and used the leg residues from the pig study. All trials in Cambodia (Papers I-III) were conducted under a 12-h light regime in an outdoor climate, in facilities covered with a roof. Temperature and humidity in cages or pens were recorded four times a day (06:00, 12:00, 18:00 and 00:00). Sprinklers were mounted on the top of the zinc roof of the housing to counteract the huge variations in ambient temperature and humidity, especially at 12:00 during the strong sunshine in March and April. The temperature and humidity were, respectively, 29.0±1.7 °C and 69±5% in Paper I, 28.8±0.7 °C and 70.3±3% in Paper III, and 28±1.0 °C and 70 ± 3% in Paper IV over the period of each experiment.

Capture and rearing

The wild field crickets were caught live by hand in July 2014 from light traps at night. About 2000 individuals per location were collected from two different locations. The crickets were nursed in four concrete block pens. Egg trays were placed inside the pens as hiding places and the pens were covered with nylon net to prevent crickets escaping and predators entering. A water channel was placed around the base of the pens to protect the crickets from ants. The crickets had free access to chicken feed, but cassava plants and other plants were also offered. Feed was placed on feed trays. Water was available for 24 hours/day in water trays. The crickets laid eggs after 2 weeks. The eggs were collected for 48 h in plastic bowls filled with wet biochar. The eggs hatched within 8-13 days. The same rearing procedure was applied for the next generation.

Experimental design, preparation and management

Paper I. Nymphs of the second generation (n=1040, 7 days old, 0.013±0.008 g/cricket) were allocated to 13 dietary treatments in a completely randomized design (4 replicates/diet). Cricket survival and growth on 12 experimental diets consisting of weeds, agricultural and food by-products were studied for 70 days, with chicken feed used as a control. Each diet was fed to six replicate groups consisting of 20 individuals per replicate at day 0. Each replicate was kept in a plastic cage (60 m x 42 m x 31 cm) with a lid covered by a net to allow air exchange. No other materials were present in the cages, to make sure that crickets had nothing to eat besides the feed offered. Water was offered on plates and was available 24 hours/day. Feed was offered every two days. Cages were cleaned every seventh day.

Paper II. Field crickets used in this study were obtained from Paper I. However, only crickets fed chicken feed and the best diets (highest weight and survival, similar or better than on chicken feed) were included, *i.e*. crickets fed cassava tops and the weeds *C. rutidosperma* and *S. nodiflora*. All crickets still alive after 70 days on each diet were euthanized by drowning, dried (at 60 °C for 48 hours) and stored at room temperature from 28 December 2014 to 23 October 2015. Thereafter they were kept at -20 °C for 7 months until further analyses in the laboratory. To increase the volume of the samples, crickets from all replicates from one diet were pooled.

Paper III. Castrated male piglets of the local multi blood-strain breed (n=21, 30-45 days, 13.0±0.3 kg/pigs) were allocated to three diets (7 piglets/treatment in a fixed block design), containing whole cricket meal (WC), cricket body meal (BC) or fish meal (control) as the main protein source. Diets were fed *ad libitum*. Prior to the study, the piglets were vaccinated and also dewormed. All piglets were kept in an outdoor shelter, in individual bamboo/wooden stalls with a slatted floor. Piglets and cages were washed and cleaned every day. Piglets were adapted to the feeds and the cages for 5 days before the experiment. Plastic film was fitted under the slatted floor to collect the urine. Each stall was equipped with a nipple connected to a water container and piglets had water access 24 hours/day.

Paper IV. Native Cambodian chickens (n=180, 40-50 days, 414±2 g/chicken, 50:50-male:female) were allocated to one of three dietary treatments, containing whole cricket meal (WC), cricket leg meal (LC) or fish meal (control) as the main protein source, with six replicates per treatment, in a completely randomized design. Each replicate was kept in a pen made of bamboo frame and nylon net. The concrete floor of each pen was divided into two sections, one where rice husks were spread and one with coarse sand. Prior to the study, the chickens were vaccinated and fed a commercial chicken feed in coarse form.

Feed preparation

Paper I. Chicken feed, rice bran and spent grain were bought from local markets. Residues from mungbean sprout production were provided free by a local mungbean sprout producer. Chicken feed and rice bran were stored in a dry area during the experimental period. The fresh spent grain and mungbean sprout residues were collected from the producer every two weeks and stored in a refrigerator at 10 °C. Water spinach was harvested in the fields around the station and cassava tops were harvested from a farm about 7 km from CelAgrid. Both were collected once a week. The weed *S. nodiflora* (grows in upland fields and wastelands at mountainous areas) was collected once every two weeks by a farmer and sent by car to the experimental site. The weeds *A. sessilis, A. spinosus, C. benghalensis, C. rutidosperma, C. viscosa* and *B. diffusa* were collected mainly on roadsides and fallow land around CelAgrid once a week. All fresh plants were prepared using same procedure throughout the experimental period, *i.e*. they were cleaned/washed, trimmed/bunched and stored at 10 °C.

Paper III. Three iso-nitrogenous diets were made based on maize meal and rice bran. Whole cricket meal (WC), cricket body meal (BC) or fish meal was used as the main protein source in the diet. Fish meal, maize meal, rice bran, salt and shell were purchased from commercial animal feed retailers. Crickets for production of cricket meal were purchased from a middleman, who bought them from cricket trap owners. The freshly caught crickets were sent by car to the experimental site and stored at 8 °C overnight. The crickets were then roasted on a zinc plate over a fire until the moisture content was about 20%, followed by sun-drying for 2-3 h to reduce the moisture content to 10%. The body and legs were separated by squeezing the dry crickets gently by hand and then sifting them through a plastic basket. The proportion of body and leg mass was 62 and 38%, respectively. Dry whole crickets and cricket bodies were milled to meal.

