Animal Feed Science and Technology 221 (2016) 174–184

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

# In vitro digestibility and fermentability of selected insects for dog foods

G. Bosch [a,*], J.J.M. Vervoort [b], W.H. Hendriks [a,c]

[a] Animal Nutrition Group, Department of Animal Sciences, Wageningen University, PO Box 338, 6700 AH, Wageningen, The Netherlands
[b] Laboratory of Biochemistry, Department of Agrotechnology and Food Sciences, Wageningen University, PO Box 8128, 6700 ET, Wageningen, The Netherlands
[c] Division of Nutrition, Faculty of Veterinary Medicine, Utrecht University, PO Box 80.151, 3508 TD, Utrecht, The Netherlands

ARTICLE INFO

*Article history:*
Received 18 January 2016
Received in revised form 12 August 2016
Accepted 22 August 2016

*Keywords:*
Amino acid
Digestibility
Dog
Fermentation product
In vitro
Insect

ABSTRACT

Insects are considered as a sustainable protein source for future pet foods. Here we aimed to evaluate the protein quality of larvae of the black soldier fly (*Hermetia illucens*, BSF), housefly (*Musca domestica*, HF) and yellow mealworm (*Tenebrio molitor*, YMW) and to evaluate the fermentation characteristics of their indigestible fractions. Clean freeze-dried larvae were subjected to in vitro simulated canine gastric and small intestinal digestion. Undigested insect residues, shrimp chitin and fructooligosaccharides (positive control, FOS) were incubated for 48 h with inoculum with fresh feces from three dogs simulating large intestinal fermentation. The AA profiles differed among the larvae with proteins from BSF and YMW larvae containing more Val and less Met and Lys than HF larvae. The in vitro N digestibility of the HF (93.3%) and YMW (92.5%) was higher than BSF larvae (87.7%). The BSF larvae also had lower in vitro digestibility values for essential AA (92.4%) and non-essential AA (90.5%) compared to the larvae of the HF (96.6 and 96.5%) and YMW (96.9 and 95.3%). Gas production for FOS increased rapidly during the first 6 h. Low and similar amounts of gas were found for HF larvae and chitin whereas gas production slowly increased over 30 h and was slightly higher at 48 h for BSF than for chitin. Gas production for YMW increased considerably between 6 and 20 h. At 48 h, gas produced for undigested residues was comparable to shrimp chitin and lower than FOS ($P < 0.001$). Incubation with insect residues resulted in more N-acetylglucosamine than with shrimp chitin ($P < 0.05$), suggesting higher microbial degradation of insect chitin. Fecal microbiota from one dog appeared to be better able to ferment the undigested residue of YMW as gas production increased considerably between 6 and 20 h of incubation and was higher than for the microbiota from the other two dogs. The associated metabolite profile indicated that acetate, propionate and butyrate were the main fermentation products. Furthermore, formate was produced in relatively high amounts. It is concluded that the protein quality, based on the amino acid profile and digestibility, of selected insect larvae was high with the undigested insect fractions being at least partly fermented by the dog fecal microbiota. As the microbiota from one dog was better capable of fermenting the undigested residue of YMW larvae, it is of interest to further study the selective growth of intestinal microbiota in dogs fed insect-based food.



*Abbreviations:* AA, amino acid; BSF, black soldier fly; BW, body weight; CP, crude protein; DM, dry matter; FOS, fructooligosaccharide; HF, housefly; NMR, nuclear magnetic resonance; OM, organic matter; VFA, volatile fatty acid.

* Corresponding author.
*E-mail address:* guido.bosch@wur.nl (G. Bosch).

http://dx.doi.org/10.1016/j.anifeedsci.2016.08.018
0377-8401/

## 1. Introduction

The combination of a growing human population, increasing standard of living and urbanization in developing countries fuels global demand of and competition for protein sources for consumption by humans and animals (Aiking, 2011; Boland et al., 2013). The pet food industry is tightly interlinked with the human food industry and livestock and fish production systems and, as such, also competes for the resources (Swanson et al., 2013). With over 140 million dogs and 160 million cats living in households in Europe and the USA (FEDIAF, 2012; Pet Food Institute, 2014) and growing populations particularly in Asia and Latin America, the availability of high quality and sustainable protein sources for pet food production is increasing in importance. Consequently, the search for and development of additional protein sources for human foods and animal feeds is intensifying (Aiking, 2011; Boland et al., 2013). Insects have been proposed as a high quality alternative protein source for both humans and animals (Rumpold and Schlüter, 2013; Van Huis et al., 2013) and can be reared on various sources of organic waste, which contributes to their sustainability (Van Huis et al., 2013).

Among insects, larvae of the black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae BSF), housefly (*Musca domestica* (L.), Diptera: Muscidae; HF) and mealworm (*Tenebrio molitor* (L.), Coleoptera: Tenebrionidae; MW) can be reared on various sources of organic waste contributing to their sustainability as feed ingredients for pig and poultry (Veldkamp and Bosch, 2015) and fish (Barroso et al., 2014). Also application of insects in pet foods is gaining attention (Bosch et al., 2014; McCusker et al., 2014). Few data are, however, available regarding the nutritional quality of insects as alternative protein sources for various animal species (Veldkamp and Bosch, 2015). The protein quality of these alternative sources is mainly dependent on the amino acid (AA) composition as well as AA bioavailability. The AA composition of larvae of the BSF, HF and MW has been described (Bosch et al., 2014; Makkar et al., 2014; McCusker et al., 2014), but information regarding AA bioavailability in various animal species is highly limited. Three studies evaluated the AA bioavailability of dehydrated HF larvae in turkey chickens (Zuidhof et al., 2003) and broiler chickens (Hwangbo et al., 2009; Pieterse and Pretorius, 2013) using the apparent fecal digestibility approach. These estimates of AA bioavailability have, however, limited accuracy due to large intestinal or cecal microbial AA metabolism (Hendriks et al., 2012). Recently, De Marco et al. (2015) reported an average apparent ileal AA digestibility of 85% for a MW larvae meal and 66% for a BSF larvae meal in 5-week old broiler chickens.

Apart from the pre-cecal digestible components, the non-digestible components of insects are important for pet food applications. Dogs have a relatively simple large intestine, but potential fermentation of non-digested components affect microbial activity and composition, host intestinal and general health and fecal attributes like consistency and odor. The cuticle of insects contains chitin, a linear polymer of β-(1–4) *N*-acetyl-D-glucosamine units, which is embedded in a matrix with proteins (Andersen et al., 1995a). The proteins and sclerotization largely determine the mechanical properties of the cuticle and may vary between body regions of, and among insects (Andersen et al., 1995a). For BSF and MW larvae, the amount of chitin was estimated to be 5.4 and 2.8% of dry matter (DM) (Finke, 2013). Chitin may be degraded using intestinal chitinases, which are found in humans, mice (Boot et al., 2005) and several bat species (Strobel et al., 2013). Although dogs originate from wolves that have diets low in invertebrates (Bosch et al., 2015), their genome also contains chitinase protein-coding genes (Bussink et al., 2007) and chitinolytic activity has been found in extracts of the gastric mucosa of dogs (Cornelius et al., 1975) suggesting gene expression and secretion of chitinase. Degradation of chitin from squid pen in the gastrointestinal tract of dogs, however, was low (Okamoto et al., 2001), indicating a low digestive efficiency of chitinase for this specific source. In addition, microbial chitinolytic activity in the dogs was low, which may categorize chitin a non-fermentable fiber. Given the role of fermentable and non-fermentable dietary fibers in intestinal microbial activity and health (Fahey et al., 2004), it is of interest to study the potential fermentation characteristics of indigestible parts of insects containing chitin.

