



Experimental feeding studies with crickets and locusts on the use of feed mixtures composed of storable feed materials commonly used in livestock production

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ABSTRACT

Insects such as the Mediterranean field cricket, *Gryllus bimaculatus* and the Desert locust, *Schistocerca gregaria*, are emerging as potential sources of human food and feed for livestock. High nutritive value and efficient feed conversion make them attractive for commercial production as novel livestock, but these properties strongly vary with the insects' diet. Current mass-rearing protocols are based on fresh, non-storable feed materials. This requires constant supply and makes the systems sensitive to fluctuations regarding nutritional quality and safety. Hence there is a need to find storable, readily available feeds. Therefore, experimental diets were composed from the five different feed materials, corn meal; soya extracts; dried cowpea leave; corn stover; dried carrot; and a vitamin supplement. The diets were formulated such as to vary in macro-nutrient and vitamin content. Effects of these diets on consumption, biomass gain, feed conversion and nutritional composition of the insects were assessed. Crickets were fed a combination of corn meal and cowpea leave ("Starch") and a combination of soya extract and corn stover ("Protein/fiber"). Locusts were fed "Starch" and "Protein/fiber" and variations of these, supplemented with vitamins and carrot ("Protein/fiber/carrot" and "Starch/carrot"). Additionally, a combination of cowpea leaves and soya extract, supplemented with vitamins and carrot ("Protein/carrot"), was tested on locusts. Crickets grew and gained biomass relatively well when fed "Starch" but struggled with digestion of the high-fiber diet "Protein/fiber". Locusts fed "Starch" and "Protein/fiber" failed to gain biomass or performed poorly. When supplementing these diets with vitamins and carrot, locusts on "Starch/carrot" failed to grow while locusts fed "Protein/fiber/carrot" could gain biomass and showed excellent feed conversion. Accordingly, vitamin supplementation of this diet had a positive impact. Locusts fed "Protein/carrot" showed the best results regarding feeding efficiency and production figures. All tested feed materials were accepted by the insects. Therefore, these feed materials may replace fresh feed materials and may thus improve efficiency and safety of insect production systems. Indeed, certain diet formulations

Abbreviations: ADF, acidic detergent fiber expressed inclusive of residual ash; CP, crude protein; DM, dry matter; ECD, efficiency of conversion of apparently digested feed into body matter; ECI, efficiency of conversion of ingested feed into body matter; ECP, efficiency of conversion of feed protein into body protein; EE, ether extract; NDF, neutral detergent fiber assayed without a heat stable amylase and expressed inclusive of residual ash; NFC, non-fiber carbohydrates; SEM, standard error of mean

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revealed nutritional limitations. They might serve as model diets to derive nutritional requirements of insects e.g. for protein, amino acids or vitamins.

1. Introduction

Due to a fast-growing world population, demand for food, especially high-quality protein is increasing drastically (Henchion et al., 2017). Insects as food and feed, in particular as a new and sustainable source of high-quality protein, are increasingly attracting the attention to science. There are several reasons why insects are very interesting as possible sources of human food or feed for livestock production.

About 2000 edible insect species are well documented so far (Jongema, 2015). Although the nutritional value of insects varies according to species, either fresh or processed, they are generally comparable to beef and fish in many nutritive aspects. The protein contents are ranging from 350 to 700 g/kg DM and the protein quality in edible insects is considered outstanding, as indicated by rich amino acid profiles and digestibility characteristics. They have adequate amounts of all essential amino acids, especially lysine, threonine and methionine, which are limited in cereal- and legume-based diets (Thompson, 1973; Finke et al., 1989; Bernard et al., 1997; Bukkens, 1997; Ramos-Elorduy et al., 1997; Barker et al., 1998; Wang et al., 2004; Ayieko et al., 2010; Fontaneto et al., 2011; Kinyuru et al., 2013; Rumpold and Schlüter, 2013; Makkar et al., 2014). They are high in energy as well as rich in other nutrients such as fat; unsaturated fatty acids (Thompson, 1973; Fontaneto et al., 2011); important mineral salts and vitamins (Thompson, 1973; Finke et al., 1989; Bernard et al., 1997; Bukkens, 1997; Ramos-Elorduy et al., 1997; Barker et al., 1998; Wang et al., 2004; Ayieko et al., 2010; Fontaneto et al., 2011; Kinyuru et al., 2013; Rumpold and Schlüter, 2013; Makkar et al., 2014). Feed conversion efficiency of insects is also considered as being higher compared to conventional livestock such as pigs, cattle and chicken (Nakagaki and Defoliart, 1991; Van Huis, 2013; Ooninx et al., 2015a). Correspondingly, there is some indication that insect production could be lower in greenhouse gas emissions than production of conventional livestock (Ooninx et al., 2010; Halloran et al., 2016; Smetana et al., 2016). Additionally, some insects may nourish on substrates of inferior quality, such as compost or manure (El Boushy, 1991; Sheppard et al., 1994).

However, it is very important to be aware that most of these characteristics, such as the nutritional composition of the insects and their efficiency of feed conversion, strongly depend on the composition of the diet consumed by the insects (Simpson and Raubenheimer, 2001; Ooninx and Van der Poel, 2011; Ooninx et al., 2015a). Only through efficient production systems, including optimized feeds, the high potential insects bear as new and innovative source of food and feed can be fully exploited. Current systems for large scale production of insects such as crickets and locusts mostly depend on fresh feed materials including wheat seedlings, fresh grass or other crop residues (Ochieng-Odero et al., 1994). Constant supply of these materials with uniform nutritional profiles is required which makes such production systems more vulnerable and inflexible. Also, in view of pathogenic pressure within the production system as well as quality and food/feed safety of the end-product, frequent import of materials of varying quality and often uncontrolled origin into the production system is a critical challenge faced.

Thus, objective of this study is to identify feed materials which are nutritious to crickets and locusts to ensure high productivity and at the same time make certain that a long shelf-life and storability of the products is provided. Through that, production of these insects can be uncoupled from fresh and hence perishable feeds. This is furthermore critical regarding production systems in temperate climates where availability of fresh feed materials is subject to seasonal cycles. Storable materials would simplify the feed management and enables better monitoring and quality assurance of feed import to reduce risks of quality fluctuations or imported pathogens.

2. Materials and methods

The study comprised two feeding experiments with crickets (*Gryllus bimaculatus*) and locusts (*Schistocerca gregaria*), respectively referred to as experiment 1 and 2. All experiments were conducted at facilities of the Animal Rearing and Containment Unit at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya, between September 2016 and February 2017. The study aimed to identify feed materials which are nutritious to crickets and locusts and, at the same time, storable to uncouple production of these insects from fresh and hence perishable feeds. The study on crickets was considered a pilot effort, which was linked to further differentiated feeding regimens with locusts.

2.1. Feed materials

Five different feed materials were examined in the study: soya extracts, corn meal, dried cowpea leave, corn stover and dried carrot powder supplemented with vitamins. Sufficient amounts of these materials were obtained to ensure availability of each substrate from one batch with the same source and constant quality for the entire duration of the study (3 months). Therefore, corn meal and soya extracts were obtained from an ICIPE contractor. Fresh cowpea leaves were obtained from the local market. Excess soil and sand were removed roughly and the plant material sundried for four days. The after-harvest corn stover was collected from a field of ICIPE in Kitale, Kenya and brought to Nairobi, where it was sundried for another four days. The dry cowpea leaves and corn stover were ground with a Munch hammer mill (Munch, Wuppertal, Germany) and sieved (2 mm mesh size), to ensure a suitable particle

size for incorporation into insect diets. Carrots obtained from a local supplier were chopped into smaller sizes and placed on plastic sheets under moving dry air ($28 \pm 2^\circ\text{C}$) at ambient temperature for 2 days using a Xpelair® heater (WH30, 3KW Wall Fan Heater, United Kingdom). Thereafter, the semi-dried carrots were oven-dried at 60°C for 72 h and then ground with a costumery blender to obtain carrot powder. All substrates were stored individually in tightly closed sterilized plastic containers in a clean cupboard at room temperature until they were needed for the respective experiments.

The nutritional compositions of the different feed materials are shown in Table 1. Through combination of the substrates, five different diets were created. The portion of each feed material in the respective diet formulations and their estimated nutritional compositions are shown in Table 2. Focus of the dietary formulation was, to create diets with different nutritional emphasis that vary in protein, starch and fiber content. In the first formulation, corn meal is a starch-rich feed component, while cowpea leaves provide protein and fiber. This diet is more starch accentuated and therefore in this study referred to as “Starch” diet. In the second formulation, soybean extracts are a rich source of high-quality protein, while corn stover provides high levels of fiber. Hence, this diet is more protein and fiber accentuated and therefore it is referred to as “Protein/fiber” diet. Two more diets were composed the same way as the “Starch” and “Protein/fiber” diets, but additionally supplemented with 20% carrot powder and vitamins to create two feed formulations referred to as “Starch/carrot” and “Protein/fiber/carrot”. Also, a fifth feed formulation was created containing soya extracts, cowpea leaf powder, carrot powder and vitamin supplementation. As this formulation is very rich in protein it will be referred to as “Protein/carrot”.