Paper IV. The same procedure as in Paper III was performed, except that one of the three diets included cricket leg meal (LC) as the main protein source. Thus, the three diets were whole cricket meal (WC), LC and control (fish meal).

Feeds and feeding

Paper I. Chicken feed was used as the control, since this is the feed commonly used on commercial cricket rearing farms (Megido *et al*., 2016; Lundy & Parrella, 2014; Hanboonsong *et al*., 2013; Nakagaki & DeFoliart, 1991). Agricultural and food industry by-products were rice bran (RiceB), cassava tops (CassaT), water spinach (WateS) and spent grain (SpenG), residue from mungbean sprout (Mung). The weeds were *A. sessilis, A. spinosus, C. benghalensis, Cleome rutidosperma, C. viscosa, B. diffusa and S. nodiflora*. The crickets were offered feed in the morning every second day. Chicken feed, RiceB, SpenG and Mung were offered *ad libitum* on small plastic plates. CassaT, WateS and weeds were also offered *ad libitum*, but were laid in the bottom of the cages across the water plates. The amount of feed offered and rejected was recorded every two days.

Paper III. The diets were offered at 8:00, 12:00 and 17:00 h. Feed allowance (approximately 5% of body weight) was semi-*ad libitum* but below maximal feed intake so that refusals were minimized. Amounts of feed offered and refusals were weighed every day and analyzed for DM content.

Paper IV. Chickens were trained to eat a fine form of meal for 20 days prior to the study. Feed was offered *ad libitum* during the training period. Feed intake was measured during the last three days of the training period to get an indication of lowest percentage feed intake per kg body weight, in order to minimize feed refusals during the experiment. The amount of feed offered was 95% of the amount consumed by the group with the lowest feed intake. Thus, feed was offered at a level of 9.6% body weight. The chickens were fed twice a day (at 9:00 and 17:00) and had free access to drinking water. Feed offered and refusals were weighed every day and analyzed for DM content.

Survival and weight measurement

Paper I and *Paper IV*. Once a week, the number of live crickets/chickens was counted and weighed in each replicate. Survival rate in a replicate was calculated by dividing the number of live crickets/chickens at each time point by the initial number of crickets/chickens (n=20/n=10). Mean individual cricket/chicken weight for each replicate was calculated by dividing replicate weight by the number of live crickets/chickens at the time of measurement.

Paper I. Individual weight gain was calculated by subtracting the mean individual weight of crickets at the start (day 0) from the mean individual weight at day 49 or day 70. Feed intake per week was calculated on a DM basis by subtracting the weekly refusals from the feed offered and dividing this by the mean number of crickets alive at the time of measurement and the number of crickets in the previous week. Feed conversion ratio (FCR) was calculated by dividing the total mean individual feed consumption by the total mean individual weight gain.

Paper III. Body weight was measured every 5 days. Total collection of faeces and urine was performed during the last 5 days of the experiment.

Total collection, digestibility and feed conversion

Paper III. Feed refusals, urine and faeces were collected every day and 5% was sampled and stored at -4 °C until the end of the collection period. Representative samples of the collected feed, refusals and faeces from each treatment were mixed thoroughly by hand and then homogenized in a mill (0.5 mm) for analysis of DM, ash, organic matter (OM), CP, crude fat (CFat) and crude fiber (CF). Urine was collected in a plastic bucket, to which sulphuric acid (20 mL concentrated 98% H_2SO_4) was added to maintain pH below 4.0. Digestibility was calculated as intake minus faecal losses divided by intake. Feed conversion ratio was calculated as feed intake (DM) divided by weight gain from start to 25 days. Nitrogen (N) retention was calculated as N intake minus losses in urine and faeces. Retention (%) of N digested was calculated as: (N retention (g/day) / N digested (g/day)) × 100.

Chemical analysis of feeds and refusals

The DM content in feed and refusals was measured by oven drying at 70 °C for 48 h or until constant weight was achieved. Analysis of nitrogen (N) was performed by the Kjeldahl digestion method, and ash was determined by combustion in a muffle furnace at 550 °C for 4.5 h (AOAC, 1990). The CP content was calculated as N×6.25 and OM as DM less ash. CFat and CF were analyzed according to AOAC (1990) and metabolizable energy (MJ/kg DM) was calculated as: $ME = 4{,}168 - 12.3 \times ash + 1.4 \times CP + 4.1 \times CFat - 6.1 \times CF$ (Noblet & Perez, 1993). Amino acid analyses (Paper II) were performed by Eurofins Food and Feed AB using Method/ref SS-EN ISO 13903:2005. The method analyses free (synthetic and natural) and total (peptide-bound and free) amino acids using an amino acid analyzer or HPLC equipment. Analysis of mineral concentrations was carried out by high-resolution inductively coupled plasma mass spectrometry (HR-ICPMS) after microwave-assisted acid digestion (ALS Global Umeå, Sweden).

Sample preparation

Paper II. The material was prepared for chemical analysis by removing wings and the large pair of hind legs (femur, tibia and tarsus) from the body by hand. These were analyzed separately from the bodies. The length of the insect was recorded for each individual. The weight of the entire insect, the body, the wings and the hind legs was recorded with an electronic scale (model Sartorius 1702 MP8; capacity: 202 g, readability: 0.1 mg; Uppsala, Sweden). The material was milled into a fine powder before being sent off for chemical analysis.