The objectives of this study were to evaluate the bioavailability of AA in BSF, HF and MW larvae and to study the fermentation characteristics of the indigestible fractions of BSF, HF and MW larvae by the canine large intestinal microbiota. In vitro methods were applied to simulate the dog's digestive processes in the stomach and small intestine (Hervera et al., 2007) and in the large intestine (Bosch et al., 2013). As potential microbial metabolites derived from these substrates are not extensively described in literature, non-targeted and comprehensive $^{1}$H nuclear magnetic resonance (NMR) spectroscopy was applied to detect a variety of different fermentation metabolites (Jacobs et al., 2008).

## 2. Materials and methods

### 2.1. Substrates

Live BSF larvae (Koppert B.V., Berkel en Rodenrijs, the Netherlands) and YMW larvae (Kreca, Ermelo, the Netherlands) were washed with demineralized water on a metal filter screen (1.25 mm), dried with a towel and stored frozen (−20 °C). Live HF larvae (Jagran B.V., Hillegom, the Netherlands) were distributed in buckets forming thin layers (∼1 cm) and frozen (−20 °C). Once frozen, larvae were sieved (1.25 mm screen) to remove the growing substrate and excreta. All frozen insects were freeze-dried to a constant weight after which remnants of the growing substrate and excreta were manually removed. Cleaned insects were ground using an ultra-centrifugal mill at 10,000 rpm without a sieve (ZM100, Retch BV, Ochten, The Netherlands). Substrates were stored at −20 °C until further analyses. Control substrates were chitin from shrimp shells

(Sigma-Aldrich, Saint Louis, MO, US) and fructooligosaccharides (Raftifeed IPS, Orafti, Tienen, Belgium; positive control, FOS).

### 2.2. In vitro digestion

Substrates were digested according to an up-scaled Boisen two-step in vitro method (Boisen and Fernández, 1997) with modifications in order to simulate canine digestive physiology characteristics (Hervera et al., 2007) and to collect the undigested residue (Jha et al., 2011). Amount of substrate incubated was standardized to 10 g air-dry material. Substrates were incubated in beakers with a phosphate buffer solution (250 ml, 0.1 M, pH 6.0) and a HCl solution (100 ml, 0.2 M). The pH was adjusted to 2.0 with 1 M HCl or 10 M NaOH. Fresh pepsin solution (10 ml, 25 g/l, porcine pepsin 2000 FIP U/g, Merck 7190) and chloramphenicol solution (0.005 g/ml ethanol) were added. Each beaker was covered with a glaze and placed in a heating chamber at 39 °C under constant magnetic stirring. Fresh pancreatin solution was prepared by mixing 140 g of pancreatin (Porcine pancreas grade VI, SigmaP-1750) in 140 ml phosphate buffer (0.2 M, pH 6.8) under continuous magnetic stirring for 15 min. Non-solubilized material was removed by centrifugation (10 min at 3000 rpm/min, 20 °C). After 2 h of incubation, 90 ml phosphate buffer (0.2 M, pH 6.8) and 50 ml of 0.6 M NaOH were added to the solution. The pH was adjusted to 6.8 with 1 M HCl or 10 M NaOH and 10 ml of the supernatant of the pancreatin solution was added. The hydrolysis was continued for 4 h under the same conditions. After hydrolysis, the undigested residues were collected by filtration of the slurries on a nylon gauze (pore size 37 μm, permeability 24%, Kabel Zaandam B.V., Zaandam, the Netherlands), washed twice with 99.5% acetone and 95% ethanol to remove lipids and free sugars. Residues were allowed to dry overnight and transferred to pre-weighed plastic containers. Containers with residues were oven-dried to a constant weight (70 °C, 18 h) and weighed again. The number of replicates differed per insect type and was 6 for BSF, 4 for HF and 12 for YMW larvae. The intra-assay coefficient of variation of the digestion procedure for DM digestibility was 1.4% for BSF and 0.4% for HF and YMW larvae. The dried residues were pooled per insect species and mildly crushed to a fine powder with a mortar and pestle.

### 2.3. In vitro fermentation

All experimental procedures were approved by Wageningen University. Fresh feces were collected from three privately-owned retriever-type dogs: one intact male (9 mo, 31 kg body weigh (BW); dog 1), one intact female (12 yr, 31 kg BW; dog 2), one castrated male (11 yr, 40 kg BW; dog 3). Dogs were fed a nutritionally complete canned diet (Pedigree pate with beef, Masterfoods GMBH, Verden, Germany) with a gross composition (g/kg as is basis) of 185 DM, 70 crude protein (CP), 55 crude fat, 5 crude fiber, and 23 crude ash (values provided by the manufacturer). Dogs were fed approximately 460 kJ of energy/kg of $BW^{0.75}$ per day in two equal portions for at least 2 wk prior to feces collection. Feces were collected directly after voiding in sterilized plastic bottles prefilled with $CO_2$. After placing the feces into the bottle, a second 500 ml bottle containing $CO_2$ was poured into the bottle to ensure anaerobic conditions. The bottle with feces was closed and transported within 5 min to the laboratory where the feces were manually homogenized, weighed and diluted 1/9 (w/v) in a 39 °C anaerobic sterile physiological saline solution (9 g/l NaCl). The diluted mixture was homogenized for 60 s using a hand-blender and filtered through nylon gauze (pore size 40 μm, permeability 30%; PA 40/30, Nybolt, Switzerland). All procedures were carried out under a constant stream of $CO_2$. The filtrate was mixed with a pre-warmed N-containing medium (Williams et al., 2005) in a 5/84 (vol/vol) and flushed for 5 min with $CO_2$. For each dog, two batches of incubation bottles were prepared, i.e. one for 6 h of incubation and one for 48 h of incubation. All incubations were done in simplo. The medium/inoculum mixture (44.5 ml) was dispensed into each bottle containing 0.25 g of substrate. For each dog and incubation time, one blank bottle containing only 44.5 ml of the inoculum was incubated. Inoculated bottles were immediately attached to fully automated gas production measurement equipment (Cone et al., 1996). At t = 0, a sample (1 ml) was taken from each of the three medium/inoculum mixtures and frozen at −80 °C. After 6 h of incubation, the first batch of bottles were detached from the gas measurement equipment and 1 ml of fermentation liquid was sampled and frozen at −80 °C pending further analyses of fermentation product concentrations. The same sampling procedure was performed after 48 h of incubation.

### 2.4. Chemical analyses

Dry matter content of substrates was determined by drying to a constant weight at 103 °C (ISO, 1999b) and crude ash by combusting at 550 °C (ISO, 2002). Nitrogen content of substrates and residues was determined using the Kjeldahl method (ISO, 2005). Substrates were analyzed for crude fat according to the Berntrop method (ISO, 1999a), total starch according to an enzymatic procedure (ISO, 2004) and sugar as described by Van Vuuren et al. (1993) without a segmented flow analyzer. Amino acids were analyzed as described by Hendriks et al. (1996) and Van Rooijen et al. (2014). Phenylalanine and histidine of substrates were not reported, as the peak areas in the chromatograms for these AA appeared to be overlapping with likely glucosamine or galactosamine.