Each portion (10 g) of the vitamin supplemented diets was enriched with 0.3 ml of a liquid multivitamin preparation (Mixavit®, Julphar Gulf Pharmaceutical Industries, United Arab Emirates). According to the manufacturer, 1 ml of the multivitamin preparation contains the following active ingredients: Vitamine A: 8332 I.U.; Vitamine D: 667 I.U.; Vitamine B1: 2.5 mg; Vitamine B2: 2 mg; Vitamine C: 83 mg; Nicotinamide: 17 mg.

To obtain the nutritional composition of the feed materials, each one was sampled at the beginning of the study, around the half way stage and at the end. The samples were filled into airtight ziploc bags and stored at -20°C until further analysis. For determination of the nutritional composition, equal parts of these subsamples of each substrate were pooled to each one sample and then transferred for analysis.

2.2. Experimental setup

The crickets (*Gryllus bimaculatus*) and desert locusts (*Schistocerca gregaria*) were kept according to the regular ICIPE rearing protocols for the respective specie in a room with constant climate conditions (average of $30.0 \pm 1.7^\circ\text{C}$ temperature, $36.8 \pm 6.6\%$ relative humidity and 12:12 photoperiod) (Launois-Luong et al., 1991).

Experiment 1: Crickets were held in plastic containers ($48 \times 32 \times 35$ cm, L x W x H) equipped with six cardboard egg trays to provide shelter and cotton wool balls soaked in water for hydration. Cricket eggs obtained from the Animal Rearing and Containment Unit, held on wet cotton wool were introduced to each cage and allowed to hatch. Onset of the feeding study (day 1) was marked for each cage individually in the moment when hatching in the respective cage began. Upon hatching, about 250–350 nymphs were present in each cage.

Twelve cages were randomly placed on shelves in the experimental room. Crickets in 6 cages were fed “Starch”, while in another 6 cages they were fed “Protein/fiber”. Insects in cages on the same diet were referred to as one treatment and insects of each cage were referred to as an experimental replicate.

Experiment 2: Desert Locusts were held in metal cages, each one equipped with a 60 W lightbulb with wire mesh fence wrapped around it, to provide another source of heat for the locusts to gather around. Temperature inside the cage was around 36°C during the light phase and corresponded with ambient temperature during the dark phase (Launois-Luong et al., 1991). Water soaked cotton wool balls were placed in each cage to provide hydration (Ochieng-Odero et al., 1994). Locust eggs obtained from the Animal Rearing and Containment Unit at ICIPE, contained in sand filled metal tubes were introduced to each cage and allowed to hatch. Onset of the feeding study (day 1) was marked for each cage individually when hatching in the respective cage began. About 220–330 nymphs were present in each cage upon hatching. Twenty-one cages were randomly placed on shelves in the experimental room. As with in

Table 1
Nutritional composition of feed materials used in the study.

Nutritional composition	Feed material				
	Corn meal	Soya extract	Cowpea leave	Corn stover	Carrot powder
DM (g/kg)	905	925	898	915	865
Ash (g/kg DM)	25	57	160	49	69
CP (g/kg DM)	97	447	239	41	73
EE (g/kg DM)	36	184	25	08	12
NDF (g/kg DM)	233	204	322	787	116
ADF (g/kg DM)	50	167	267	519	139
NFC (g/kg DM)	514	34	151	30	596

DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber, NFC: non-fiber carbohydrates (DM – Ash – CP – EE – NDF).

Table 2

Portion of each feed material in respective diet formulations and estimated nutritional composition of the formulated experimental diets.

Feed component	Diet type				
	“Starch”	“Protein/fiber	“Starch/carrot”	“Protein/fiber/ carrot”	“Protein/carrot”
Corn meal	500 g/kg		400 g/kg		
Soya extracts		500 g/kg		400 g/kg	400 g/kg
Cowpea leave	500 g/kg		400 g/kg		400 g/kg
Corn stover		500 g/kg		400 g/kg	
Carrot			200 g/kg	200 g/kg	200 g/kg
Nutritional composition					
DM (g/kg)	902	920	894	909	903
Ash (g/kg DM)	93	53	88	56	101
CP (g/kg DM)	168	244	149	210	289
EE (g/kg DM)	30	96	27	79	86
NDF (g/kg DM)	278	496	246	420	234
ADF (g/kg DM)	158	343	154	302	202
NFC (g/kg DM)	333	32	385	145	193

DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber, NFC: non-fiber carbohydrates (DM – Ash – CP – EE – NDF).

the previous Cricket Study, insects in 6 cages were fed the “Starch” diet, while in another 6 cages they were fed “Protein/fiber”. Further, locusts in respectively 3 cages were fed “Starch/carrot”, “Protein/fiber/carrot” and “Protein/carrot” diet.

The total numbers of insects per cage were subject to high variation due to an unpredictable number of eggs.

2.3. Feeding

For feeding, 10 g of the formulated diet was weighed into a Petri dish (10 cm diameter). Then, depending on the respective water holding capacity of the different feed materials, 20 to 40 ml of water was added to the diet to form clumps. The Petri dish prevented excessive spreading of feed material in the cage. Freshly prepared diets were introduced daily into each cage. Beginning with one petri dish containing 10 g of diet, the amount of daily served petri dishes was increased, depending on the rate of consumption, to up to 6 petri dishes containing 10 g each. The petri dishes were replaced daily and the leftovers from each petri dish collected. Leftover feed was dried in an air-circulation oven at 135 °C for 3 h to obtain the dry matter weight. Daily consumption was calculated by subtracting weight of the feed leftovers from that of the initial serving.

2.4. Individual liveweight

As soon as the insects reached a reasonable size and body mass (Crickets: 28 days from hatching; Locusts: 12 days from hatching), the individual live weight of the insects was recorded in intervals of 3 days until maturity. Therefore, 30 insects of each cage were randomly picked and weighed. The crickets and locusts were returned to their respective cages after every measurement exercise was completed.

2.5. Harvest of insects

Final sampling of the crickets took place after 45 days from hatching, when they could be expected to have reached a reasonable body size without having reached sexual maturity; and of the locusts after 29 days from hatching or as soon as the last molting from 5th instar to pre-adult stage occurred. Therefore, all living insects of each cage were caught, filled in airtight ziploc bags, weighed and killed by freezing at –20 °C. Thereafter, the dead insects were filled into brown paper bags and dried in an air-circulation oven at 60 °C for 72 h to reach constant sample weight. Then the samples were removed from the oven and allowed to reach room temperature in ambient air. Subsequently, the insects were weighed, counted and ground into fine powder (particle size below 2 mm) using a laboratory blender. The insect powder from each replicate was kept separately in airtight ziploc bags and the samples were stored at –20 °C for further analysis.

2.6. Fecal wastes

After sampling the living insects, all the remaining waste was removed from the cages, filled in airtight ziploc bags and weighed. Dry matter content of the samples was obtained by drying at 135 °C in an air-circulation oven for 2 h. In case of the fecal waste samples of locusts, the sand imported into the cages as hatching substrate prior to the onset of the study was estimated by analysis of acid insoluble ash (method 920.08) content according to the methodology described by the Association of Official Analytical Chemists AOAC (1990), and deducted from total sample dry matter.

2.7. Analysis of nutritional composition

The nutritional analysis of individual feeding substrates, feed mixtures, and insects was performed according to the methodology described by AOAC (1990). Measured parameters included: content of dry matter (DM, method 930.15), ash and organic matter (method 942.05), ether extract (EE; method 920.29), and crude protein (CP; method 984.13). Neutral detergent fiber (NDF) assayed without a heat stable amylase and expressed inclusive of residual ash was performed according to Mertens (2002), and acidic detergent fiber (ADF) expressed inclusive of residual ash according to AOAC (1990; method 973.18). Non-fiber carbohydrates (NFC) was calculated as DM – Ash – CP – EE – NDF. All analyses were performed in duplicates.

2.8. Calculation of indices of feed consumption and utilization

Different indices to evaluate and compare feed consumption and utilization were calculated as described by Waldbauer (1968) and the standard formulae as reported by several authors (Hung et al., 1993; Luo et al., 2005; Haghghi et al., 2009). As a standard procedure, all calculations were based on DM values if not indicated otherwise.

$$\text{Consumption index (\%)} = (\text{Average daily feed consumption (DM)} / \text{Average individual life weight (DM)}) \times 100$$

$$\text{Efficiency of conversion of ingested feed into body matter (ECI, \%)} = (\text{Weight gained by insects during feeding period (DM)} / \text{Weight of feed consumed (DM)}) \times 100$$

$$\text{Apparent digestibility of feed (\%)} = ((\text{Weight of feed consumed (DM)} - \text{Weight of fecal waste (DM)}) / \text{Weight of feed consumed (DM)}) \times 100$$

$$\text{Efficiency of conversion of apparently digested feed into body matter (ECD, \%)} = (\text{Weight gained by animals during feeding period (DM)} / (\text{Weight of feed consumed (DM)} - \text{Weight of feces (DM)})) \times 100$$

$$\text{Efficiency of conversion of feed protein into body protein (ECP, \%)} = (\text{Total CP in harvested insect biomass} / \text{Total CP quantity in consumed feed}) \times 100$$

As measurements started with hatching, the eggs imported into the cages reflected initial body mass and protein of insects. These amounts were considered as negligible and were set to zero.

