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Bioconversion of fresh chicken excreta by housefly larvae (*Musca domestica* L.) in relation to excreta sterilisation and carbohydrate addition

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RESEARCH ARTICLE

Abstract

Housefly larvae can be reared on manure and used as animal feed. Larvae can utilise proteins and easily digestible carbohydrates and have a largely unknown relation with microorganisms. The hypothesis addressed in this study was that larvae compete with microorganisms for easily digestible carbohydrates such as starch. This was tested by adding starch or (non-digestible) fructo-oligosaccharides (FOS) to unsterilised or heat-sterilised fresh chicken excreta. Experimental substrates were unsterilised excreta (E), sterilised excreta (sE) with either starch (E + star, sE + star) or FOS (E + FOS, sE + FOS). The highest wet yield (9.7 g) and heaviest larvae (13.2 mg) were on sE + star, followed by E (7.2 g and 8.1 mg). Both E + FOS and sE + FOS had minimal yields and larval weights (0.3 and 0.2 g, 2.9 and 1.7 mg) with E + star intermediate (3.3 g and 7.5 mg). Survival differed between diets, sE and E averaged 70%, sE + star 57%, E + star 33% with 9% for E + FOS and sE + FOS. DM bioconversion decreased from E (3.5%), sE + star (3.1%), sE (2.6%), E + star (1%) to E + FOS and sE + FOS (0.1%). Nitrogen bioconversion was highest on sE + star (9.9%) and E (9.3%), lower on sE (6.6%), E + star (4%) and the lowest on E + FOS and sE + FOS (0.5%). Different substrate temperature profiles during the larval growth period and different larval size distributions were found for the different substrates. The results support the hypothesis that larvae compete with microorganisms for an easily digestible carbohydrate (starch).

Keywords: insect nutrition, manure, optimisation

1. Introduction

Population growth and rising per-capita income are predicted to lead to increased global consumer demand for animal-derived protein by 50 to 70% over the period 2005-2050 (Alexandratos and Bruinsma 2012; Henchion *et al.,* 2017). This will drive both the demand for livestock feed and emission and pollution problems associated with increased production of livestock manure (Godfray *et al.,* 2018; Nicholson *et al.,* 2005). Production of poultry has expanded fivefold over the last half century, with 2018 production estimated at 114 million tons of meat and 76 million tons of eggs by 23 billion animals (FAOSTAT, 2020; Statista 2020). Global excretion of nitrogen (N) from poultry manure in 2017 has been estimated at 5.65 million tons for broilers and 2.36 million tons for layers (FAOSTAT, 2020). Mass rearing of insects on manure could provide protein for livestock feed and a partial solution to the environmental burden of the growing manure surplus. Housefly larvae (*Musca domestica* L.) have been reared successfully on chicken, pig and cattle manure at a laboratory and industrial scale (Barnard *et al.,* 1998; Calvert *et al.,* 1970; Cickova *et al.,* 2012; Hussein *et al.*, 2017; Miller *et al.,* 1974; Zhang *et al.*, 2014). These larvae are rich in crude protein (30-60% of the dry matter; DM) and fat (14-30% DM), with an amino acid profile comparable to fish meal (e.g. Gadzama and Ndudim, 2019; Pieterse and Pretorius, 2014). Larvae and defatted larvae meal can be used as an alternative for fish meal in diets of poultry, pigs and fish (Hashizume *et al.*, 2019; Ogunji *et al.*, 2008; Pretorius, 2011; Veldkamp *et al.*, 2012). Despite the suitability and bioconversion potential of chicken manure, surprisingly little is known about its

nutritional value for larvae and how manure should be treated to optimise yield and bioconversion.

Fresh chicken excreta is a mixture of undigested dietary remains, endogenous material (gut and urinary mucous and secretions) and microbial mass. Fresh chicken manure commonly has a carbon to nitrogen ratio (C/N) of approximately 8/1, a total N content varying from 3 to 8% of DM with up to 60% of the N present as uric acid (Chen *et al.*, 2005; Nahm, 2003; Wang *et al.*, 2015). Digestible carbohydrate content is low and consists of dietary remains especially fibre and bacterial glycogen (Weurding *et al.*, 2001; Wilson *et al.*, 2010). Recalcitrant plant fibre content (hemicellulose, cellulose and lignin) has been found to constitute up to 35% of excreta dry mass (Chen *et al.*, 2005; Rehman *et al.*, 2017).