### 2.5. $^{1}$NMR spectroscopy

For measurement of metabolites, in vitro fermentation liquid samples collected and stored at −20 °C were thawed and centrifuged at 14,000 rpm for 5 min at 5 °C. The supernatant was collected, equilibrated to room temperature and diluted

**Table 1**
Chemical composition (in g/kg of dry matter) of freeze-dried and ground black soldier fly larvae (BSF), housefly larvae (HF) and yellow mealworm larvae (YMW).

| Parameter | Insect larvae | | |
|---|---|---|---|
| | BSF | HF | YMW |
| Dry matter, g/kg | 966 | 963 | 987 |
| Nitrogen | 82 | 93 | 88 |
| Crude fat | 216 | 208 | 342 |
| Crude ash | 80 | 93 | 41 |
| Essential AA | | | |
| Arginine | 20.5 | 26.8 | 25.3 |
| Isoleucine | 20.4 | 21.8 | 23.5 |
| Leucine | 31.4 | 35.4 | 38.8 |
| Lysine | 28.4 | 40.4 | 29.5 |
| Methionine | 6.9 | 11.1 | 6.6 |
| Threonine | 18.7 | 24.4 | 21.3 |
| Valine | 28.7 | 28.7 | 33.3 |
| Nonessential AA | | | |
| Alanine | 32.4 | 33.7 | 40.2 |
| Aspartic acid | 40.7 | 53.8 | 42.8 |
| Cysteine | 4.7 | 6.5 | 4.8 |
| Glutamic acid | 53.7 | 83.4 | 57.2 |
| Glycine | 25.3 | 25.1 | 27.5 |
| Serine | 19.2 | 24.0 | 22.9 |
| Tyrosine | 30.2 | 29.7 | 38.1 |
| AA-N/total N[a], % | 65.0 | 71.6 | 69.0 |

[a] The partial contribution of proteins to total N in insects (i.e. chitin also contains N) was calculated using the molar N per molar AA. For aspartic acid and glutamic acid 1.5 mol N per mol AA was used.

1:1 in phosphate buffer (75 mM, pH 7.4) containing 1 mM maleic acid as internal standard. Subsequently, a 200 μl sample was transferred to 3 mm NMR tubes (Bruker Match System, Germany), and measured at 300 K in an Avance III NMR spectrometer with a 600 MHz/54 mm UltraShielded Plus magnet equipped with a CryoPlatform cryogenic cooling system, a BCU-05 cooling unit, an ATM automatic tuning and matching unit as described previously (Haenen et al., 2013). Assignment of metabolite resonances was performed by comparing with published literature, the Human Metabolome Database online library (http://hmdb.ca/), and internal standards. Two dimensional homo- and heteronuclear NMR measurements were also performed in order to support the assignments. The NMR spectra were baseline corrected, aligned and calibrated to the internal standard. The integrals of the metabolites identified in the samples were converted into concentrations using the intensity of the internal standard as well as using calibration curves of volatile fatty acid (VFA) molecules under identical conditions as the fermentation liquid measurements. Integrals of interesting metabolites were carefully selected in order to avoid overlap of resonances. The assigned resonances were checked with the help of 2D NMR spectra.

### 2.6. Calculations and statistical analyses

Organic matter (OM) was calculated as DM—crude ash content and CP as N × 6.25. As proteins contribute partially to the analyzed total N in insects, i.e. chitin also contains N, the percentage N from all analyzed AA of total N was calculated using the molar N per molar AA. For aspartic acid and glutamic acid, 1.5 mol N per mol AA was used. In vitro DM, OM, N and AA digestibility coefficient values for the insect substrates was calculated as the amount of residue collected (in g DM) × content in residue (in percentage of DM) divided by the amount of substrate incubated (in g DM) × content in substrate (in percentage of DM). Amounts of gas and metabolites produced from substrates were expressed on a per g OM basis.

Differences between substrates for gas produced after 48 h and fermentation metabolites at 6 h and at 48 h of incubation were tested for significance using a 1-way ANOVA by the GLM procedure (SAS 9.2, SAS Institute, Cary, NC, USA). The statistical model included substrate as a fixed effect. In case of a significant effect of substrate, differences between substrates were analyzed using Tukey–Kramer's multiple comparison procedure in the LSMEANS statement in SAS. The level of significance was set at $P < 0.05$.

## 3. Results

### 3.1. Composition

Chemical composition of insect larvae is provided in Table 1. The HF larvae were slightly higher in CP than BSF and YMW larvae. BSF and HF larvae were lower in crude fat and higher in crude ash than YMW larvae. In general, the HF larvae and YMW larvae contained on a DM basis, more essential and non-essential AA than BSF larvae. Also the AA-N/total N percentage was higher for the larvae from the HF and YMW than for BSF larvae. The HF larvae were in particular rich in

**Table 2**
In vitro digestibility coefficients of freeze-dried and ground black soldier fly larvae (BSF), housefly larvae (HF) and yellow mealworm larvae (YMW).

| Parameter | Insect larvae | | |
|---|---|---|---|
| | BSF | HF | YMW |
| Dry matter | 0.814 | 0.886 | 0.923 |
| Organic matter[a] | 0.824 | 0.901 | 0.922 |
| Nitrogen | 0.877 | 0.933 | 0.925 |
| Essential AA | | | |
| Arginine | 0.935 | 0.975 | 0.979 |
| Isoleucine | 0.917 | 0.961 | 0.956 |
| Leucine | 0.916 | 0.963 | 0.965 |
| Lysine | 0.932 | 0.971 | 0.977 |
| Methionine | 0.936 | 0.967 | 1.000 |
| Threonine | 0.921 | 0.959 | 0.966 |
| Valine | 0.911 | 0.964 | 0.940 |
| Nonessential AA | | | |
| Alanine | 0.910 | 0.969 | 0.957 |
| Aspartic acid | 0.929 | 0.972 | 0.973 |
| Cysteine | 0.869 | 0.943 | 0.947 |
| Glutamic acid | 0.926 | 0.975 | 0.974 |
| Glycine | 0.866 | 0.959 | 0.911 |
| Serine | 0.907 | 0.965 | 0.946 |
| Tyrosine | 0.931 | 0.974 | 0.961 |

[a] Organic matter of original substrate and undigested residue calculated as dry matter − crude ash.

**Table 3**
Chemical composition (in g/kg of dry matter) of undigested residues of freeze-dried and ground black soldier fly larvae (ur-BSF), housefly larvae (ur-HF) and yellow mealworm larvae (ur-YMW).

| Parameter | Insect larvae | | |
|---|---|---|---|
| | ur-BSF | ur-HF | ur-YMW |
| Dry matter, g/kg | 935 | 939 | 950 |
| Nitrogen | 54 | 55 | 85 |
| Crude ash | 137 | 159 | 28 |
| Essential AA | | | |
| Arginine | 7.2 | 5.9 | 6.8 |
| Isoleucine | 9.1 | 7.5 | 13.7 |
| Leucine | 14.1 | 11.4 | 17.9 |
| Lysine | 10.3 | 10.1 | 8.9 |
| Methionine | 2.4 | 3.2 | 0.0 |
| Threonine | 8.0 | 8.8 | 9.6 |
| Valine | 13.7 | 9.0 | 26.5 |
| Nonessential AA | | | |
| Alanine | 15.7 | 9.2 | 22.8 |
| Aspartic acid | 15.6 | 13.3 | 15.3 |
| Cysteine | 3.3 | 3.2 | 3.4 |
| Glutamic acid | 21.4 | 17.9 | 19.4 |
| Glycine | 18.2 | 8.9 | 32.2 |
| Serine | 9.6 | 7.4 | 16.3 |
| Tyrosine | 11.2 | 6.7 | 19.7 |
| AA-N/total N[a], % | 43.5 | 32.7 | 35.7 |

[a] The partial contribution of proteins to total N in insects (i.e. chitin also contains N) was calculated using the molar N per molar AA. For aspartic acid and glutamic acid 1.5 mol N per mol AA was used.

lysine, methionine, threonine, aspartic acid, cysteine, glutamic acid and serine whereas YMW larvae contained relatively large amounts of isoleucine, leucine, valine, alanine, glycine, serine and tyrosine.