2.9. Statistical analysis

Feed dry matter consumption and development of insect live weight in the time course of both experiments are presented as graphs showing respective treatment means and standard errors of means (SEM). Endpoint measurements such as cumulative feed intake, data on harvested insects, performance indices, etc., were analyzed using one-way analysis of variance (ANOVA) using feeding groups as treatments and data on individual cages as replicates within treatments (R Core Team, 2016). Differences in $P < 0.05$ were considered significant. To reveal significant differences between group means, Student-Newman-Keuls test was performed (Torrie and Steel, 1997; Mendiburu, 2016). Means which are not significantly different are indicated with the same superscript.

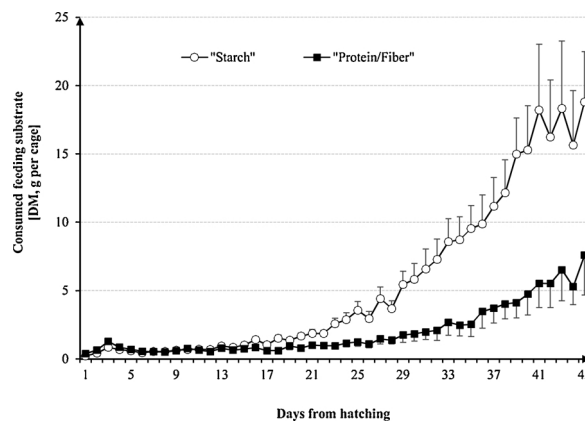


Fig. 1. Means and standard errors of daily feed consumption of *Gryllus bimaculatus* fed on different diets in experiment 1. “Starch”, Corn meal: 500 g/kg, Cowpea leave: 500 g/kg; “Protein/fiber”, Soybean extracts: 500 g/kg, Corn stover: 500 g/kg.

3. Results

3.1. Acceptance of diets and yield of analyzable sample material

Experiment 1: Crickets of all 6 replicates of the “Starch” treatment accepted the diet and showed growth, hence sufficient sample material for further analysis could be obtained from all replicates of this treatment. The same applied to 4 out of 6 replicates from the treatment “Protein/Fiber”.

Experiment 2: In case of the “Starch” treatment, only 2 out of 6 replicates showed consumption of the diet and growth, while locusts of the remaining four replicates showed retarded growth and mortality rates up to 100%. By feeding locusts “Protein/fiber”, also retarded growth and development as well as pale abdominal color, accompanied by high death rates eventuated. Mortality rates of 100% by molting from 3th to 4th instar could be observed in all replicates; hence no further data was obtained from this treatment. Due to insufficient gain of biomass or high mortality rates within locusts fed “Protein/fiber” and “Starch/carrot”, not enough or no sample material for analysis of nutritional composition could be obtained from these treatment groups.

3.2. Daily feed consumption and individual liveweight

Experiment 1: Daily consumption of crickets fed “Starch” was distinctly higher compared to that fed “Protein/fiber” and disparities become even more pronounced towards the end of the study (Fig. 1). Furthermore, the individual live weight of crickets on “Starch” increased noticeably faster than on “Protein/fiber” (Fig. 2). After 43 days from hatching, the average individual live weight of crickets fed “Starch” was 0.59 g compared to 0.17 g of crickets fed “Protein/fiber”.

Experiment 2: Daily feed consumption of locusts on “Starch”, “Protein/fiber” and “Starch/carrot” did not increase appreciably, while daily consumption of locusts fed “Protein/fiber/carrot” and “Protein/carrot” increased noticeably over time (Fig. 3).

The increase in individual live weight of locusts fed “Starch” and “Starch/carrot” was comparatively smaller. Weight increase of locusts fed “Protein/fiber” could not be recorded, as this treatment group showed a mortality rate of 100% by the 15th day after hatching, whereas individual live weight of locusts on “Protein/fiber/carrot” increased sharply. Locusts fed “Protein/carrot” molted to the 6th instar (pre-adult stage) already after 26 days from hatching and thus were harvested; consequently, the last measuring point of individual live weight for this treatment was at the 24th day from hatching (Fig. 4). After 27 days from hatching, locusts fed “Starch/carrot” showed the lowest individual live weight (0.26 g), followed by locusts fed “Starch” (0.48 g) and “Protein/fiber/carrot” (1.08 g). Highest individual live weight gain of locusts was obtained with “Protein/carrot” as diet (1.29 g) after 24 days.

3.3. Production figures and zoo technical indices

Experiment 1: Table 3 presents the production figures and zoo technical indices of crickets fed “Starch” and “Protein/fiber”. Feed DM consumption per cage of crickets on “Starch” was significantly higher than on “Protein/fiber” (243 g vs. 88 g; $F_{1,10} = 9.18$, $P < 0.013$); so was the total DM biomass gain per cage (36.3 g vs. 9.2 g; $F_{1,10} = 9.57$, $P < 0.011$).

Crickets fed “Protein/fiber” resulted a consumption index of 12.3%, which was significantly higher of those fed “Starch” (9.5%; $F_{1,8} = 10.12$, $P < 0.013$). Crickets on “Starch” had a significantly higher ECI than those fed “Protein/fiber” (14.5% vs. 10.4%; $F_{1,8} = 17.31$, $P < 0.003$). The same applied to apparent digestibility of feed DM (55.2% vs. 42.2%; $F_{1,8} = 13.41$, $P < 0.006$), whereas ECD did not show significant differences between the treatments. Also, ECP of crickets fed “Starch” was significantly higher than with “Protein/fiber” as diet (43.0% vs. 27.3%; $F_{1,8} = 106.90$, $P < 0.001$).

Experiment 2: Table 4 presents production figures and zoo technical indices of locusts fed “Starch”, “Protein/fiber/carrot” and “Protein/carrot”. Statistical analysis revealed significant differences between the treatments in all compared production figures. Locusts fed “Protein/carrot” showed significantly the highest results for total feed DM consumption per cage (504 g; $F_{2,9} = 38.77$, $P < 0.001$), total DM biomass gain per cage (102 g; $F_{2,9} = 68.06$, $P < 0.001$), number of animals per cage (294 animals/cage; $F_{2,9}$

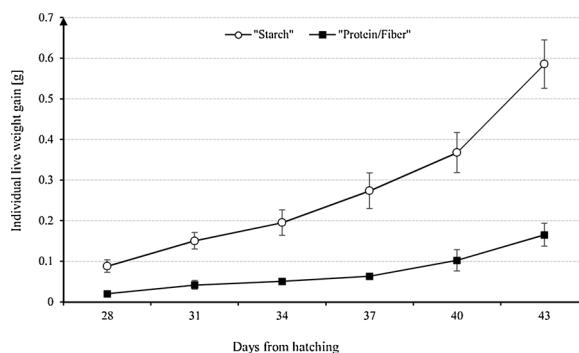


Fig. 2. Means and standard errors of individual live weight gain of *Gryllus bimaculatus* fed on different diets in experiment 1. “Starch”: corn meal, cowpea leaves (50:50), “Protein/fiber”: soybean extract, corn stover (50:50). “Starch”, Corn meal: 500 g/kg, Cowpea leaf: 500 g/kg; “Protein/fiber”, Soybean extracts: 500 g/kg, Corn stover: 500 g/kg.

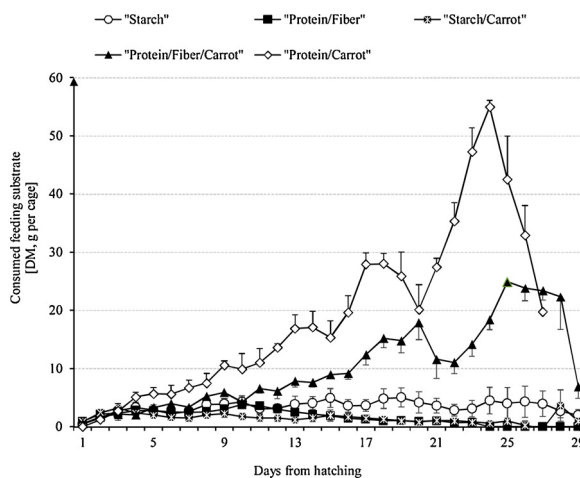


Fig. 3. Means and standard errors of daily feed consumption of *Schistocerca gregaria* fed on different diets in experiment 2. “Starch”, Corn meal: 500 g/kg, Cowpea leave: 500 g/kg; “Protein/fiber”, Soybean extracts: 500 g/kg, “Protein/fiber/carrot”, Soybean extracts: 400 g/kg, Corn stover: 400 g/kg, Carrot: 200 g/kg; “Protein/carrot”, Soybean extracts: 400 g/kg, Cowpea leave: 400 g/kg, Carrot: 200 g/kg.