Statistical analysis

All data were analyzed using ANOVA after evaluation of normal distribution. The model used was $y_{ijk} = \mu + a_i + \beta_j + \tau_k + \beta_{\tau jk} + e_{ijk}$, where μ is general mean value, a_i is the random effect of individual/replicate i, β_j is the effect of treatment j, τ_k is the effect of time point k, $\beta_{\tau jk}$ is an interaction between treatment and time, allowing for different time developments for the different treatments, and e_{ijk} is the residual. The residuals were assumed to be correlated within individual according to an autoregressive AR (1) model. For data where time was not a factor, the following model was used: $y_{ij} = \mu + a_i + \beta_j + e_{ij}$. A Tukey test was used for comparison. The level of statistical significance was set to $P<0.05$. Data are presented as least square means ± standard error. All statistical analyses were performed in R software (version 3.3.3, R Development Core Team 2015; https://www.r-project.org).

Additional studies

At the start of the work described in this thesis, an attempt was made to rear two-spotted cricket in captivity. Wild individuals were collected in the same way as described for the field cricket and the second generation was used for two feed experiments, hereafter called 'Additional study A' and 'Additional study B.

Additional study A was conducted at the same time as the work in Paper I and used the same feeds and the same procedures. This experiment was considered a failure due to very high mortality rate of the two-spotted crickets. For instance, only one cricket fed water spinach survived until 56 days. Two crickets fed chicken feed, six crickets fed rice bran and one cricket fed *C. rutidosperma* survived until 42 days. Thus, only the results on survival and growth up to 35 days are presented in this thesis.

In Additional Study B, both cricket species (field cricket and two-spotted cricket) were fed grass/sedge weeds including *C. dactylon, S. halepense, B. reptans, E. colona, B. mutica, E. indica* and *D. aegyptium*. The experiment was conducted in May-October 2016, again using the same procedures as in Paper I. This experiment was also considered a failure due to high mortality rate of both cricket species and only results until 35 days are presented.

6 Summary of the results

Paper I: Growth and survival of reared Cambodian field crickets fed weeds and agricultural and food industry by-products

The survival of field crickets fed weeds and agricultural and food industry by-products did not differ from that of crickets fed chicken feed, except that crickets fed *B. diffusa* from day 14 to day 49 and *A. sessilis* from day 35 to day 49 had lower survival (*P*<0.05) than crickets fed chicken feed. The weight of crickets fed CassaT and *C. rutidosperma* was not different from that of crickets fed chicken feed throughout the study (*P*>0.05). However, from day 35 to day 70, the weight of crickets fed *C. rutidosperma,* Mung, SpenG and *S. nodiflora* was lower than that of crickets fed chicken feed. At day 63, the weight of crickets fed *C. rutidosperma* and *S. nodiflora* did not differ from that of crickets fed chicken feed. The weight of crickets fed *C. benghalensis*, RiceB and WateS from day 28 and until day 70 was lower (*P*<0.05) than that of crickets fed chicken feed, except at day 63, when *C. benghalensis* was not different from chicken feed. In crickets fed *A. sessilis, A. spinosus and B. diffusa* from day 21 to day 70, body weight was lower (*P*<0.05) than in crickets fed chicken feed in the same period.

Feed consumption at day 49 was lower for WateS and all weeds except *C. rutidosperma* than for chicken feed. Feed conversion ratio was not different at day 42, except for RiceB, for which FCR was higher than for chicken feed. Feed consumption and FCR were not different after 70 days, but weight gain was lower than with chicken feed for all test feeds except *C. rutidosperma* and CassaT. Weight gain showed no correlation to content of crude protein, ash, crude fiber, crude fat and gross energy in feed.

Paper II: Amino acid and mineral composition

The chemical composition of whole cricket and of the body and leg fractions of field crickets caught from the wild are shown in Table 3. These preliminary analyses were performed in laboratory facilities at CelAgrid.

Table 3. *Chemical composition (dry matter (DM) basis) of whole cricket, body (legs removed) and legs of field cricket collected from the wild*

Field cricket	% in DM				ME (MJ/kg) in DM
	CP	Ash	CF	Cfat	
Whole	62.5	4.9	10.4	13.5	18.1
Body (legs removed)	60.3	4.5	9.4	17.9	19.0
Leg	67.5	5.2	13.2	8.2	16.8

CP = crude protein, CF = crude fibre, CFat = crude fat, ME = metabolisable energy

Methionine content was higher in crickets fed cassava tops than in crickets fed chicken feed, *C. rutidosperma* or *S. nodiflora* (Table 4).

Table 4. *Amino acid content (g/kg DM) and iron (Fe, mg/kg DM) of Cambodian field crickets fed chicken feed, cassava tops and the weeds Cleome rutidosperma and Synedrella nodiflora. SE = standard error*

AA/Minerals	Chicken	CassaT	*C. rutido*	*S. nodif*	SE	P
Cysteine + Cystine	2.8	4.3	3.9	3.0	0.03	0.09
Lysine	15	26	22	18	0.2	0.08
Methionine	5.5[a]	9.0[b]	8.0[a]	5.4[a]	0.1	0.04
Fe (mg/kg)	241	163	415	200	77	0.28

Means with different superscripts within rows are significantly different (P<0.05) [1]Values <0.3 are below the detection limit and in statistical analysis zero was used.

Paper III: Apparent faecal digestibility and nitrogen retention

There was a difference in live weight between piglets on the different diets from day 10, with piglets fed BC and WC being heavier than piglets fed the control diet. From day 10, rolling five-day weight gain was also higher for BC and WC piglets than for the control piglets except for days 20-25, when there was no difference between WC and control.