### 3.2. In vitro digestibility

In vitro DM and OM digestibility values of YMW larvae were higher than that of HF and BSF larvae and BSF larvae showed the lowest N digestibility values (Table 2). On average, the in vitro AA digestibility of BSF, HF and YMW larvae was respectively 3.8, 3.3 and 3.6% higher than N digestibility. In particular in BSF and YMW larvae, cysteine and glycine showed lower in vitro digestibility values than for the other AA. The undigested residues collected after in vitro digestion were lower in N for BSF and HF larvae than the original substrates whereas for the YMW larvae the N content of the residue was approximately similar (Table 3). Furthermore, the AA-N/total N percentage of the residues was lower than the original substrates with

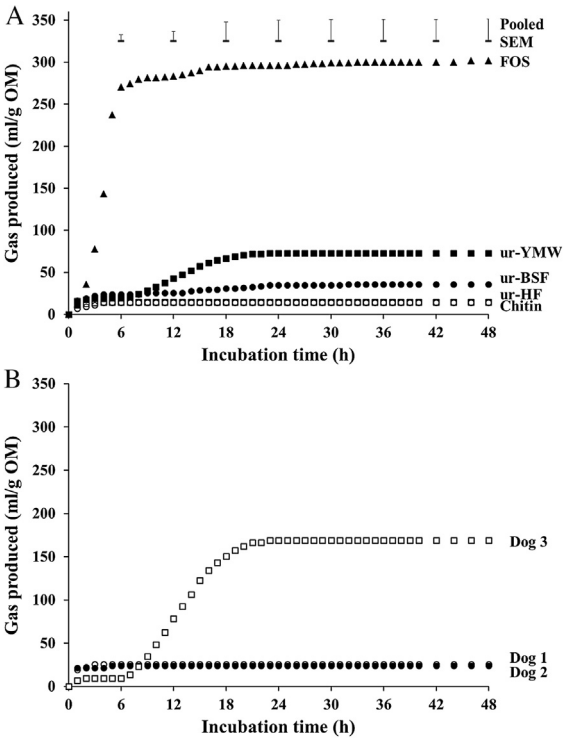


**Fig. 1.** Mean amount of gas produced from undigested residues of black soldier fly larvae (ur-BSF, ○), housefly larvae (ur-HF, ●), yellow mealworm larvae (ur-YMW, □), chitin (■), and from fructooligosaccharides (FOS, ▲) using fecal inoculum from dogs (n = 3) (panel A) and specifically from ur-YMW by fecal inoculum from dogs 1 (○), 2 (●) and 3 (□) (panel B).

the residue of BSF larvae having a higher value than the larvae of HF and YMW. The undigested residues of YMW larvae contained less crude ash than the residues of BSF and HF larvae.

### 3.3. *In vitro* fermentability

The DM and OM contents of FOS (i.e. positive control) were respectively 96.5% and 100% of DM and for chitin the contents were respectively 92.9% and 99.7% of DM (results not shown). Gas production from FOS increased rapidly from 0 to approximately 6 h, slightly increased again from 12 to 18 h of incubation and remained stable until the end of incubation (i.e. 48 h) (Fig. 1A). For the undigested residues of the BSF, HF and YMW larvae, gas production increased during approximately the first 3 h of incubation. For chitin and ur-HF larvae, no additional gas was thereafter produced whereas for the ur-BSF larvae the gas produced slightly increased until approximately 30 h of incubation and that for the ur-YMW increased considerably between 6 and 20 h of incubation. At 48 h, the amounts of gas produced from the undigested residues of the BSF, HF and YMW larvae were not different from incubation with chitin ($P > 0.05$) and lower than FOS ($P < 0.001$). Chitin and the undigested residue of HF larvae resulted in the lowest amount of gas and incubation with the undigested residues of BSF larvae resulted in slightly higher values. Gas production from the undigested residue of YMW larvae increased between 6 and 20 h and was at 48 h of incubation numerically higher than the undigested residues of BSF and HF larvae. This difference in gas production kinetics appeared to be the result of one of the three fecal donors. The inocula from dogs 1 and 2 gave low amounts of gas but incubation with fecal inoculum from dog 3 resulted in considerable amounts of gas (Fig. 1B). Incubation of blanks resulted in low amounts of gas ranging from 0.0 to 2.9 ml after 48 h of incubation (results not shown), which was similar to the absolute amounts of gas produced (i.e. not expressed on a per g OM basis) from chitin and the ur-HF larvae.

Eleven components were identified in the fermentation liquids (Table 4). At 6 h of incubation with FOS, the main metabolites in fermentation liquids were acetate, propionate, butyrate and ethanol, which were all present in larger amounts ($P < 0.001$) than for the undigested insect residues and chitin (Table 5). Lactate concentration at 6 h of incubation was numerically higher for FOS than for other substrates, which was mainly due to the considerably high concentration for dog 3 (5.06 mmol/g OM, result not shown). Formate concentration was higher for incubation with FOS compared to chitin ($P = 0.045$) whereas ethanol and propanol concentrations were higher for incubation with FOS than with all other substrates ($P < 0.001$). Incubation with the ur-BSF larvae resulted in higher succinate concentration than for FOS ($P = 0.049$). Furthermore, higher phenylalanine concentrations after 6 h of incubation with ur-BSF, ur-HF and ur-YMW larvae compared to FOS ($P = 0.004$, $P = 0.004$ and $P = 0.020$, respectively). For succinate, valerate and N-acetylglucosamine no differences ($P > 0.05$) were observed between the substrates. At 48 h of incubation, concentrations of acetate, propionate and butyrate further

**Table 4**
$^{1}$H NMR chemical shifts and concentration ranges (mmol/l) of identified compounds measured in fermentation liquids.

| Compound | Chemical shift and assignment | Concentration range |
|---|---|---|
| Acetate | 1.90 (singlet) | 2.12–41.02 |
| Propionate | 1.05 (triplet) | 1.02–31.89 |
| Butyrate | 1.54 (multiplet) | 0.23–7.76 |
| Propanol | 3.55 (triplet) | 0.00–0.67 |
| Lactate | 1.32 (doublet) | 0.00–27.36 |
| Ethanol | 1.17 (triplet) | 0.00–4.61 |
| Formate | 8.44 (singlet) | 0.10–1.55 |
| Succinate | 2.44 (singlet) | 0.00–0.04 |
| Phenylalanine | 7.30 (multiplet) | 0.03–0.63 |
| Valerate | 0.84 (triplet) (tentative) | 0.00–0.63 |
| *N*-acetylglucosamine | 2.06 (singlet) | 0.06–0.68 |

**Table 5**
Mean amount of compounds in fermentation liquids (mmol/g organic matter incubated) for undigested residues of black soldier fly larvae (ur-BSF), housefly larvae (ur-HF), yellow mealworm larvae (ur-YMW), chitin, and from fructooligosaccharides (FOS) at 6 and 48 h of incubation with fecal inoculum from dogs (n = 3).