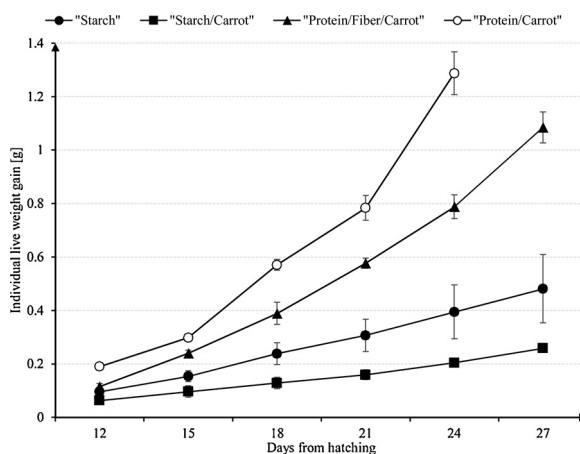


Fig. 4. Means and standard errors of individual live weight gain of *Schistocerca gregaria* fed on different diets in experiment 2. “Starch”, Corn meal: 500 g/kg, Cowpea leave: 500 g/kg; “Protein/fiber/carrot”, Soybean extracts: 400 g/kg, Corn stover: 400 g/kg, Carrot: 200 g/kg; “Protein/carrot”, Soybean extracts: 400 g/kg, Cowpea leave: 400 g/kg, Carrot: 200 g/kg.

= 76.00, $P < 0.001$), and total CP gain per cage (65.7 g; $F_{2,5} = 48.06$, $P < 0.001$). Total feed DM consumption per cage for locusts fed “Protein/fiber/carrot” was 259 g, total DM biomass gain per cage was 48 g and total CP gain per cage was 30.2 g.

Consumption index of locusts fed “Protein/fiber/carrot” and “Protein/carrot” was significantly lower compared to “Starch” (11.5% and 6.0% vs. 18.3%; $F_{2,5} = 21.55$, $P < 0.003$). ECI was significantly higher when locusts were fed “Protein/fiber/carrot” and “Protein/carrot” compared to “Starch” (16.3% and 20.2% vs. 11.0%; $F_{2,5} = 13.44$, $P < 0.010$), whereas no significant difference in digestibility of feeding substrate could be determined between the experimental treatments. Moreover, ECD of locusts fed “Starch” was significantly lower than of locusts fed “Protein/fiber/carrot” and “Protein/carrot” (15.8% vs. 27.5% and 38.5%; $F_{2,5} = 8.04$, $P < 0.027$). Obtained results suggest a tendency to a higher ECP of locusts fed “Protein/fiber/carrot” compared to those fed “Starch” (48.7% vs. 36%; $F_{2,5} = 5.80$, $P < 0.050$).

3.4. Nutritional analysis of crickets and locusts reared on different diet treatments

Experiment 1: Crude protein content of crickets fed “Protein/fiber” was significantly higher than of those fed “Starch” (650 g/kg vs. 500 g/kg; $F_{1,8} = 32.08$, $P < 0.001$). Furthermore, obtained EE portions were significantly higher in crickets fed “Starch” compared to those fed “Protein/fiber” (396 g/kg vs. 265 g/kg, $F_{1,7} = 17.92$, $P < 0.004$). No significant difference between the treatments in the other analyzed nutritional components DM, Ash, NDF and ADF were determined (Table 5).

Experiment 2: No significant differences between treatments regarding nutritional composition of the locusts were determined (Table 6). Though, the results suggest a tendency of higher crude protein contents of locusts fed “Protein/fiber/carrot” and “Protein/carrot” compared to locusts fed “Starch” (630 g/kg and 645 g/kg vs. 560 g/kg; $F_{2,5} = 5.30$, $P = 0.058$).

Table 3
Production figures and zoo technical indices of *Gryllus bimaculatus* fed different diets obtained in experiment 1.

Production figures	Diet type		SEM	F Value	Significance level
	“Starch”	“Protein/fiber”			
Feed DM consumption/cage (g)	243 ₆	89 ₆	33.6	F _{1,10} = 9.18	P = 0.013
Total DM biomass gain/cage (g)	36.3 ₆	9.2 ₆	5.84	F _{1,10} = 9.57	P = 0.011
Total fresh biomass gain/cage (g)	114 ₆	34 ₆	17.2	F _{1,10} = 10.02	P = 0.010
Total CP gain/cage (g)	17.6 ₆	7.7 ₄	2.67	F _{1,8} = 4.57	P = 0.065
Number of animals/cage	204 ₆	201 ₆	22.3	F _{1,10} < 0.01	P = 0.953
Total DM feces/cage (g)	108 ₆	68 ₄	15.4	F _{1,8} = 1.81	P = 0.216
Final DM body weight of animals (mg)	177 ₆	39 ₆	23.8	F _{1,10} = 31.85	P < 0.001
Final fresh body weight of animals (mg)	562 ₆	146 ₆	69.9	F _{1,10} = 41.73	P < 0.001
Zoo technical indices					
Consumption index (%)	9.5 ₆	12.3 ₄	0.60	F _{1,8} = 10.12	P = 0.013
ECI (%)	14.5 ₆	10.4 ₄	0.82	F _{1,8} = 17.31	P = 0.003
Apparent digestibility of feed (%)	55.2 ₆	42.2 ₄	2.68	F _{1,8} = 13.41	P = 0.006
ECD (%)	26.3 ₆	26.1 ₄	1.89	F _{1,8} < 0.01	P = 0.958
ECP (%)	43.0 ₆	27.3 ₄	2.66	F _{1,8} = 106.90	P < 0.001
Diet component					
Corn meal	500 g/kg				
Soybean extracts		500 g/kg			
Cowpea leave	500 g/kg				
Corn stover		500 g/kg			

CP, crude protein; DM, dry matter; ECI, efficiency of conversion of ingested feed into body matter; ECD, efficiency of conversion of apparently digested feed into body matter, ECP, efficiency of conversion of feed protein into body protein; SEM, standard error of mean. “Starch”, Corn meal: 500 g/kg, Cowpea leave: 500 g/kg; “Protein/fiber”, Soybean extracts: 500 g/kg, Corn stover: 500 g/kg.

*The composition of the treatment diets is given at the table bottom.

**The number of observations is indicated as subscript for each experimental value.

Table 4
Production figures and zoo technical indices of *Schistocerca gregaria* fed different diets obtained in experiment 2.

Production figures	Diet type			SEM	F Value	Significance level
	“Starch”	“Protein/fiber/ carrot”	“Protein/ carrot”			
Feed DM consumption/cage [g]	98 ₆ c	295 ₃ b	504 ₃ a	53.4	F _{2,9} = 38.77	P < 0.001
Total DM biomass gain/cage [g]	9 ₆ c	48 ₃ b	102 ₃ a	11.8	F _{2,9} = 68.06	P < 0.001
Total fresh biomass gain/cage [g]	33 ₆ c	179 ₃ b	374 ₃ a	43.2	F _{2,9} = 89.32	P < 0.001
Total CP gain/cage [g]	11.9 ₂ c	30.2 ₃ b	65.7 ₃ a	8.49	F _{2,5} = 48.06	P < 0.001
Number of animals/cage	50 ₆ c	162 ₃ b	294 ₃ a	31.1	F _{2,9} = 76.00	P < 0.001
Total DM feces/cage [g]	57 ₂ b	113 ₃ b	239 ₃ a	30.9	F _{2,5} = 13.86	P = 0.009
Final DM body weight per animal [mg]	131 ₆ b	297 ₃ a	347 ₃ a	35.6	F _{2,9} = 9.51	P = 0.006
Final fresh body weight per animal [g]	0.49 ₆ b	1.10 ₃ ab	1.27 ₃ a	0.126	F _{2,9} = 11.79	P = 0.003
Zoo technical indices						
Consumption index [%]	18.3 ₂ a	11.5 ₃ b	6.0 ₃ b	1.91	F _{2,5} = 21.55	P = 0.003
ECI [%]	11.0 ₂ b	16.3 ₃ a	20.2 ₃ a	1.48	F _{2,5} = 13.44	P = 0.010
Apparent digestibility of feed [%]	74.8 ₂	61.6 ₃	52.5 ₃	4.91	F _{2,5} = 1.96	P = 0.235
ECD [%]	15.8 ₂ b	27.5 ₃ ab	38.5 ₃ a	3.82	F _{2,5} = 8.04	P = 0.027
ECP [%]	36.0 ₂ b	48.7 ₃ a	45.1 ₃ ab	2.24	F _{2,5} = 5.80	P = 0.050
Diet component						
Corn meal	500 g/kg					
Soybean extracts		400 g/kg	400 g/kg			
Cowpea leave	500 g/kg		400 g/kg			
Corn stover		400 g/kg				
Carrot		200 g/kg	200 g/kg			

CP, crude protein; DM, dry matter; ECI: Efficiency of conversion of ingested feed into body matter, ECD: Efficiency of conversion of apparently digested feed into body matter, ECP: Efficiency of conversion of feed protein into body protein; SEM, standard error of mean. “Starch”, Corn meal: 500 g/kg, Cowpea leave: 500 g/kg; “Protein/fiber/carrot”, Soybean extracts: 400 g/kg, Corn stover: 400 g/kg, Carrot: 200 g/kg; “Protein/carrot”, Soybean extracts: 400 g/kg, Cowpea leave: 400 g/kg, Carrot: 200 g/kg.