Microbial mass of chicken excreta has not been quantified but bacterial densities of 10^7 to 10^{11} microorganisms per g excreta have been reported (Apajalahti *et al.*, 2004; Nodar *et al.*, 1990). Typically, bacteria (i.e. *Bacillus* and *Escherichia* sp.) found in the chicken colon contain protein (50-80% DM), ribonucleic acids from RNA or DNA (10-20% DM), a limited amount of fat (<9% DM) and glycogen (<5% DM) (Kurbanoglu and Algur, 2002; Metcalf *et al.*, 2004; Ritala *et al.*, 2017; Sekar *et al.*, 2020; Wilson *et al.*, 2010). When excreta leave the body, a die-off of obligate anaerobic microorganisms takes place, releasing microbial cell content into the faecal mass (Ben-Amor *et al.*, 2005). Fresh excreta exposed to oxygen at room temperature are rapidly colonised by different microorganisms. These microorganisms rapidly utilise easily digestible carbohydrates and protein (Steger *et al.*, 2005) potentially resulting in a competition between housefly larvae and microorganisms for nutrients.

Microbial protein, along with the undigested dietary and endogenous protein, is a potential source of amino acids for larvae. The protein (and uric acid) in excreta can also be used for novel microbial growth and energy, with excess N emitted as ammonia (Maeda *et al.*, 2011). Ammonia production due to microbial hydrolysis of proteins and breakdown of uric acid in chicken manure can be substantial, leading to high volatilisation of N (Nahm, 2003). Microbial use of N highly depends on the C/N, moisture content, temperature and aeration (Kutzner, 2008). Generally, a low C/N (below 20/1) favours bacterial protein breakdown and conversion of (non-)protein N to ammonia while higher C/N (above 25/1 to 30/1) favour incorporation of (non-)protein N into new microbial mass (Ekinci *et al.*, 2000; Kutzner, 2008).

In the midgut of housefly larvae, different amylases and maltases have been identified (Pimentel *et al.*, 2018; Terra and Jordão, 1989) indicating a capability to utilise carbohydrates including starch as an energy source. Housefly larvae have a complex trophic relationship with microorganisms and were found to ingest and digest microorganisms but it is not known what constituents of which species are essential (Espinosa-Fuentes and Terra, 1987; Nayduch and Burrus, 2019; Zurek *et al.*, 2000). The current study hypothesised that housefly larvae reared on fresh chicken excreta compete with microorganisms for easily digestible carbohydrates. This hypothesis was tested experimentally by addition of easily digestible carbohydrates that can be used by both larvae and microorganisms (gelatinised starch, fructo-oligosaccharides (FOS)) to sterilised and unsterilised fresh chicken excreta. The effect of sterilisation and carbohydrate additions on larval performance (survival, growth) and substrate parameters (DM, nitrogen, uric acid, carbon, ammonia content) were determined.

2. Materials and methods

Housefly culture

A housefly culture was established at Wageningen University & Research in January 2019, using the Dutch GK strain provided by Groningen University. Adult flies were kept in mesh Bugdorm™ cages (47.5×47.5×93 cm) at 1000-3,000 per cage densities in a climate room (27 °C., 60% RH, 16:8 light-darkness) and fed *ad libitum* with a 50/50 mixture (v/v) of full-cream milk powder (De Zuidmolen, Groesbeek, the Netherlands) and deactivated yeast (BioToday, Ulvenhout, the Netherlands) provided in a thin layer in plastic plates. Pure sucrose was supplied separately as sugar cubes (Albert Heijn home brand, Zaandam, the Netherlands) in flat plastic plates. Tap water was supplied in polypropylene pots with cotton wicks inserted through the lids. Fresh food and water were provided every 48 hours. Larvae were reared in the same room in 1 l food grade polypropylene trays with mesh lids using a bran-based substrate: coarse wheat bran (Meneba, Rotterdam, the Netherlands), wheat flour (Albert Heijn home brand), milk powder, deactivated yeast and tap water in weight (as is) ratio of 100:15:10:10:120, respectively. The population was sustained by allowing flies to oviposit on wheat bran substrate and partitioning the substrate and young larvae into the 1 L trays at 400-500 larvae to 300 g fresh substrate. After pupation, trays with pupae were placed in clean cages for eclosion.