Dry matter, OM, CP, CF, CFat and ME intake of the piglets was significantly higher for the WC and BC diets than for the control. Feed conversion ratio was lower for the WC and BC diets than for the control. Dry matter digestibility was highest in pigs fed diet WC, while ash, CF and CFat digestibility were higher in piglets fed BC and WC than in control piglets. There were no differences in OM digestibility. Nitrogen retention was higher in piglets fed diets WC and BC compared with the control. Faecal N excretion was higher for diets BC and WC than for the control diet, but there was no difference in urinary excretion between diets.

Paper IV: Growth performance of native chicken

The body weight of native chickens fed diet WC was higher than in the control from day 7 to day 35, while the body weight of chickens fed LC was higher from day 7 to day 21 but lower from day 21 to day 35, compared with the control. Daily weight gain and daily feed intake of chickens fed WC was higher, and LC was lower, than for the control diet. Feed conversion ratio of chickens fed WC was lower, and LC was higher, compared with the control.

Additional study A: Growth and survival of two-spotted crickets fed weeds and agricultural and food industry by-products

The survival of two-spotted crickets fed spent grain, cassava tops, *C. rutidosperma*, rice bran, *C. viscosa* and residue from mungbean sprout production did not differ from that of crickets fed chicken feed from day 7 to day 35 (Figure 3). However, two-spotted crickets fed *C. benghalensis*, water spinach, *A. sessilis*, *A. spinosus* and *B. diffusa* showed significantly lower(P<0.001) survival from day 14 to day 21 compared with those fed chicken feed.

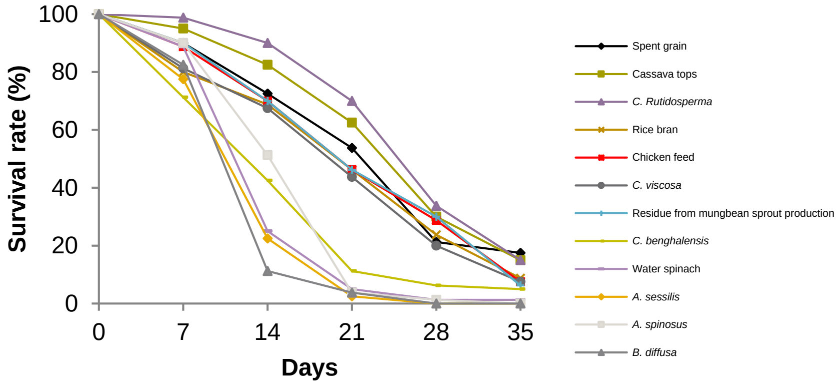

Figure 3. Survival rate of two-spotted crickets fed chicken feed (control), spent grain, cassava tops, *Cleome rutidosperma*, rice bran, *Cleome viscosa*, and residues from mungbean sprout production, *Commelina benghalensis*, water spinach, *Alternanthera sessilis*, *Amaranthus spinosus* and *Boerhavia diffusa* for 35 days.

The weight of two-spotted crickets fed cassava tops and residues from mungbean sprout production did not differ from that of crickets fed chicken feed at day 21 (Figure 4), but crickets fed the other feeds weighed significantly less (P<0.001) than crickets fed chicken feed both at day 21 and day 28 (P<0.01). At day 35, however, there were no weight differences (Figure 4).

Feed intake and feed conversion ratio of two-spotted crickets could not be calculated due to insufficient weight gain data.

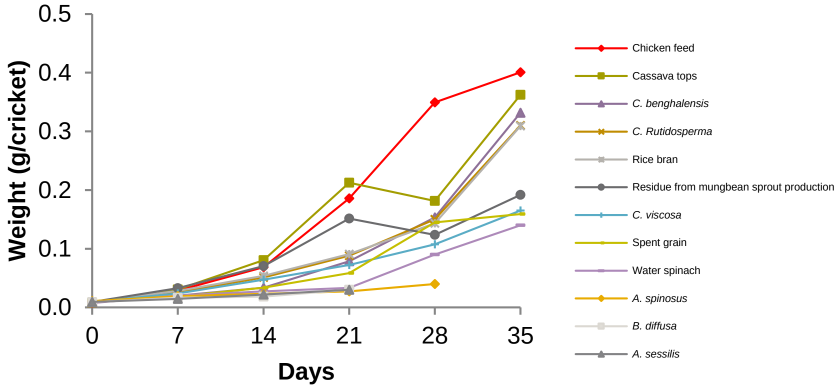

Figure 4. Weight of two-spotted cricket fed chicken feed (control), spent grain, cassava tops, *Cleome rutidosperma*, rice bran, *Cleome viscosa*, and residue from mungbean sprout production, *Commelina benghalensis*, water spinach, *Alternanthera sessilis*, *Amaranthus spinosus* and *Boerhavia diffusa* for 35 days.

Additional study B: Growth and survival of reared field crickets and two-spotted crickets fed grass/sedge weeds

Field crickets and two-spotted crickets fed grass/sedge weeds showed significantly lower (P<0.001) survival compared with those fed chicken feed (Figures 5 and 6). Field crickets fed *S. halepense, B. reptans E. colona, E. indica* and *D. aegyptium* all died after 7 days (Figure 5). Two-spotted crickets fed *S. halepense, B. reptans* and *E. colona* all died after 7 days and two-spotted crickets fed *E. indica* and *D. aegyptium* all died after 21 days (Figure 6).