*Composition of the treatment diets is given at the table bottom.

**The number of observations is indicated as subscript for each experimental value.

***Means which are significantly different, are labeled with different lower-case letters.

Table 5
Nutritional composition of *Gryllus bimaculatus* fed different diets in experiment 1.

Nutritional composition	Diet type		SEM	F Value	Significant level
	“Starch”	“Protein/ fiber”			
DM [g/kg]	308 ₆	281 ₄	9.9	F _{1,8} = 1.27	P = 0.293
Ash [g/kg DM]	75 ₆	58 ₄	8.1	F _{1,8} = 1.15	P = 0.315
CP [g/kg DM]	500 ₆	650 ₄	27.5	F _{1,8} = 32.08	P = 0.001
EE [g/kg DM]	396 ₆	265 ₃	25.7	F _{1,7} = 17.92	P = 0.004
NDF [g/kg DM]	244 ₆	285 ₁	15.1	F _{1,5} = 0.88	P = 0.391
ADF [g/kg DM]	148 ₆	165 ₁	17.5	F _{1,5} = 0.09	P = 0.771
Diet component	“Starch”	“Protein/ fiber”			
Corn meal	500 g/kg				
Soybean extracts		500 g/kg			
Cowpea leave	500 g/kg				
Corn stover		500 g/kg			

DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber, NFC, non-fiber carbohydrates (DM – Ash – CP – EE – NDF); SEM, standard error of mean. “Starch”, Corn meal: 500 g/kg, Cowpea leave: 500 g/kg; “Protein/fiber”, Soybean extracts: 500 g/kg, Corn stover: 500 g/kg.

*Composition of the treatment diets is given at the table bottom.

**The number of observations is indicated as subscript for each experimental value.

Table 6
Nutritional composition of *Schistocerca gregaria* fed different diets obtained in experiment 2.

Nutritional composition	Diet type			SEM	F Value	Significance level
	“Starch”	“Protein/fiber/ carrot”	“Protein/carrot”			
DM (g/kg)	280 ₂	267 ₃	272 ₃	3.9	F _{2,5} = 0.92	P = 0.458
Ash (g/kg DM)	40 ₂	37 ₃	33 ₃	1.6	F _{2,5} = 1.63	P = 0.286
CP (g/kg DM)	560 ₂	630 ₃	645 ₃	15.6	F _{2,5} = 5.30	P = 0.058
EE (g/kg DM)	265 ₂	230 ₃	227 ₃	11.0	F _{2,5} = 1.08	P = 0.408
NDF (g/kg DM)	NA*	172 ₃	148 ₃	8.9	F _{1,4} = 2.06	P = 0.224
ADF (g/kg DM)	NA*	117 ₃	127 ₃	7.3	F _{1,4} = 0.42	P = 0.553
Diet component	“Starch”	“Protein/fiber/ carrot”	“Protein/carrot”			
Corn meal	500 g/kg					
Soybean extracts		400 g/kg	400 g/kg			
Cowpea leave	500 g/kg		400 g/kg			
Corn stover		400 g/kg				
Carrot		200 g/kg	200 g/kg			

DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; NFC, non-fiber carbohydrates (DM – Ash – CP – EE – NDF); SEM, standard error of mean. “Starch”, Corn meal: 500 g/kg, Cowpea leave: 500 g/kg; “Protein/fiber/carrot”, Soybean extracts: 400 g/kg, Corn stover: 400 g/kg, Carrot: 200 g/kg; “Protein/carrot”, Soybean extracts: 400 g/kg, Cowpea leave: 400 g/kg, Carrot: 200 g/kg.

*Not analyzed due to lack of sample material.

**Composition of the treatment diets is given at the table bottom.

***The number of observations is indicated as subscript for each experimental value.

4. Discussion

The results of this study show, that it is possible to rear locusts and crickets on dried and therefore storable feed materials commonly used in livestock production. Also, some tested diets resulted in remarkably high feed and protein conversion efficiency ratios; and changes in efficiency of feed conversion and nutritional composition of the insects could be attributed to nutritional properties of the tested diets. Furthermore, macro-nutritional limitations could be identified by reversing them through dietary supplementation.

The value-added part of a commercial production cycle of insects includes the entire phase from hatching of the eggs until the harvest of living biomass. In this study, crickets and locusts were kept and fed under experimental conditions from introducing the eggs and hatching (day 1) until harvesting the insects as sample material at a pre-defined age of 45 days (crickets) and 29 days (locusts). Furthermore, during the experiments, nothing else but the refused feed which remained on the petri dishes was removed and therefore each cage can be considered as a closed system. Skins from molting, lost body parts such as legs or even dead animals remained in the cages and were mostly eaten by the other insects of the same cage. Focus of this study was not the physiological capability of feed or protein conversion of the individual insect, but of the combined one of all insects per cage which therefore was considered as one production unit. Therefore, the production figures and zoo technical indices which were obtained in these

experiments are highly application oriented in view of commercial production of insects and insect protein.

4.1. Experiment 1

All results concerning daily feed consumption, development of individual live weight, further production figures and zoo technical indices have shown that it is possible to feed crickets with the storable feed materials corn meal, cowpea leave, soybean extract and corn stover. Crickets of both treatments accepted the experimental diets and yielded biomass. However, the daily consumption and individual live weight gain as well as end-point measurements of aggregated consumption per cage and total gain of biomass per cage was higher for crickets fed “Starch” compared to “Protein/fiber”. Yet, the biomass gain for crickets of both treatments is relatively low, considering reported individual weights higher than 0.8 g for crickets fed chicken feed after a rearing period of only 4 weeks (Dobermann et al., 2018).

The apparent digestibility of feed dry matter of “Starch” was significantly higher than of “Protein/fiber”. Furthermore, crickets fed “Starch” could transform feed intake more efficiently into body mass gain than crickets fed “Protein/fiber”, but efficiency of transformation of apparently digested of feed dry matter into body mass gain did not show significant differences between the treatments. Evidently, the difference in feed quality between “Starch” and “Protein/fiber” resulted mainly from a significantly lower digestibility of feed dry matter, probably due to high fiber contents, rather than from imbalances or limitations among the absorbed nutrients from the diets.

This is also in line with the values reported by Dobermann et al. (2018), who obtained an ECI of 33.15% for crickets fed a diet with similar protein and fat contents as the “Starch” diet, but a remarkably lower fiber content (40 g/kg vs. 278 g/kg). In this study as well, a diet with higher protein and fat contents, but also a higher fraction of fiber resulted in a significantly lower ECI of 8.65%, which is comparable to the results for the “Protein/fiber” treatment with 10.8%. Oonincx et al. (2015a) conducted feeding trials with the house cricket, *Acheta domesticus*, and reported an ECI of 12% for crickets fed a diet of similar composition regarding protein and fat content as the tested “Starch” diet, which resulted in an ECI of 14.5%. Furthermore, ECI with a high-protein high-fat diet was lower (8%), as it was the case in the present study with “Protein/fiber” (10.4%). However, in this study no further information on nutritional composition of the used diets e.g. regarding ADF content is provided. Values for House Cricket ECI as reported by other authors (20–38%) were remarkably higher. Though, not only deviations in the nutritional values of respectively fed diets, but also observation periods of different durations might explain diverging results (Woodring et al., 1977, 1979; Clifford and Woodring, 1990; Nakagaki and Defoliart, 1991). Significantly higher consumption rates of crickets fed “Protein/fiber” might have resulted from higher feed intakes as reaction of the insects to a low density of available nutrients in the diet caused by low digestibility. Such increased feed intake as reaction to nutrient-deficient diets is a commonly observed insect behavior and in accordance with the concept of growth, nutritional and intake targets proposed by Simpson and Raubenheimer (1993). The increased flow rate might also explain a significantly lower ECP of crickets fed “Protein/fiber”.

To estimate the protein content of the insects, crude protein (N x 6.25) method was applied. This method does not differentiate between N derived from protein and that derived from chitin, the structural component of the exoskeleton which is built up of a long-chain polymer of an N-acetylglucosamine ((C₈H₁₃O₅N)_n). For this reason, this method may be suspected to overestimate the true protein content of insects. Janssen et al. (2017), for example, proposes a nitrogen-to-protein conversion factor of 5.60 for insects. However, Finke (2007) analyzed the fraction of N that actually derives from amino acids and found that the crude protein method provides a reasonable estimate of the total content of true protein in insects. The digestibility of the chitin-bound true protein, however, remains to be assessed in comparison to protein from the inside of the exoskeleton hull, which may be considered to be highly digestible. Crude protein content was the lowest in *G. bimaculatus* when fed “Starch” compared to “Protein/fiber” (500 g/kg vs. 650 g/kg), whereas insects showed significantly higher contents of EE on “Starch” than on “Protein/fiber” (396 g/kg vs. 265 g/kg). These values are in the same range as reported by Young (2010) regarding CP content (507 g/kg–683 g/kg) and EE content (226 g/kg–237 g/kg) in DM for *G. bimaculatus* reared on three different diets. In this study as well, crickets with the highest CP contents showed the lowest EE contents and vice versa. However, no specific information on the nutritional composition of the diets fed is provided.