| Compound | Substrate | | | | | Pooled SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | ur-BSF | ur-HF | ur-YMW | Chitin | FOS | | |
| 6 h incubation | | | | | | | |
| Acetate | 1.591$^{a}$ | 1.482$^{a}$ | 1.254$^{a}$ | 1.179$^{a}$ | 5.442$^{b}$ | 0.149 | <0.001 |
| Propionate | 0.890$^{a}$ | 0.944$^{a}$ | 0.787$^{a}$ | 0.696$^{a}$ | 3.846$^{b}$ | 0.278 | <0.001 |
| Butyrate | 0.271$^{a}$ | 0.294$^{a}$ | 0.221$^{a}$ | 0.149$^{a}$ | 0.507$^{b}$ | 0.033 | <0.001 |
| Propanol | 0.004$^{a}$ | 0.005$^{a}$ | 0.002$^{a}$ | 0.002$^{a}$ | 0.089$^{b}$ | 0.010 | <0.001 |
| Lactate | 0.031 | 0.004 | 0.004 | 0.001 | 2.023 | 0.678 | 0.213 |
| Ethanol | 0.273$^{a}$ | 0.262$^{a}$ | 0.233$^{a}$ | 0.178$^{a}$ | 0.778$^{b}$ | 0.027 | <0.001 |
| Formate | 0.032$^{ab}$ | 0.040$^{ab}$ | 0.032$^{ab}$ | 0.028$^{a}$ | 0.075$^{b}$ | 0.010 | 0.040 |
| Succinate | 0.002 | 0.002 | 0.002 | 0.002 | 0.000 | 0.000 | 0.053 |
| Phenylalanine | 0.100$^{a}$ | 0.102$^{a}$ | 0.084$^{a}$ | 0.071$^{ab}$ | 0.026$^{b}$ | 0.011 | 0.003 |
| Valerate | 0.037 | 0.041 | 0.049 | 0.040 | 0.020 | 0.021 | 0.894 |
| *N*-acetylglucosamine | 0.062 | 0.077 | 0.069 | 0.057 | 0.037 | 0.020 | 0.700 |
| 48 h incubation | | | | | | | |
| Acetate | 3.453$^{a}$ | 2.934$^{a}$ | 3.349$^{a}$ | 1.347$^{a}$ | 6.902$^{b}$ | 0.664 | 0.002 |
| Propionate | 1.885$^{a}$ | 1.403$^{ab}$ | 1.236$^{ab}$ | 0.734$^{b}$ | 5.632$^{c}$ | 0.145 | <0.001 |
| Butyrate | 0.511 | 0.315 | 0.627 | 0.102 | 0.755 | 0.222 | 0.317 |
| Propanol | 0.002$^{a}$ | 0.003$^{a}$ | 0.002$^{a}$ | 0.002$^{a}$ | 0.095$^{b}$ | 0.007 | <0.001 |
| Lactate | 0.001 | 0.006 | 0.015 | 0.001 | 0.004 | 0.006 | 0.486 |
| Ethanol | 0.042$^{a}$ | 0.057$^{a}$ | 0.066$^{a}$ | 0.153$^{ab}$ | 0.438$^{b}$ | 0.064 | 0.007 |
| Formate | 0.080 | 0.065 | 0.127 | 0.028 | 0.024 | 0.046 | 0.521 |
| Succinate | 0.001 | 0.001 | 0.002 | 0.005 | 0.000 | 0.001 | 0.167 |
| Phenylalanine | 0.117$^{ab}$ | 0.123$^{a}$ | 0.097$^{ab}$ | 0.074$^{bc}$ | 0.031$^{c}$ | 0.010 | <0.001 |
| Valerate | 0.133$^{a}$ | 0.122$^{a}$ | 0.100$^{ab}$ | 0.062$^{bc}$ | 0.043$^{c}$ | 0.009 | <0.001 |
| *N*-acetylglucosamine | 0.138$^{a}$ | 0.137$^{a}$ | 0.124$^{a}$ | 0.091$^{b}$ | 0.047$^{c}$ | 0.007 | <0.001 |

Mean values with different superscript letters within a row were significantly ($P < 0.05$) different.

increased for all substrates. Production of acetate and propionate from FOS was higher than from other substrates but substrates did not differ in butyrate concentrations. Incubation with ur-BSF resulted in more propionate than with chitin ($P = 0.002$). Valerate concentrations were also higher at 48 h of incubation and were higher for the undigested residues of the larvae substrates than for FOS ($P < 0.010$). Lactate concentrations for incubation with FOS at the end of incubation were low and not different from other substrates. Similarly, lower ethanol concentrations were noted for all substrates compared to 6 h of incubation, with higher concentrations for incubation with FOS relative to the ur-BSF, ur-HF and ur-YMW larvae ($P = 0.009$, $P = 0.012$ and $P = 0.014$, respectively). Propanol concentrations were higher for incubation with FOS than with all other substrates ($P < 0.001$). Substrates did not differ in succinate and formate concentrations. Concentrations of phenylalanine were higher for undigested residues of the insect larvae substrates than for FOS ($P < 0.001$). Incubation with the undigested residues of the insect larvae substrates resulted in more N-acetylglucosamine than for incubation with chitin ($P < 0.05$) and with FOS ($P < 0.001$). For dog 3, the main microbial metabolites from ur-YMW at 48 h of incubation were acetate (5.95 mmol/g OM), propionate (1.57 mmol/g OM) and butyrate (1.51 mmol/g OM) (results not shown). In addition, the concentration of formate increased from 0.09 mmol/l before incubation to 0.47 mmol/l at 48 h of incubation.

## 4. Discussion

This study evaluated aspects of nutritional quality of BSF, HF and YMW larvae, which are potential sustainable alternative protein sources for pet foods. The composition of the insect larvae varies among studies (reviewed by Makkar et al., 2014).

The BSF larvae used here were slightly higher in CP and lower in crude ash than in other studies (Makkar et al., 2014; McCusker et al., 2014) although a slightly higher CP content was found in a previous study (Bosch et al., 2014). The lower crude ash content of BSF larvae might be related to a lower amount of calcium carbonate covering the integument (Johannsen, 1922) and to a low ash content of the growing substrate present in the gut at harvest as observed in other insect species (Finke and Oonincx, 2013). The AA composition of the HF and YMW larvae was within the ranges reported in the scientific literature (Makkar et al., 2014). The AA profile of BSF larvae was very close to the profile (CP basis) reported in Bosch et al. (2014), although the valine contents were lower in the present study (5.6 vs. 6.3% of CP). The valine contents of BSF and YMW larvae were higher compared to the reference data for conventional protein sources like meat and bone meal (4.4%, n = 1230), fish meal (5.0%, n = 10,409) and soybean meal (4.8%, n = 1198) (Sauvant et al., 2004). Furthermore, compared to these conventional sources, studied insects contained relatively high amounts of histidine and tyrosine and low amounts of arginine. In foods for adult dogs that are based on soybean or rendered meat meals, methionine is generally the first limiting AA (NRC, 2006). Proteins from BSF and YMW larvae contained similar amounts of methionine as those in meat and bone meal and soybean meal but lower amounts than fish meal. Intermediate amounts of methionine were found for the HF larvae. The protein of HF larvae contained less lysine than fish meal (7.5%) but more than BSF larvae, YMW larvae, meat and bone meal (5.0%) and soybean meal (6.1%).