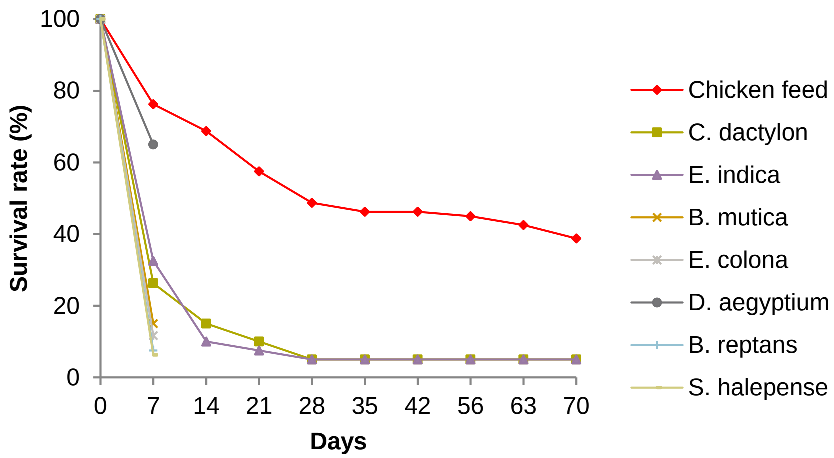

Figure 5. Survival rate of field crickets fed chicken feed (control), *Cynodon dactylon*, *Sorghum halepense*, *Brachiaria reptans*, *Echinochloa colona*, *Brachiaria mutica*, *Eleusine indica* and *Dactyloctenium aegyptium* for 35 days.

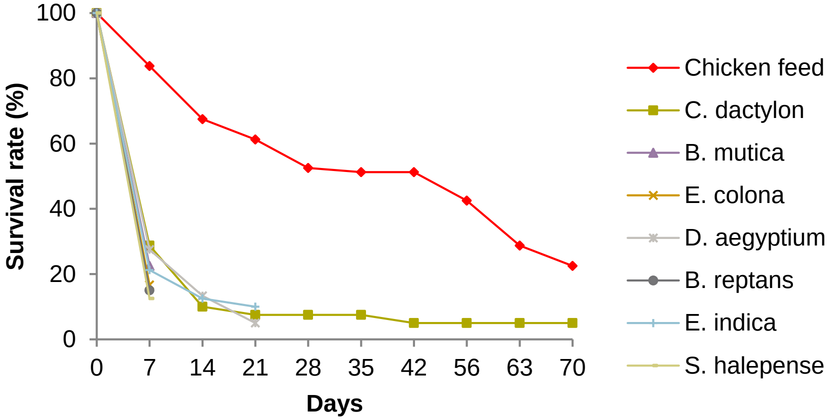

Figure 6. Survival rate of two-spotted crickets fed chicken feed (control), *Cynodon dactylon*, *Sorghum halepense*, *Brachiaria reptans*, *Echinochloa colona*, *Brachiaria mutica*, *Eleusine indica* and *Dactyloctenium aegyptium* for 35 days.

The weight of field crickets fed *C. dactylon* and *E. indica* was significantly lower ($P<0.001$) from day 21 to day 35, compared with crickets fed chicken feed (Figure 7).

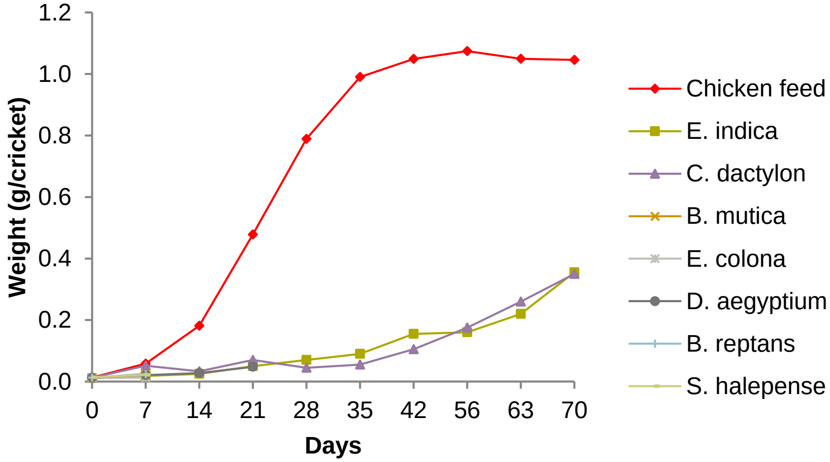

Figure 7. Weight of field crickets fed chicken feed (control), *Cynodon dactylon*, *Sorghum halepense*, *Brachiaria reptans*, *Echinochloa colona*, *Brachiaria mutica*, *Eleusine indica* and *Dactyloctenium aegyptium* for 35 days.

The weight of two-spotted crickets fed *C. dactylon* and *E. indica* was significantly lower than for crickets fed chicken feed (P<0.001) from day 14 to day 35 of the feeding trial (Figure 8).