These significantly higher CP and lower EE contents of crickets on “Protein/fiber” compared to “Starch” on the one hand; and low individual live weight gain and overall biomass gain of crickets on “Protein/fiber” on the other hand imply, that crickets on “Protein/fiber” suffered from starvation, and therefore could not build up more fat reserves. The lower biomass gain per cage was associated by lower individual body weight, while the number of animals per cage remained fairly unchanged. Therefore, it can be concluded that the “Protein/fiber” diet was low in general quality, but obviously not deficient in a particular, crucial nutrient entailing death of the animals. Even though, the feed protein content of “Protein/fiber” was higher than that of “Starch” (244 g/kg vs. 168 g/kg), “Protein/fiber” was found to be inferior for nutrition of *G. bimaculatus*. This is remarkable, as the dietary protein content was reportedly suggested as a mayor limiting factor for growth and development of crickets (Merkel, 1977; Dobermann et al., 2018). Also this points to digestibility of the feeding substrate as limiting factor on utilization of the feed materials for growth of crickets fed “Protein/fiber”.

Apart from low digestibility, other factors might have prevented better utilization of the feeds. All insects need a range of essential dietary components such as essential amino acids or vitamins, e.g. at least 6 different B vitamins, to ensure normal metabolic function, development and growth (McFarlane, 1976, 1983). Even though the capability of synthesizing such micro nutrients can vary among different species, a lack of these nutrients in the diet might have considerable impacts, depending on the species ranging from retarded growth to death (Ritchot and McFarlane, 1961; Vanderzant and Richardson, 1963; Chippendale, 1975; Oonincx et al., 2018). Therefore, further research is required to identify possible limitations through these (micro)nutrients and their impact on feeding efficiency and production parameters of the insects.

4.2. Experiment 2

The previously performed Cricket Study on *G. bimaculatus*, dealt with “Starch” and “Protein/fiber” as experimental diets. Results of this study suggested that some of the tested substrates (e.g. soybean extracts and corn stover) might be insufficient in micronutrient content and therefore did not meet the insects nutritional needs. For this reason, the different feeding formulations were supplemented with carrot powder and vitamins to indicate eventual improvements of these diets when fed to the locust *S. gregaria* compared to the initial dietary formulations.

The results for daily feed consumption, development of individual live weight and further production figures as well as zoo technical indices have shown that it is possible to feed *S. gregaria* with “Protein/fiber/carrot” and “Protein/carrot”, both carrot and vitamin supplemented diets. When feeding locusts “Starch”, only 2 of 6 replicates yielded sufficient sample material for nutritional analysis and no replicate of locusts on “Starch/carrot” yielded sufficient sample material for nutritional analysis.

Determined efficiency ratios for feed conversion into body mass are similar to values reported by several authors for *S. gregaria* reared on natural, fresh diets such as cabbage, lettuce or cauliflower leaves with ECI between 6.7–17.3% and ECD between 20.7–60.0% (Hill, 1970; Mehrotra et al., 1972; Bernays and Woodhead, 1984; Rao and Subrahmanyam, 1986). These values however, may vary strongly with the physiological age and stage of development in which the feed efficiency was determined as well as the nutritional quality of feed materials used. For example, Mehrotra et al. (1972) reported an ECD of 38.3% for 3rd instar locusts and 60.0% for 5th instar locusts. Therefore, comparison of our results with respective data from literature is difficult, the more so as to our knowledge never before data was collected over the entire timespan from hatching until harvest. Furthermore, our study aimed into testing potentially imbalanced feed mixtures rather than optimizing the animals' performance.

Reported values for nutritional composition of locusts are in line with those of the current study. Das (1945) and Ooninx et al. (2015b) analyzed the nutritional composition of *S. gregaria* and measured CP contents of respectively 618 g/kg and 655 g/kg, and fat contents of 170 g/kg and 176 g/kg, which are similar to the results of locusts fed “Protein/fiber/carrot” and “Protein/carrot”, whereas locusts fed “Starch” showed remarkably lower CP and higher EE contents. Also Grabowski et al. (2008) reported a CP content of 607 g/kg and EE content of 162 g/kg for locusts reared under optimal laboratory conditions, whereas locusts exposed to high levels of stress contained lower CP contents (579 g/kg) and higher EE contents (309 g/kg).

Supplementation of “Protein/fiber” with carrot and vitamins showed drastic improvements. Locusts fed “Protein/fiber” failed to develop into adults in all the experimental replicates. This treatment resulted in retarded growth and development as well as pale abdominal color, accompanied by high death rates with 100% mortality across all replicates at the time of molting from 3th to 4th instar. These symptoms have been documented by Dadd (1957) and Dadd (1960b) due to a lack of ascorbic acid and beta-carotene in the insects diet. Several studies showed, that ascorbic acid plays an important role in the nutrition and physiology of different plant-feeding insects (Thorsteinson, 1958; Vanderzant et al., 1962; Vanderzant and Richardson, 1963; Chippendale, 1975). However, in most plant components except fruits and green vegetable parts, ascorbic acid is present only in very low concentrations or completely absent. This explains, why in the present studies supplementation of “Protein/fiber” with carrot and ascorbic acid amongst other vitamins, to create the “Protein/fiber/carrot” diet, all symptoms described above were immediately corrected and dramatic improvement in development and growth was evident. Therefore, a lack of vitamins and/or carotenoids in the diet may be a limiting factor in the nutrition of *S. gregaria* with feed materials commonly used in livestock production. However, processing and storing such substrates can have a significant effect on their vitamin and carotenoid content. Mulokozi and Svanberg (2003) for example reported a beta-carotene content of 296 µg/g DM for cowpea leaves that were sundried similarly to the ones used in the present study, which was significantly lower than for cowpea leaves dried in a solar cabinet dryer with a reported content of 426 µg/g DM. Hence, establishing appropriate methods of preserving feeding substrates by drying may drastically improve their suitability for insect nutrition.

As mentioned above, feeding “Starch” to locusts resulted in poor development and growth with resultant high mortality rates. Unlike in case of “Protein/fiber” and “Protein/fiber/carrot”, the addition of 20% carrot and vitamin supplementation to “Starch” (“Starch/carrot”) did not improve growth and development of *S. gregaria*. Growth limiting properties might be suspected in corn meal, as cowpea leave did not cause negative effects on locusts within the “Protein/carrot” treatment. Locusts fed “Starch” were not able to transform ingested feed efficiently into body mass gain. Even though, digestibility of “Starch”, “Protein/fiber/carrot” and “Protein/carrot” did not vary significantly, the ECD of locusts fed “Starch” was relatively low. Indeed, “Starch” could be digested by the locusts, but the nutrients not efficiently metabolized. This might explain the lower CP and relatively high EE contents of locusts on “Starch” compared to “Protein/fiber/carrot” and “Protein/carrot”. Inferentially, besides from growth inhibitors or lack of vitamins there might have been other limiting factors affecting the metabolism of insects fed “Starch”. Low body protein and, simultaneously high body fat accretion suggests an undersupply with feed protein (absolutely or relatively in terms of limiting essential amino acids). Furthermore, the role of other micronutrients on development and survival of *S. gregaria* warrants urgent attention.

Locusts on “Protein/carrot” developed relatively faster and reached the 6th instar stage (pre-adult stage) 3 days earlier than those of the other treatments including “Protein/fiber/carrot”. It can therefore be assumed, that the “Protein/carrot” diet met the nutritional requirements of *S. gregaria* to a higher extend and contained all required nutritional components needed to accelerate development. A major difference in terms of macro-nutrient composition between “Protein/fiber/carrot” and “Protein/carrot” lied in portions of NDF (420 g/kg vs. 234 g/kg) and NFC (145 g/kg vs. 193 g/kg). Dadd (1960a) reported non-fiber carbohydrates as essential nutrients for *S. gregaria* with an optimum content of around 260 g/kg dietary NFC. Therefore, a higher NFC content in “Protein/carrot” might have contributed to superior performance of this treatment. However, more research is required to prove this assumption.

Furthermore, the high digestibility of feed dry matter of “Protein/fiber/carrot” (61.6%) by the locusts is remarkable, as it

contained corn stover and consequently high NDF and ADF contents. NDF has been widely reported as a hard digestible feed component, but can be digested by Desert Locusts to a high extend (up to 600 g/kg of dietary NDF) as ascertained by Cazemier et al. (1997). Given that *S. gregaria* was able to efficiently convert corn stover into body mass implies, that such a low-value by-product can be used as feed component for locusts. Insects might therefore form a more sustainable food or feed source, with the potential of production with little food competition to human and livestock nutrition. Without any optimization in terms of protein utilization, *S. gregaria* showed an ECP of 45% (“Protein/carrot”) and even 49% (“Protein/fiber/carrot”). Evidently, large portions of feed protein could be transformed into insect protein, which is remarkable since dietary CP contents in the experimental diets were relatively high (e.g. 280 g/kg in “Protein/carrot”). These results are comparable to ECP values reported for the Argentinian Cockroach or Black Soldier Fly larvae (51% each); and remarkably higher than for the Yellow Mealworm (22%), when reared on a high-protein/low-fat diet (Ooninx et al., 2015a). This strongly suggests that improvement of feeding formulations can further raise transformation efficiency of protein from feed to the harvested insect.