Experimental substrates

Fresh (<24 h old) excreta of layers fed a commercial soy-based feed were obtained from Rondeel farm at Barneveld (the Netherlands). Portions (~400 g) were either directly frozen at -20 °C (E) or directly sterilised for 30 min in an autoclave at 121 °C (core temperature) and frozen (sE). Before use, frozen excreta were allowed to thaw overnight at 5 °C. Thawed 400 g portions were sampled for chemical analyses, after which 100 g was used as is, or mixed with either 32 g gelatinised corn starch (Research Diet Services,

Wijk bij Duurstede, the Netherlands) or 32 g fructo-oligosaccharides (Lamberts Healthcare, Kent, United Kingdom). To samples with additions, 50 ml tap water was also added to set moisture at ~33%. The added quantities were chosen to increase the C/N of pure excreta from 9/1 to approximately 20/1. Substrates were individually mixed by hand in 770 ml transparent polypropylene pots (PP Joni bekers, art. 003829, Gédé verpakkingen, Limmen, the Netherlands) fitted with a mesh Bugdorm™ lid (Megaview Science Co., Ltd, Taichung, Taiwan). Six replicates were made per substrate. Substrate composition and chemical characteristics are provided in Table 1.

Egg collection and partitioning

Oviposition pots were made by fitting black cotton socks, cut to 20% length, with the tip down into 300 ml round polypropylene pots which were half filled with semi-skimmed milk (Albert Heijn home brand) with the tip of the sock submersed in the milk and covered with an aluminium foil lid containing a slit at the container edge to allow access. Oviposition pots were placed in cages with adult flies (7 to 10 days post eclosion) and left for 4-6 h. After removal, pots were kept in a larger closed container lined with wet (tap water) paper tissue to maintain high air humidity. Portions of eggs (~75 mg, ~1,250 eggs), were deposited on the experimental substrates, in a crevice close to the container wall directly after weighing and lightly spraying (<0.5 ml) with demineralised water. Inoculated substrates were placed on shelves in the same climate room as the adult cages for 5 days.

Harvesting, sampling and measuring of larvae and substrates

During the 5-day larval growth period, temperature of the inoculated substrates was measured daily using a probe thermometer (TFA Dostmann, Wertheim-Reicholzheim, Germany). On day 5, all inoculated substrates were placed on melting ice to slow down larval and microbial metabolism. Portions (20-30 g) of left-over inoculated substrates (substrate residue) without larvae were stored in polypropylene pots at -20 °C before freeze drying and chemical analysis. Larvae were quantitatively collected from the remaining substrate residues by floatation. This involved submerging the substrate residue in 2 l 14% NaCl solution at room temperature under gentle stirring, with difference in buoyancy causing larvae to float and substrate residue to sink. Floating larvae were collected from the top of the solution using a fine sieve, rinsed with tap water, transferred to filter paper to absorb excess water. All larvae were transferred to small polypropylene pots before being frozen at -20 °C.

For determination of total and individual larval weight, larvae were thawed and evenly spread out on clean filter paper. After removal of remaining substrate particles, 25 to 30 larvae were randomly selected (by selection along a line form the operator to the far side of the paper) and individually weighed. Total wet larvae mass was determined by weighing all collected larvae of a single substrate residue together, including individually weighed larvae. After weighing larvae were frozen again at -20 °C for freeze drying and chemical analyses.

Table 1. Ingredient and chemical composition of chicken excreta substrates.[1]

Item	Unsterilised chicken excreta			Sterilised chicken excreta		
	None	Starch	FOS	None	Starch	FOS
Ingredient composition (% as is)						
Excreta	100	55	55	100	55	55
Starch	0	18	0	0	18	0
FOS	0	0	18	0	0	18
Water	0	27	27	0	27	27
Chemical composition (% DM)[2]						
DM (% as is)	29.7	33.0	33.	32.4	34.5	34.5
Starch	1.7	51.6	0.8	1.9	50.2	1.0
Nitrogen	4.0	2.0.1	2.0	4.1	2.1	2.1
Uric acid	5.3	2.6	2.5	2.8	1.5	1.5
NH_3	0.3	-	-	0.2	-	-
NDF	36.7	20.1	20.1	34.1	18.9	18.9
ADF	21.1	3.7	3.7	20.4	3.6	3.6
C/N	9.3	21.3	20.1	9.0	19.8	18.7

[1] ADF = acid detergent fibre; C/N = carbon to nitrogen ratio; DM = dry matter; FOS = fructo-oligosaccharides; NDF = neutral detergent fibre; NH_3 = ammonia.
[2] Nitrogen, uric acid, starch, NDF and ADF were analysed for sterilised and unsterilised excreta and calculated for the other substrates based on inclusion level.