The protein quality of ingredients depends not only on the amounts of AA, but also on their AA bioavailability, for which limited data are currently available for insects. Bioavailability of AA was assessed using an in vitro method simulating digestive processes in the stomach and small intestine (Hervera et al., 2007). The method has been applied in several studies evaluating OM and N digestibility of single ingredients and complete pet foods. Similar in vitro OM and N digestibility values for BSF and YMW larvae were found in a previous study (84.3 and 89.7% for BSF and 91.5 and 91.3% for YMW, respectively) (Bosch et al., 2014). Although these insects were minimally processed and not heat-treated like proteins in commercial pet foods, the in vitro N digestibility values are high and in the range of values found for 54 commercial dog and cat foods (84.7–99.7%) using similar laboratory procedures (Van Zelst et al., 2015). The higher in vitro AA digestibility values for YMW compared to BSF larvae were in line with apparent ileal digestibility findings in broiler chickens of De Marco et al. (2015), although the values in the present study were greater (96 vs. 85% and 91 vs. 66%, respectively). These differences can in part be explained by the different insect processing procedures and assays used. The considerably low AA digestibility value for the BSF larvae in the latter study was related to values for isoleucine (45%), lysine (46%), methionine (42%) and tyrosine (43%), although it remained unclear what caused these low values. The high AA content in combination with the high in vitro AA digestibility values found in the present study indicate that minimally processed insect larvae have a high protein quality, which is an important characteristic. For application of insect protein meals in pet foods, it is of interest to use processing techniques that minimally impact the bioavailability of the AA but also sanitize the insects in particular when these are produced from organic waste streams.

The fraction from the food that is not digested and absorbed in the small intestine, is potentially substrate for the microbiota in the large intestine. Based on the chemical characterization of the undigested residues collected after in vitro digestion, these fractions contain at least N-containing compounds and minerals. Part of the N in the residues would originate from protein but also from chitin, a linear polymer of β-(1–4) *N*-acetylglucosamine units, which is embedded in a matrix with proteins of the cuticle (Andersen et al., 1995a). The proteins and sclerotisation largely determine the mechanical properties of the cuticle and may vary between body regions of insects and vary among insects (Andersen et al., 1995a). Chitin may be degraded using intestinal chitinases, which are found in humans and mice (Boot et al., 2005). Although dogs originate from wolves that have diets low in invertebrates (Bosch et al., 2015), their genome also contains chitinase protein-coding genes (Bussink et al., 2007) and chitinolytic activity has been found in extracts of the gastric mucosa of dogs (Cornelius et al., 1975) suggesting gene expression and secretion of chitinase. Degradation of chitin from squid pen in the gastrointestinal tract of dogs, however, was low (Okamoto et al., 2001), indicating a low digestive efficiency of chitinase for this specific source. In addition, microbial chitinolytic activity in the dogs was low, which may make chitin a non-fermentable fiber. The BSF and YMW larvae contain approximately 5.4 and 2.8% chitin of DM (Finke, 2013). When applying the fractions of N from chitin of total N for these larvae from that study and assuming a 0% digestibility of chitin (Okamoto et al., 2001), the in vitro digestibility of chitin-corrected N would be 92.7% for BSF larvae and 95.6% for YMW larvae. These estimates are in close agreement with the average AA digestibility values of respectively 91.5 and 96.1% in the present study. Based on the N content of the BSF and MW larvae and their residues and on the chitin content of the larvae (Finke, 2013), the chitin content in the residue would approximately be respectively 32.9 and 51.5% of DM and protein (chitin-corrected N × 6.25) 19.9 and 31.7% of DM. Resistance to proteolysis of these proteins was likely, in part, related to disulphide linkages between two cysteine molecules. This is substantiated by the lower in vitro digestibility of cysteine of the insect larvae than that for the other AA. Cuticular proteins of the integument, however, tend to be low in cysteine (Andersen et al., 1995a; Willis, 2010), suggesting that cysteine is likely part of other structural components of insects such as the peritrophic matrix. This non-cellular semipermeable membrane lines the gut of most insects (Tellam et al., 1999). It is mainly composed of class III and IV peritrophins, which are proteins rich in cysteine that show proteinase resistance and contribute to structure of the matrix by covalently cross-linking to other peritrophins and constituents such as chitin or proteoglycans (Tellam et al., 1999). The lower in vitro glycine digestibility for BSF and MW larvae was likely related to cuticle proteins (Andersen et al., 1995a). In particular the epicuticle consists of a tightly cross-linked network of proteins and lipids suggested to be resistant to degradation (Andersen, 1979). The proteins in the hard and stiff cuticle of MW larvae are, next to glycine, richer in alanine, tyrosine, valine and proline compared to the soft larval cuticles of dipteran species (Andersen et al., 1995b). The latter is in

line with the high amounts of alanine, tyrosine and valine in the undigested residues of YMW larvae relative to the residues of BSF and HF larvae with soft cuticles. The structural and elastic protein in insects named resilin is also rich in glycine, but is likely not a substrate for the large intestinal microbiota as it is suggested to be well digestible (Weis-Fogh, 1960).

The in vitro fermentation of the undigested insect residues resulted in, relative to the positive control FOS, little gas production and metabolite formation. The amount of gas produced was in line with the values for FOS in two previous studies in the same laboratory (Bosch et al., 2008, 2013). The production of acetate and butyrate from FOS was close to that observed in Bosch et al. (2008) after 72 h of incubation but propionate production was higher in the present study. Such differences in metabolite production may be related to variation in the composition of microbial communities among donors as affected by genetics (Hand et al., 2013) or diet (Sunvold et al., 1995). The difference between FOS and residues of the insect larvae was larger for the amount of gas produced than for the amount of the main fermentation metabolite acetate. The amount of gas produced originates from direct gas production during substrate fermentation but also from $CO_2$ released from the carbonate buffer (indirect gas production) when VFA are produced. In case AA are fermented, $NH_3$ is produced that binds H+ ions and as such prevents the release of $CO_2$ from the buffer (Cone and Van Gelder, 1999). The fermentability of undigested residues of insects can therefore be better derived by the formed metabolites. The fermentation of AA like proline and hydroxyproline in structural proteins yields acetate, butyrate, and to a lesser extent, propionate but also fatty acids like valerate (Macfarlane and Macfarlane, 2003). The amounts of VFA formed from the undigested residues of insects were less than from FOS but in the range of VFA from poultry meat meal after 72 h of incubation (Bosch et al., 2013) indicating that the insect residues were relatively well fermentable. The intermediate fermentation metabolites lactate, ethanol and formate (Macfarlane and Macfarlane, 2003) were in particularly found after 6 h of incubation with FOS. Fucose was likely present in FOS, as indicated by its fermentation product propanol (Louis et al., 2007). Little information is available regarding the fermentation of chitin. Microbial degradation of marine chitin yields acetate, $NH_3$ and fructose-6-phosphate (Keyhani and Roseman, 1999). Fructose-6-phosphate is further catabolized to pyruvate, which can be further degraded to acetate, propionate, butyrate and gasses (Macfarlane and Macfarlane, 2003). Crab shell chitin incubated with isolated fecal microbes from pigs, horses and takins yielded in particular propionate and butyrate (Šimůnek et al., 2001), although no amounts were presented. The levels of fermentation products found for chitin from shrimp shells were in line with those for the blanks (results not shown), indicating that this chitin source appeared to be non-fermentable by fecal microbiota from dogs. The levels of N-acetylglucosamine were, however, higher in the undigested residues of the insect larvae than that of chitin after 48 h of incubation, suggesting at least some microbial hydrolysis of chitin. The higher valerate concentrations from some insect residues than from shrimp chitin might also reflect microbial catabolism of chitin, although valerate may also originate from microbial amino acid catabolism (Macfarlane and Macfarlane, 2003). To what extent chitin from these insects is fermentable remains unclear as chitin was not quantified. The fermentation of the indigestible fraction of YMW larvae was different for dog 3. After a lag time of approximately 6 h gas production increased and after 48 h substantial amounts of VFA were found indicating considerable higher microbial utilization of the undigested residue of this substrate than for the other two dogs. The lag time indicates that first selective growth of microbes was required before substantial fermentation occurred, which was observed for gum arabic using dog microbiota (Bosch et al., 2008) and alginate using pig microbiota (Jonathan et al., 2012). Although the N-acetylglucosamine concentration in the fermentation liquid for this dog was similar to that in the other two dogs (data not shown), a higher utilization capacity of YMW-chitin might explain the differences between the three dogs. In particular, high amounts of formate were formed. In humans, formate is formed by *Bifidobacterium breve* when carbohydrate is limited (Macfarlane and Macfarlane, 2003). Furthermore, formate has a pungent odor, has antibiotic activity against yeasts and some bacterial species and is readily absorbed and metabolized (Partanen and Mroz, 1999). Based on these findings, it is of interest to further study the selective growth of intestinal microbiota in a larger population of dogs fed a diet including insect as a protein source. Overall, these data suggest that the intestinal microbiota from the dogs can utilize the undigested AA from BSF, HF and YMW larvae. The chitin of YMW may be utilized by the canine microbiota and warrants further study.