5. Conclusion

The conducted study shows that 1) crickets and locusts can be produced on diets composed of storable feed materials commonly used in livestock production, 2) both crickets and locusts use feed equally or more efficient in converting feed to edible body mass than other edible insects, 3) on suitable diets the insects utilized protein equally or more efficiently than other edible insects, and 4) in some dietary treatments, reversible nutritional deficiencies appeared, which makes the tested diets suitable as model diets to detect limitations through certain (micro)nutrients in further studies. It is anticipated that the findings of this study will lead to further efforts in order to explore the enormous potential of insect production as novel livestock and sustainable protein source for human food and animal feed.

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References

- AOAC, 1990. Official methods of analysis of the AOAC. Methods 920.08, 920.29, 930.15, 942.05, 973.18, 984.13, 15th ed. AOAC, Arlington, Virginia (Accessed 28 May 2019). <https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf>.
- Ayieko, M., Oriaro, V., Nyambuga, I.A., 2010. Processed products of termites and lake flies: improving entomophagy for food security within the Lake Victoria region. *Afr. J. Food Agric. Nutr. Dev.* 10, 14. (Accessed 28 May 2019). <https://www.ajol.info/index.php/ajfand/article/view/53352/41930>.
- Barker, D., Fitzpatrick, M.P., Dierenfeld, E.S., 1998. Nutrient composition of selected whole invertebrates. *Zoo Biol.* 17, 123–134. [https://doi.org/10.1002/\(SICI\)1098-2361\(1998\)17:2<123::AID-ZOO7>3.0.CO;2-B](https://doi.org/10.1002/(SICI)1098-2361(1998)17:2<123::AID-ZOO7>3.0.CO;2-B).
- Bernard, J.B., Allen, M.E., Ullrey, D.E., 1997. Feeding captive insectivorous animals: nutritional aspects of insects as food NAG Handbook. Fact Sheet 3, 1–7. (Accessed 28 May 2019). <https://nagonline.net/wp-content/uploads/2014/01/NAG-FS003-97-Insects-JONI-FEB-24-2002-MODIFIED.pdf>.
- Bernays, E.A., Woodhead, S., 1984. The need for high levels of phenylalanine in the diet of *Schistocerca gregaria* nymphs. *J. Insect Physiol.* 30, 489–493. [https://doi.org/10.1016/0022-1910\(84\)90029-5](https://doi.org/10.1016/0022-1910(84)90029-5).
- Bukkens, S.G., 1997. The nutritional value of edible insects. *Ecol. Food Nutr.* 36, 287–319. <https://doi.org/10.1080/03670244.1997.9991521>.
- Cazemier, A.E., den Camp, H.J.O., Hackstein, J.H., Vogels, G.D., 1997. Fibre digestion in arthropods. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 118, 101–109. [https://doi.org/10.1016/S0300-9629\(96\)00443-4](https://doi.org/10.1016/S0300-9629(96)00443-4).
- Chippendale, G.M., 1975. Ascorbic acid: an essential nutrient for a plant-feeding insect, *Diatraea grandiosella*. *J. Nutr.* 105, 499–507. <https://doi.org/10.1093/jn/105.4.499>.
- Clifford, C.W., Woodring, J.P., 1990. Methods for rearing the house cricket, *Acheta domesticus* (L.), along with baseline values for feeding rates, growth rates, development times, and blood composition. *J. Appl. Entomol.* 109, 1–14. <https://doi.org/10.1111/j.1439-0418.1990.tb00012.x>.
- Dadd, R.H., 1957. Ascorbic acid and carotene in the nutrition of the desert locust, *Schistocerca gregaria* Forsk. *Nature* 179, 427.
- Dadd, R.H., 1960a. The nutritional requirements of locusts—III carbohydrate requirements and utilization. *J. Insect Physiol.* 5, 301–316. [https://doi.org/10.1016/0022-1910\(60\)90012-3](https://doi.org/10.1016/0022-1910(60)90012-3).
- Dadd, R.H., 1960b. Some effects of dietary ascorbic acid on locusts. *Proc. R. Soc. Lond. Ser. B* 153, 128–143. <https://doi.org/10.1098/rspb.1960.0091>.
- Das, S., 1945. Locusts as food and manure. *Indian Farm.* 6 (412).
- Dobermann, D., Michaelson, L., Field, L.M., 2018. The effect of an initial high-quality feeding regime on the survival of *Gryllus bimaculatus* (black cricket) on bio-waste. *J. Insects Food Feed* 5, 117–123. <https://doi.org/10.3920/JIFF2018.0024>.
- El Boushy, A.R., 1991. House-fly pupae as poultry manure converters for animal feed: a review. *Bioresour. Technol.* 38, 45–49. [https://doi.org/10.1016/0960-8524\(91\)90220-E](https://doi.org/10.1016/0960-8524(91)90220-E).
- Finke, M.D., DeFoliart, G.R., Benevenga, N.J., 1989. Use of a four-parameter logistic model to evaluate the quality of the protein from three insect species when fed to rats. *J. Nutr.* 119, 864–871. <https://doi.org/10.1093/jn/119.6.864>.
- Finke, M.D., 2007. Estimate of chitin in raw whole insects. *Zoo Biol.* 26, 105–115. <https://doi.org/10.1002/zoo.20123>.
- Fontaneto, D., Tommaso-Ponzetta, M., Galli, C., Risé, P., Glew, R.H., Paoletti, M.G., 2011. Differences in fatty acid composition between aquatic and terrestrial insects used as food in human nutrition. *Ecol. Food Nutr.* 50, 351–367. <https://doi.org/10.1080/03670244.2011.586316>.
- Grabowski, N.T., Nowak, B., Klein, G., 2008. Proximate chemical composition of long-horned and short-horned grasshoppers (*Acheta domesticus*, *Schistocerca gregaria* and *Phymateus saxosus*) available commercially in Germany. *Arch. Lebensmittelhyg.* 59, 204–208.
- Haghighi, D.T., Saad, C.R., Hosainzadeh Sahafi, H., Mansouri, D.A., 2009. The effect of dietary lipid level on the growth of kutum fry (*Rutilus frisii kutum*). *Iran. J. Fish. Sci.* 8, 13–24. <http://aquaticcommons.org/id/eprint/22330>.
- Halloran, A., Roos, N., Eilenberg, J., Cerutti, A., Bruun, S., 2016. Life cycle assessment of edible insects for food protein: a review. *Agron. Sustain. Dev.* 36, 57. <https://doi.org/10.1007/s11367-016-0400-0>.