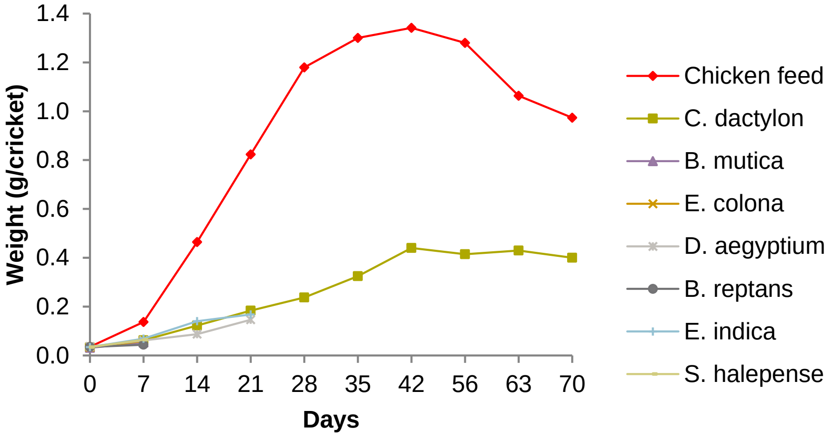

Figure 8. Weight of two-spotted crickets fed chicken feed (control), *Cynodon dactylon*, *Sorghum halepense*, *Brachiaria reptans*, *Echinochloa colona*, *Brachiaria mutica*, *Eleusine indica* and *Dactyloctenium aegyptium* for 35 days.

7 General discussion

Cricket rearing in smallholder farming systems can play an important role in producing cheap, high quality food for family consumption and in improving nutritional status in rural communities, especially in women and children under 5 years. In addition, smallholder cricket farming can be a means to supply the local market with high-quality food in a sustainable manner. Thus, cricket farming can be a way to alleviate malnutrition in the short term and in the long term.

Major outcomes of this thesis

This thesis showed that rearing of native Cambodian field crickets is possible using simple means. Pens and housing can be simple and this species can be fed on some weeds and agricultural and food industry by-products for their survival and growth. Cassava tops and *C. rutidosperma* showed great potential as feed and the other weeds and agricultural by-products also showed potential, except *A. sessilis, A. spinosus* and *B. diffusa*. The results presented in this thesis also indicate that the amino acid content in crickets can be altered with diet and that cassava tops seem to be a good feed resource with respect to amino acid quality in crickets. This is supported by the high nitrogen retention observed in the study on pigs. Field crickets are likely to be very beneficial as a feedstuff for monogastric animals and most likely also for humans, due to their composition and nutritional value. Removal of legs was found not to facilitate or improve the digestibility values and nitrogen retention in piglets. Thus, in order to minimize food residues, crickets should not be peeled if processed into meal. However, if there is a need to peel, residues such as cricket leg meal can be used as feedstuff for native Cambodian chickens without any major adverse effects, thereby replacing fish meal.

Cricket rearing as smallholder farming

Like other livestock animals, quality stockbreeding is required for cricket rearing to be successful. Inbreeding depression is a potential challenge to farmers in Cambodia. Inbreeding has a significant effect on the survival of sand cricket (*Gryllus firmus*) (Roff, 1998) and bush cricket (*Teleogryllus commodus*) (Drayton *et al.*, 2010). Similar problems can be expected to occur in field cricket and two-spotted cricket when reared under confined conditions, because they are members of the same family, the Gryllidae. The populations currently kept at CelAgrid, as a result of the present thesis, can be an important resource in helping cricket farmers avoid inbreeding depression. Inbreeding occurs because farmers rear crickets for the demands of the market and keep reproducing from same population for many generation in small pens, whereas the population produced at CelAgrid has a recorded history and there are no more than 3-4 generations in a rotation of nursery pens. However, the greatest challenge for smallholder farmers is that they depend mainly on feedstuffs from feed companies and are therefore vulnerable to feed price increases (Hanboonsong *et al.*, 2013). The data provided in this thesis on weeds and by-products are therefore of great importance.

Crickets may practise cannibalism when starving (McFarlane, 1962). Cannibalism may also be a result of the feeds offered not being nutritionally balanced or not preferred. The rapid increase in weight observed in this thesis for two-spotted crickets fed cassava tops, *C. benghalensis* and *C. rutidosperma* between days 28-35 may be a result of cannibalism and/or the moulting stage. According to Clifford and Woodring (1990), house crickets double their weight in the last instar, and a similar trend can be expected for two-spotted crickets. On the other hand, the rapid increase in weight may be caused by the non-linear increase that occurs in female crickets in the egg development stage, while male crickets show no such increase.

Nutritional values of field cricket

Crude protein level in whole cricket meal was within the range reported previously for crickets (58-78%) (Moreki *et al.*, 2012; Barker *et al.*, 1998; Finke *et al.*, 1989; Nakagaki *et al.*, 1987). In this thesis the CP content in whole field cricket meal was 62.5%, which was higher than that reported for house crickets *(Brachytrupes portentosus)* (Abdul Razak *et al.*, 2012), Mormon cricket (DeFoliart *et al.*, 1982) and two-spotted cricket (Wang *et al.*, 2005). The whole cricket meal diet also resulted in greater weight in piglets than fish meal, which is considered an excellent protein source for growing pigs (Kim & Easter, 2001). The CP level in cricket meals used in this thesis was similar to that in fish meal and soy protein concentrate (NRC, 2012).

The fat content of crickets in this thesis was in the upper part of the range reported for fat (3-22% fat) (Moreki *et al.*, 2012; Wang *et al.*, 2005; Finke *et al.*, 1989; Nakagaki *et al.*, 1987; DeFoliart *et al.*, 1982). The amino acid profile of crickets compares favourably with that of fish meal on a CP basis (Wang et al., 2005; Finke, 2002). According to WHO (2007), the daily protein requirement of a child under 5 years is 0.66 g/kg for maintenance plus 0.20 g/kg for growth, of which the amino acid requirement is 45 and 22 mg/kg for lysine and sulphur-containing amino acids, respectively. In this thesis, the content of lysine and methionine + cysteine/cystine (sulphur-containing amino acid) was high in field crickets fed cassava tops and most suitable for utilization as a protein food sources for humans, especially for children under 5 years of age.