## 5. Conclusions

The protein quality of BSF, HF and YMW larvae was high with larvae containing large amounts of bioavailable protein and essential AA. The AA profiles differed among these insects with proteins from BSF and YMW larvae containing more valine and less methionine and lysine than HF larvae. The high bioavailability of the AA should be ensured when insects are processed to protein meals and incorporated in pet foods. The proteins in undigested fractions of the insect larvae were fermented by the canine fecal microbiota and the fermentability of chitin of insects require further study. The microbiota from one dog was better capable of fermenting the undigested residue of YMW larvae. It is, therefore, of interest to further study the selective growth of intestinal microbiota in a larger population of dogs fed a diet including insect as protein source.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

## Acknowledgements

The study was part of the strategic research program of Wageningen UR "Customized Nutrition" and financed by Wageningen UR and an industrial partner. The funding sources were not involved in the study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the article for publication. The authors thank R. Wormgoor and M. Hendriks for care of the dogs, S. van Laar-Schuppen and M. Breuer for their technical assistance during this study.

## References

Aiking, H., 2011. Future protein supply. Trends Food Sci. Technol. 22, 112–120.
Andersen, S.O., Hojrup, P., Roepstorff, P., 1995a. Insect cuticular proteins. Insect Biochem. Mol. Biol. 25, 153–176.
Andersen, S.O., Rafn, K., Krogh, T.N., Hojrup, P., Roepstorff, P., 1995b. Comparison of larval and pupal cuticular proteins in *Tenebrio molitor*. Insect Biochem. Mol. Biol. 25, 177–187.
Andersen, S.O., 1979. Biochemistry of insect cuticle. Annu. Rev. Entomol. 24, 29–61.
Barroso, F.G., de Haro, C., Sánchez-Muros, M.J., Venegas, E., Martínez-Sánchez, A., Pérez-Bañón, C., 2014. The potential of various insect species for use as food for fish. Aquaculture 422–423, 193–201.
Boisen, S., Fernández, J.A., 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. Feed Sci. Technol. 68, 277–286.
Boland, M.J., Rae, A.N., Vereijken, J.M., Meuwissen, M.P.M., Fischer, A.R.H., van Boekel, M.A.J.S., Rutherfurd, S.M., Gruppen, H., Moughan, P.J., Hendriks, W.H., 2013. The future supply of animal-derived protein for human consumption. Trends Food Sci. Technol. 29, 62–73.
Boot, R.G., Bussink, A.P., Verhoek, M., De Boer, P.A.J., Moorman, A.F.M., Aerts, J.M.F.G., 2005. Marked differences in tissue-specific expression of chitinases in mouse and man. J. Histochem. Cytochem. 53, 1283–1292.
Bosch, G., Pellikaan, W.F., Rutten, P.G.P., van der Poel, A.F.B., Verstegen, M.W.A., Hendriks, W.H., 2008. Comparative in vitro fermentation activity in the canine distal gastrointestinal tract and fermentation kinetics of fiber sources. J. Anim. Sci. 86, 2979–2989.
Bosch, G., Wrigglesworth, D.J., Cone, J.W., Pellikaan, W.F., Hendriks, W.H., 2013. Effect of preservation conditions of canine faeces on in vitro fermentation. J. Anim. Sci. 91, 259–267.
Bosch, G., Zhang, S., Oonincxs, D.G.A.B., Hendriks, W.H., 2014. Protein quality of insects as potential ingredients for dog and cat foods. J. Nutr. Sci. 3, 21–24 (e29).
Bosch, G., Hagen-Plantinga, E.A., Hendriks, W.H., 2015. Dietary nutrient profiles of wild wolves: insights for optimal dog nutrition? Br. J. Nutr. 113, S40–S54.
Bussink, A.P., Speijer, D., Aerts, J.M.F.G., Boot, R.G., 2007. Evolution of mammalian chitinase(-like) members of family 18 glycosyl hydrolases. Genetics 177, 959–970.
Cone, J.W., Van Gelder, A.H., 1999. Influence of protein fermentation on gas production profiles. Anim. Feed Sci. Technol. 76, 251–264.
Cone, J.W., Van Gelder, A.H., Visscher, G.J.W., Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Technol. 61, 113–128.
Cornelius, C., Dandrifosse, G., Jeuniaux, C., 1975. Biosynthesis of chitinases by mammals of the order Carnivora. Biochem. Syst. Ecol. 3, 121–122.
De Marco, M., Martínez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L., Belforti, M., Bergero, D., Katz, H., Dabbou, S., Kovitvadhi, A., Zoccarato, I., Gasco, L., Schiavone, A., 2015. Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens Apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. Anim. Feed Sci. Technol. 209, 211–218.
FEDIAF, 2012. Facts & Figures http://www.fediaf.org/the-european-pet-food-industry/facts-figures/ (accessed 17 December).
Fahey Jr., G.C., Flickinger, A.E., Grieshop, C.M., Swanson, K.S., 2004. The role of dietary fibre in companion animal nutrition. In: Van der Kamp, J.W., Asp, N.-G., Miller Jones, J., Schaafsma, G. (Eds.), Dietary Fibre: Bio-active Carbohydrates for Food and Feed. Wageningen Academic Publishers Wageningen, The Netherlands, pp. 295–328.
Finke, M.D., Oonincx, D.G.A.B., 2013. Insects as food for insectivores. In: Morales-Ramos, J.A., Rojas, M.G., Shapiro-Ilan, D.I. (Eds.), Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens. Elsevier Science, London, UK, pp. 583–616.
Finke, M.D., 2013. Complete nutrient content of four species of feeder insects. Zoo Biol. 32, 27–36.
Haenen, D., Souza da Silva, C., Zhang, J., Koopmans, S.J., Bosch, G., Vervoort, J., Gerrits, W.J.J., Kemp, B., Smidt, H., Müller, M., Hooiveld, G.J.E.J., 2013. Resistant starch induces catabolic but suppresses immune and cell division pathways and changes the microbiome in proximal colon of male pigs. J. Nutr. 143, 1889–1898.
Hand, D., Wallis, C., Colyer, A., Penn, C.W., 2013. Pyrosequencing the canine faecal microbiota: breadth and depth of biodiversity. PLoS One 8, e53115.
Hendriks, W.H., Moughan, P.J., Tarttelin, M.F., 1996. Gut endogenous nitrogen and amino acid excretions in adult domestic cats fed a protein-free diet or an enzymatically hydrolyzed casein-based diet. J. Nutr. 126, 955–962.
Hendriks, W.H., van Baal, J., Bosch, G., 2012. Ileal and faecal protein digestibility measurement in monogastric animals and humans: a comparative species view. Br. J. Nutr. 108, S247–S257.
Hervera, M., Baucells, M.D., Blanch, F., Castrillo, C., 2007. Prediction of digestible energy content of extruded dog food by in vitro analyses. J. Anim. Physiol. Anim. Nutr. 91, 205–209.
Hwangbo, J., Hong, E.C., Jang, A., Kang, H.K., Oh, J.S., Kim, B.W., Park, B.S., 2009. Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. J. Environ. Biol. 30, 609–614.
ISO, 1999a. Animal Feeding Stuffs—Determination of Fat Content (ISO 6492). International Organization for Standardization, Geneva, Switzerland.
ISO, 1999b. Animal Feeding Stuffs—Determination of Moisture and Other Volatile Matter Content (ISO 6496). International Organization for Standardization, Geneva, Switzerland.
ISO, 2002. Animal Feeding Stuffs—Determination of Crude Ash (ISO 5984). International Organization for Standardization, Geneva, Switzerland.
ISO, 2004. Animal Feeding Stuffs—Enzymatic Determination of Total Starch Content. International Organization for Standardization, Geneva, Switzerland.
ISO, 2005. Animal Feeding Stuffs—Determination of Nitrogen Content and Calculation of Crude Protein Content—Part 1: Kjeldahl Method (ISO 5983-1). International Organization for Standardization, Geneva, Switzerland.
Jacobs, D.M., Deltimple, N., van Velzen, E., van Dorsten, F.A., Bingham, M., Vaughan, E.E., van Duynhoven, J., 2008. $^{1}$H NMR metabolite profiling of feces as a tool to assess the impact of nutrition on the human microbiome. NMR Biomed. 21, 615–626.
Jha, R., Bindelle, J., Van Kessel, A., Leterme, P., 2011. In vitro fibre fermentation of feed ingredients with varying fermentable carbohydrate and protein levels and protein synthesis by colonic bacteria isolated from pigs. Anim. Feed Sci. Technol. 165, 191–200.
Johannsen, O.A., 1922. Stratiomyiid larvae and puparia of the North Eastern States. J. N.Y. Entomol. Soc. 30, 141–153.
Jonathan, M.C., Van Den Borne, J.J.G.C., Van Wiechen, P., Souza Da Silva, C., Schols, H.A., Gruppen, H., 2012. In vitro fermentation of 12 dietary fibres by faecal inoculum from pigs and humans. Food Chem. 133, 889–897.
Keyhani, N.O., Roseman, S., 1999. Physiological aspects of chitin catabolism in marine bacteria. Biochim. Biophys. Acta 1473, 108–122.
Louis, P., Scott, K.P., Duncan, S.H., Flint, H.J., 2007. Understanding the effects of diet on bacterial metabolism in the large intestine. J. Appl. Microbiol. 102, 1197–1208.