- doi.org/10.1007/s13593-016-0392-8.
- Henchion, M., Hayes, M., Mullen, A., Fenelon, M., Tiwari, B., 2017. Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods* 6, 53. <https://doi.org/10.3390/foods6070053>.
- Hill, L., 1970. The utilisation of food by the adult female desert locust, *Schistocerca gregaria*. *Entomol. Exp. Appl.* 13, 352–358. <https://doi.org/10.1111/j.1570-7458.1970.tb00119.x>.
- Van Huis, A., 2013. Potential of insects as food and feed in assuring food security. *Annu. Rev. Entomol.* 58, 563–583. <https://doi.org/10.1146/annurev-ento-120811-153704>.
- Hung, S.S., Lutes, P.B., Shqueir, A.A., Conte, F.S., 1993. Effect of feeding rate and water temperature on growth of juvenile white sturgeon (*Acipenser transmontanus*). *Aquaculture* 115, 297–303. [https://doi.org/10.1016/0044-8486\(93\)90144-N](https://doi.org/10.1016/0044-8486(93)90144-N).
- Janssen, R.H., Vincken, J.P., van den Broek, L.A., Fogliano, V., Lakemond, C.M., 2017. Nitrogen-to-protein conversion factors for three edible insects: *tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. *J. Agric. Food Chem.* 65, 2275–2278. <https://doi.org/10.1021/acs.jafc.7b00471>.
- Jongema, Y., 2015. World List of Edible Insects. Wageningen University, pp. 75. (Accessed 28 May 2019). https://www.wur.nl/upload_mm/7/4/1/ca8baa25-b035-4bd2-9fdc-a7df1405519a_WORLD%20LIST%20EDIBLE%20INSECTS%202015.pdf.
- Kinyuru, J.N., Konyole, S.O., Roos, N., Onyango, C.A., Owino, V.O., Ouwor, B.O., Estambale, B.B., Friis, H., Aagaard-Hansen, J., Kenji, G.M., 2013. Nutrient composition of four species of winged termites consumed in western Kenya. *J. Food Anal.* 30, 120–124. <https://doi.org/10.1016/j.jfca.2013.02.008>.
- Launois-Luong, M.H., Lecoq, M., Grimaux, M.F., 1991. Workshop on effective networking of research and development on environmentally sustainable locust control methods among locust-affected countries. Workshop, ICIPE, Nairobi. pp. 77–79. (Accessed on 28 May 2019). <http://www.icipe.org/publications/conference-presentation-posters?page=1>.
- Luo, Z.H., Liu, Y.J., Mai, K.S., Tian, L.X., Liu, D.H., Tan, X.Y., Lin, H.Z., 2005. Effect of dietary lipid level on growth performance, feed utilization and body composition of grouper *Epinephelus coioides* juveniles fed isonitrogenous diets in floating netcages. *Aquac. Int.* 13, 257–269. <https://doi.org/10.1007/s10499-004-2478-6>.
- Makkar, H.P., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. *Anim. Feed Sci. Technol.* 197, 1–33. <https://doi.org/10.1016/j.anifeedsci.2014.07.008>.
- McFarlane, J.E., 1976. Vitamin K: a growth factor for the house cricket (Orthoptera: Gryllidae). *Can. Entomol.* 108, 391–394. <https://doi.org/10.4039/Ent108391-4>.
- McFarlane, J.E., 1983. Relationship between vitamins E, K1 and K3 (menadione) in their effects on growth of *Acheta domestica* (L.). *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 74, 387–389. [https://doi.org/10.1016/0300-9629\(83\)90620-5](https://doi.org/10.1016/0300-9629(83)90620-5).
- Mehrotra, K.N., Rao, P.J., Ferooqi, T.N., 1972. The consumption, digestion and utilization of food by locusts. *Entomol. Exp. Appl.* 15, 90–96. <https://doi.org/10.1111/j.1570-7458.1972.tb02086.x>.
- Mendiburu, F., 2016. *Agricolae: Statistical Procedures for Agricultural Research*. R Package Version 1.2-4. <https://CRAN.R-project.org/package=agricolae>.
- Merkel, G., 1977. The effects of temperature and food quality on the larval development of *Gryllus bimaculatus* (Orthoptera, Gryllidae). *Oecologia* 30, 129–140.
- Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing beakers or crucibles: collaborative study. *J. Assoc. Off. Assoc. Chem. Int.* 85, 1217–1240.
- Mulokozi, G., Svanberg, U., 2003. Effect of traditional open sun-drying and solar cabinet drying on carotene content and vitamin A activity of green leafy vegetables. *Plant Foods Hum. Nutr.* 58, 1–15.
- Nakagaki, B.J., Defoliart, G.R., 1991. Comparison of diets for mass-rearing *Acheta domestica* (Orthoptera: Gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. *J. Econ. Entomol.* 84, 891–896. <https://doi.org/10.1093/jee/84.3.891>.
- Ochieng-Odero, J.P., Ndugo, S.M., El Bashir, S., Capstick, P.B., 1994. A method for rearing crowded (gregarious) and isolated (solitary) locusts (Orthoptera: Acrididae) in the laboratory. Njagi, P.G.N., Chaudhury, M.F.B. (Eds.), *Proceedings of a Workshop on Effective Networking of Research and Development on Environmentally Sustainable Locust Control Methods Among Locust Affected Countries* 33–44. (Accessed on 28 May 2019). <http://www.icipe.org/publications/conference-presentation-posters/conference-proceedings?page=1>.
- Ooninx, D.G., Van Broekhoven, S., Van Huis, A., van Loon, J.J., 2015a. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS One* 10, e0144601. <https://doi.org/10.1371/journal.pone.0144601>.
- Ooninx, D.G., Van Isterbeeck, J., Heetkamp, M.J., Van Den Brand, H., Van Loon, J.J., Van Huis, A., 2010. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS One* 5, e14445. <https://doi.org/10.1371/journal.pone.0014445>.
- Ooninx, D.G., Van Keulen, P., Finke, M.D., Baines, F.M., Vermeulen, M., Bosch, G., 2018. Evidence of vitamin D synthesis in insects exposed to UVB light. *Sci. Rep.* 8, 10807. <https://doi.org/10.1038/s41598-018-29232-w>.
- Ooninx, D.G., Van Leeuwen, J.P., Hendriks, W.H., Van der Poel, A.F., 2015b. The diet of free-roaming Australian central bearded dragons (*Pogona vitticeps*). *Zoo Biol.* 34 (3), 271–277. <https://doi.org/10.1002/zoo.21209>.
- Ooninx, D.G., Van der Poel, A.F., 2011. Effects of diet on the chemical composition of migratory locusts (*Locusta migratoria*). *Zoo Biol.* 30, 9–16. <https://doi.org/10.1002/zoo.20308>.
- Ramos-Elorduy, J., Moreno, J.M.P., Prado, E.E., Perez, M.A., Otero, J.L., De Guevara, O.L., 1997. Nutritional value of edible insects from the state of Oaxaca, Mexico. *J. Food Compos. Anal.* 10, 142–157. <https://doi.org/10.1006/jfca.1997.0530>.
- Rao, P.J., Subrahmanyam, B., 1986. Azadirachtin induced changes in development, food utilization and haemolymph constituents of *Schistocerca gregaria* Forskal. *J. Appl. Entomol.* 102, 217–224. <https://doi.org/10.1111/j.1439-0418.1986.tb00914.x>.
- R Core Team, 2016. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/> (Accessed on 28 May 2019).
- Ritchot, C., McFarlane, J.E., 1961. The B vitamin requirements of the house cricket. *Can. J. Zool.* 39, 11–15. <https://doi.org/10.1139/z61-002>.
- Rumpold, B.A., Schlüter, O.K., 2013. Nutritional composition and safety aspects of edible insects. *Mol. Nutr. Food Res.* 57 (5), 802–823. <https://doi.org/10.1002/mnfr.201200735>.
- Sheppard, D.C., Newton, G.L., Thompson, S.A., Savage, S., 1994. A value added manure management system using the black soldier fly. *Bioresour. Technol.* 50, 275–279. [https://doi.org/10.1016/0960-8524\(94\)90102-3](https://doi.org/10.1016/0960-8524(94)90102-3).
- Simpson, S.J., Raubenheimer, D., 1993. A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 342, 381–402. <https://doi.org/10.1098/rstb.1993.0166>.
- Simpson, S.J., Raubenheimer, D., 2001. The geometric analysis of nutrient-allolethical interactions: a case study using locusts. *Ecology* 82, 422–439. [https://doi.org/10.1890/0012-9658\(2001\)082\[0422:TGAONA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0422:TGAONA]2.0.CO;2).
- Smetana, S., Palanisamy, M., Mathys, A., Heinz, V., 2016. Sustainability of insect use for feed and food: life cycle assessment perspective. *J. Clean. Prod.* 137, 741–751.
- Thompson, S.N., 1973. A review and comparative characterization of the fatty acid compositions of seven insect orders. *Comp. Biochem. Physiol. B* 45, 467–482. [https://doi.org/10.1016/0305-0491\(73\)90078-3](https://doi.org/10.1016/0305-0491(73)90078-3).
- Thorsteinson, A.J., 1958. Acceptability of plants for phytophagous insects. *Proc. Xth. Int. Congr. Ent* 2, 599–602. <https://doi.org/10.1016/j.jclepro.2016.07.148>.
- Torrie, J.H., Steel, R.G., 1997. *Principles and Procedures of Statistics: a Biometrical Approach, third ed.* McGraw-Hill, New York.
- Vanderzant, E.S., Pool, M.C., Richardson, C.D., 1962. The role of ascorbic acid in the nutrition of three cotton insects. *J. Insect Physiol.* 8, 287–297. [https://doi.org/10.1016/0022-1910\(62\)90032-X](https://doi.org/10.1016/0022-1910(62)90032-X).
- Vanderzant, E.S., Richardson, C.D., 1963. Ascorbic acid in the nutrition of plant-feeding insects. *Science* 140, 989–991. <https://doi.org/10.1126/science.140.3570.989>.
- Waldbauer, G.P., 1968. The consumption and utilization of food by insects. *Adv. Insect. Phys.* 5, 229–5288. [https://doi.org/10.1016/S0065-2806\(08\)60230-1](https://doi.org/10.1016/S0065-2806(08)60230-1).
- Wang, D., Bai, Y.Y., Li, J.H., Zhang, C.X., 2004. Nutritional value of the field cricket (*Gryllus testaceus* Walker). *J. Insect Sci.* 11, 275–283. <https://doi.org/10.1111/j.1744-7917.2004.tb00424.x>.
- Woodring, J.P., Clifford, C.W., Beckman, B.R., 1979. Food utilization and metabolic efficiency in larval and adult house crickets. *J. Insect Physiol.* 25, 903–912. [https://doi.org/10.1016/0022-1910\(79\)90102-1](https://doi.org/10.1016/0022-1910(79)90102-1).
- Woodring, J.P., Roe, R.M., Clifford, C.W., 1977. Relation of feeding, growth, and metabolism to age in the larval, female house cricket. *J. Insect Physiol.* 23, 207–212. [https://doi.org/10.1016/0022-1910\(77\)90031-2](https://doi.org/10.1016/0022-1910(77)90031-2).
- Young, C.E., 2010. Proximate and Mineral Composition of Selected Whole Invertebrates and Nutritional Effects of Different Diets on the Field Cricket, *Gryllus bimaculatus*. Dissertation. Nottingham Trent University, Nottingham.