Chitin and chitosan in cricket

Chitin is found mainly in the exoskeleton of crickets. Whole field crickets and house crickets contain 7-9% chitin on a DM basis (Finke, 2007; Wang *et al.*, 2005). Chitin is considered nearly indigestible in most monogastric mammals (Ngoan & Lindberg, 2001; Ngoan *et al.*, 2000). However, Hossain and Blair (2007) report apparent digestibility values of chitin protein of around 50% and a true digestibility value of chitin protein of 87% in broilers. In this thesis, diets containing whole cricket meal and cricket body meal (legs removed) resulted in no differences in growth in pigs. Therefore, the chitin content in those diets might not necessarily have impaired nutrient utilization. Inclusion of some chitin in the diet has been shown to improve growth in broilers (Austin *et al.*, 1981).

Cricket as food

Diets containing whole cricket meal and cricket body meal both seemed palatable to the growing piglets. Since peeling causes a substantial loss of valuable nutrients, whole cricket meal should therefore be used as food. It has been suggested that the edible part of crickets comprises 80%, while in conventional livestock the edible proportion is in the range 40-60% (van Huis *et al.*, 2013). This thesis showed that crickets (as meal) are edible up to 100% without causing detrimental effects on digestive response, nutrient uptake or growth in growing pigs (Paper III). This can be considered an important step in understanding the nutritional value of crickets for humans. The gastrointestinal tract and digestive system of pigs is similar to that of humans and it has been argued that pigs are an interesting and relevant model for human responses to different diets (Nielsen *et al.*, 2014). However, faecal digestibility in pigs may be quite different from that in humans, due to more extensive hindgut fermentation in the pig (Hendriks *et al.*, 2012). Thus, the digestibility of CP in cricket meal diets (whole cricket and cricket body meal) may not be directly applicable to humans. Nevertheless, the results on nitrogen retention (which is mainly a result of amino acid retention) presented in this thesis suggest that cricket meal is definitely an interesting food opportunity.

The low investment required to create a cricket rearing system offers great possibilities to produce high-quality food, especially for countries like Cambodia where 25% of the population is food-deprived (USAID, 2017) and 36% of children suffer from stunted growth (Unicef, 2017, data from 2015). However, the content of indigestible and gut-filling chitin in crickets is a concern (DeFoliart, 1991), as this may affect the possible potential realistic daily supply of protein from crickets. Paper III showed that it is possible to include high amounts of cricket meal in the diet of monogastric animals. However, DeFoliart (1991) questioned whether insects can meet the need for calorie-dense foods in deprived children, since “so much of the insect body would be indigestible but fill up the infant’s stomach”, and therefore limit total daily intake. Calculations based on the protein requirements for children proposed by WHO (2007) indicate that a child weighing 10 kg (1-2 years) should consume 11.6 g of protein and 45 mg of lysine to meet a safe level of the daily requirements. This lysine requirement corresponds to 1.7 g DM (approx. 6.9 g of fresh crickets assuming 25% DM content) of crickets fed cassava tops (Paper II), which corresponds to 12 crickets harvested at 42 days of age (Paper I). To meet the safe level of daily protein intake (11.6 g), the child would have to consume 22 g DM of crickets fed cassava tops, corresponding to 87 g of fresh crickets. This would be similar to the size of a hamburger. Thus, the ‘gut fill’ concern seems not to be a major issue as regards diets including field crickets. Moreover, according to hearsay and observations from indigenous populations, children can thrive on diets including insects (DeFoliart, 1991).

Cricket as feed option

Besides the use as human food, crickets can also be farmed for feeding reptiles/lizards, fish, birds and other mammals. However, use for poultry production is very rare due to high prices and the high competition with human food. The major intention in the chicken study (Paper IV) was to use the leg meal, the by-product of the peeling process, for chicken feed. However, whole cricket meal was also included in that study for evaluation of the nutritional value of field crickets as feed for chicken. Some earlier studies have examined use of cricket meal as a source of protein in the diet of chickens. For example, Abdul Razak *et al.* (2012) studied broiler chickens fed house cricket meal in the diet and found higher weight gain than in chickens fed soybean meal, but slightly lower weight gain than in chickens fed fish meal. Similar findings were made in this thesis, where chickens fed whole cricket meal were heavier than chickens fed the control, while chickens fed cricket leg meal were lighter than control chickens from day 21, but there was no difference compared with chickens fed whole cricket meal. Thus, cricket meal has substantial nutritional value as a feed ingredient for poultry. Wang *et al.* (2005) studied three levels of field cricket meal to replace fish meal in maize-soybean meal-based diets. They found that cricket meal could replace up to 15% of the control diet without any adverse effects on broiler weight gain, feed intake or gain:feed ratio from 8 to 20 d post-hatching. Other studies have used house cricket meal (Nakagaki *et al.,* 1987) and Mormon cricket meal (Finke *et al.,* 1985; Defoliar *et al.,* 1982) to replace soybean meal, and have found that these cricket species are a good source of protein and comparable to soybean meal for young broiler chickens. Those studies used fish meal or soybean meal as control diets, because they are commonly used as the major protein ingredient in animal diets.

The price of whole crickets is currently high in Cambodia, because crickets are used as human food. Thus, crickets are not suitable to use as an ingredient in animal feed formulations. However, the price of cricket leg meal is not known, since this product is not on the market in Cambodia. If the crickets are going to be milled then it is not relevant to peel them (Paper III). Peeling will just be an extra cost and will not improve the nutrient quality. The EFSA recommendation on removal of cricket legs applies to crickets consumed without being milled (EFSA, 2015). If Cambodia starts to export crickets to Europe (*i.e*. crickets that must be peeled), then cricket legs can be a by-product of the peeling process and available for use as food or feed in Cambodia.