Macfarlane, S., Macfarlane, G.T., 2003. Regulation of short-chain fatty acid production. Proc. Nutr. Soc. 62, 67–72.
Makkar, H.P.S., 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.
McCusker, S., Buff, P.R., Yu, Z., Fascetti, A.J., 2014. Amino acid content of selected plant, algae and insect species: a search for alternative protein sources for use in pet foods. J. Nutr. Sci. 3, e39 31–35.
NRC, 2006. Nutrient Requirements of Dogs and Cats. National Academies Press, Washington, D.C., U.S.
Okamoto, Y., Nose, M., Miyatake, K., Sekine, J., Oura, R., Shigemasa, Y., Minami, S., 2001. Physical changes of chitin and chitosan in canine gastrointestinal tract. Carbohydr. Polym. 44, 211–215.
Partanen, K.H., Mroz, Z., 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12, 117–145.
Pet Food Institute, 2014. Pet dog and cat population http://www.petfoodinstitute.org/?page=PetPopulation (accessed 12.12.17.).
Pieterse, E., Pretorius, Q., 2013. Nutritional evaluation of dried larvae and pupae meal of the housefly (*Musca domestica*) using chemical- and broiler-based biological assays. Anim. Prod. Sci. 54, 347–355.
Rumpold, B.A., Schlüter, O.K., 2013. Potential and challenges of insects as an innovative source for food and feed production. Innov. Food Sci. Emerg. Technol. 17, 1–11.
Šimůnek, J., Hodrová, B., Bartoňová, H., Kopečný, J., 2001. Chitinolytic bacteria of the mammal digestive tract. Folia Microbiol. 46, 76–78.
Sauvant, D., Perez, J.M., Tran, G., 2004. Tables of Composition and Nutritional Value of Feed Materials: Pigs, Poultry, Cattle, Sheep, Goats, Rabbits, Horses and Fish. Wageningen Academic Publishers, Wageningen, the Netherlands.
Strobel, S., Roswag, A., Becker, N.I., Trenczek, T.E., Encarnação, J.A., 2013. Insectivorous bats digest chitin in the stomach using acidic mammalian chitinase. PLoS One 8.
Sunvold, G.D., Fahey Jr., G.C., Merchen, N.R., Reinhart, G.A., 1995. In vitro fermentation of selected fibrous substrates by dog and cat fecal inoculum: influence of diet composition on substrate organic matter disappearance and short-chain fatty acid production. J. Anim. Sci. 73, 1110–1122.
Swanson, K.S., Carter, R.A., Yount, T.P., Aretz, J., Buff, P.R., 2013. Nutritional sustainability of pet foods. Adv. Nutr. 4, 141–150.
Tellam, R.L., Wijffels, G., Willadsen, P., 1999. Peritrophic matrix proteins. Insect Biochem. Mol. Biol. 29, 87–101.
Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., Vantomme, P., 2013. Edible Insects: Future Prospects for Food and Feed Security. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
Van Rooijen, C., Bosch, G., Van der Poel, A.F.B., Wierenga, P.A., Alexander, L., Hendriks, W.H., 2014. Quantitation of Maillard reaction products in commercially available pet foods. J. Argic. Food Chem. 62, 8883–8891.
Van Vuuren, A.M., Van der Koelen, C.J., Valk, H., De Visser, H., 1993. Effects of partial replacement of ryegrass by low protein feeds on rumen fermentation and nitrogen loss by dairy cows. J. Dairy Sci. 76, 2982–2993.
Van Zelst, M., Hesta, M., Alexander, L.G., Gray, K., Bosch, G., Hendriks, W.H., Du Laing, G., De Meulenaer, B., Goethals, K., Janssens, G.P.J., 2015. In vitro selenium accessibility in dog foods is affected by diet composition and type. Br. J. Nutr. 113, 1888–1894.
Veldkamp, T., Bosch, G., 2015. Insects: a protein-rich feed ingredient in pig and poultry diets. Anim. Front. 5, 45–50.
Weis-Fogh, T., 1960. A rubber-like protein in insect cuticle. J. Exp. Biol. 37, 889–907.
Williams, B.A., Bosch, M.W., Boer, H., Verstegen, M.W.A., Tamminga, S., 2005. An in vitro batch culture method to assess potential fermentability of feed ingredients for monogastric diets. Anim. Feed Sci. Technol. 123–124, 445–462.
Willis, J.H., 2010. Structural cuticular proteins from arthropods: annotation, nomenclature, and sequence characteristics in the genomics era. Insect Biochem. Mol. Biol. 40, 189–204.
Zuidhof, M.J., Molnar, C.L., Morley, F.M., Wray, T.L., Robinson, F.E., Khan, B.A., Al-Ani, L., Goonewardene, L.A., 2003. Nutritive value of house fly (*Musca domestica*) larvae as a feed supplement for turkey poults. Anim. Feed Sci. Technol. 105, 225–230.