The results in Paper IV could have been affected by the breed of chickens used and may not be fully applicable to other breeds. In Cambodian chickens, most parts of the gastrointestinal tract are greater than in broilers and this may result in greater feed intake and DM digestibility than in broilers (Khieu Borin *et al.*, 2006). In conclusion, cricket meal is a good feedstuff for poultry and can replace fish meal or soybean meal. Therefore, cricket meal, especially cricket leg meal, can be a viable option when there is a scarcity of fish meal or soybean meal, provided it is available in sufficient volumes.

8 Conclusions and Implications

Conclusions

Based on the findings presented in this thesis, it can be concluded that:

- Native Cambodian field cricket can be domesticated and reared in captivity in concrete block pens or plastic cages and fed weeds and agricultural and food industry by-products
- Two-spotted cricket is not promising for rearing in captivity, due to high mortality for unknown reasons
- The best cricket species for rearing in smallholder farming is field cricket, due to reasonable survival and weight gain and moderate demand and price in local market places.
- Agricultural and food industry by-products, especially cassava tops and *Cleome rutidosperma* (purple cleome), have great potential to be used as feed for field cricket
- *Alternanthera sessilis* (sessile joyweed), *Amaranthus spinosus* (spiny amaranth), *Boerhavia diffusa* (punarnava) and grass/sedge weeds are not recommended for used as fresh feed for field cricket and two-spotted cricket
- Field cricket meal is a useful feed source for monogastric animals and most likely a valuable food for humans
- High nitrogen retention and digestibility of cricket meal are promising with respect to counteracting protein deficiency, while high ash digestibility and iron content may indicate potential to reduce mineral deficiency (in the rural poor in Cambodia)
- Removal of cricket legs does not facilitate or improve the digestive response and nutritive value. Therefore, there is no need to peel crickets intended to be processed to meal, in order to minimize waste
- Cricket leg meal, a by-product from cricket peeling (residue from human food processing), has potential for use as feed for native chickens.

The results in this thesis have already been used in practice by smallholder farmers in rural communities in Pursat, Mondulkiri and Prah Hear provinces, Cambodia. The key results have been compiled into a handbook (or so-called technology improvement package, TIP) written in Khmer, and used as a training module for advisory workers/field facilitators and also directly by cricket farmers. A total of 10 participants from the Association for Saving Poor Farmers from Development (ASPFD), government staff from Ministry of Environment (MoE) of Cambodia and 90 households have been trained in small-scale cricket rearing. Two projects have been conducted; i) 'Improve smallholder nutrition', funded by the Orskov foundation and implemented by ASPFD, and ii) 'Enhancing climate change resilience of rural communities living in protected areas in Cambodia', funded by the United Nations Environment Program (UNEP) and implemented by MoE. A total of 1080 egg bowls of third-generation field crickets generated in this project were distributed to farmers who participated in the two projects, in order to improve household nutrition by diversifying daily food items. The results of the thesis will also be applied in similar projects launched in mid-2018 in communities with 30 direct beneficiaries in Prey Veng, Takeo and Kampong Speu provinces, Cambodia.

9 Future research

During the work in this thesis, several new research questions were identified. For example, during the planning process it was decided not to put any materials such cardboard egg trays inside the cricket cages to avoid the risk of crickets eating them, which would have interfered with the results. The mortality rate in the studies was quite high and the possibility that this was due to some stress in the crickets as a result of lack of hiding places cannot be excluded. It was observed that the crickets tried to hide under plates of water and feed, or under feed (plants). In future research, the need for hiding places should be investigated and inedible material should be used as a hiding material under experimental conditions.

Fresh cassava leaves and tops contain cyanide and tannins, which limits the nutritive value for cattle, pig and poultry. This thesis showed that crickets can survive and grow on cassava tops, even better than on chicken feed. Further studies are needed to investigate other aspects of the reproductive cycle, such as sperm quality, number of eggs, hatching rate and life span of the crickets. Another interesting research task would be to study the digestive system in detail and determine why crickets can grow on 100% cassava tops, while other animals cannot.

Survival rate was more than twice as high for field cricket than for two-spotted cricket reared under the same conditions (*i.e.* feeds, pens). This confirmed the general opinion that two-spotted cricket is difficult to rear. In future research, the reasons behind the high mortality need to be investigated.

Food safety is a concern, both with crickets harvested from the wild and with farmed crickets. Crickets may be contaminated with germs (virus, bacterial) and/or parasites and act as an agent or induce a zoonosis. Future research should investigate whether crickets can be contaminated or transmit zoonotic diseases.

It would also be useful to conduct a survey on cricket farming techniques and economics, both in existing and non-existing (failure) cricket farms. What people think about crickets as food at present and in the future is unclear and therefore a survey on consumer preferences and attitudes to cricket eating (entomophagy) is of interest.

As a follow-up to the study on apparent faecal digestibility and nitrogen retention in piglets fed whole and peeled Cambodian field cricket meal, it would be interesting to see the development of the gastrointestinal tract and carcass properties. In future research, the length and weight of the digestive tract should be measured, and carcass properties should be determined, including deposition of fat and meat, color of fat *etc*.

The study of growth performance in native chicken fed whole meal and leg meal of Cambodian field cricket was conducted during a short period and omitted some useful indicators, such as gastrointestinal tract development and carcass proportion and color. In future research, all chickens should be slaughtered at the end of the experiment to measure digestive tract length and weight, and to inspect carcass properties.

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