Chemical analyses

Ammonia and pH were determined in freshly thawed substrate residues without larvae. Ammonia was determined using a colorimetric method after deproteinisation with trichloroacetic acid (Pellikaan *et al.*, 2011). Uric acid was analysed enzymatic-colorimetric using a commercial test kit (10694, Human GmbH, Wiesbaden, Germany). Analysis of total N and total C (Dumas method, ISO 16634-1, 2008), DM (ISO 6496, 1999), uric acid, starch (ISO 15914, 2004) and ADF/NDF content (ISO 13906, 2008) was performed on freeze dried material. All chemical analysis were performed in duplo, except for DM performed in simplo. The number of biological replicates is given as table footnotes.

Statistical analyses

To partition egg portions by weight, egg count was derived from egg weight (individual egg weight: 0.06 mg) as egg weight was found to be highly uniform (mean ± SD: 0.06±0.004 mg) in earlier tests weighing 8 clumps of 100-300 eggs followed by accurate counting. The number of larvae per replicate was estimated from the total wet larvae mass and individual larvae weights using a generalised linear model with a gamma distribution with log-link. Distributions of individual larval weight were bound by 0 and in many cases strongly skewed, making a gamma distribution (with log link) the best approximation to the density function. Model estimations of the 95% confidence intervals were checked by transforming histograms of individual larvae weights per sample (weight class width 0.5 mg) to fractions of the summed individual weights per sample with counts following from summation of weights per weight class divided by weight class means. Minimum and maximum average counts derived from histograms were highly similar to the 95% confidence interval limits generated by the model (all correlations >0.99).

Larval survival was defined as the number of inoculation eggs divided by the mean number of larvae harvested. Testing for differences in survival between substrates was conducted using a generalised linear model (binomial distribution-logit link) with substrate replicate as experimental unit followed by pairwise tests (Tukey adjustment multiple comparisons).

Testing for differences in means with normally distributed error (total wet larvae mass, larval DM, C/N, pH) was achieved by a linear model (ANOVA). For individual larval weight, a generalised linear mixed model was used (gamma-log link, substrate replicate as random effect), followed by pairwise tests (Tukey adjustment for multiple comparisons). Continuous fractions with means close to 0 (bioconversion%, ammonia%, uric acid%) were tested for differences using a generalised linear model (beta-logit link) again followed by pairwise tests (Tukey adjustment for multiple comparison). For all models, model residuals were visually inspected for normality and homoscedasticity and found to adhere to the assumptions of the statistical tests. Analysis was conducted in R (R Core Team, 2019).

3. Results

Characteristics of fresh substrates

Both unsterilised excreta (E) and sterilised excreta (sE) had a consistency like clay, making it difficult to produce homogeneous substrates. Although the same amounts of starch and FOS were added, their effect on the substrate were very different. Starch behaved as a solid while FOS dissolved in the added water, resulting in a more liquid consistency. Sterilisation led to measurable changes in the chemical composition of the manure (Table 1). In sE, the DM and N content were slightly increased, leading to a slightly lower C/N. In sE, the uric acid content was almost halved, the NH_3 content decreased by 32% while NDF and ADF decreased slightly compared to E. Both E and sE had an initial pH of 7.1.

Larval performance on different substrates

Weighing 75 mg of eggs proved to be challenging as eggs stuck together and to the metal equipment used. A wet toothpick was found to be ideal to manipulate eggs and enable counting and weighing. The number of eggs used for inoculation of the substrates varied from 1,228 to 1,286, with no marked differences in number between substrates.

Larval performance differed between substrates (Table 2). Survival of larvae was highest, around 70%, in excreta without additions, E and sE. Survival was considerably lower for the other substrates, decreasing in the order: sE + star (57%), E + star (34%), E + FOS (9%) and sE + FOS (8%). Though subject to a lower survival, larvae grown in the sE + starch substrate were substantially heavier (13.2 mg) than those from the other substrates. Larvae from E (8.1 mg), E + star (7.5 mg) and sE (6.7 mg) were similar in size. E + FOS and sE + FOS resulted in the lightest larvae, 2.9 mg and 1.7 mg respectively. Larval dry matter content was lowest for sE and E, around 140 g/kg, and significantly higher for the carbohydrate additions. Furthermore, the distribution of individual larval weight differed between substrates (Figure S1). In E, sE and sE + star, distributions were normal or close to normal, whereas in E + star, E + FOS and sE + FOS distributions were (strong) left skewed, indicating that the majority of the larvae were lighter than the substrate mean.

Substrate residues and bioconversion

Bioconversion of dry matter, C and N in to larval biomass differed between substrates (Table 3). The highest dry matter conversion (3.5, 2.6 and 3.1%) were recorded for

Table 2. Count, survival, individual wet weight, total wet weight, dry matter content and C/N of housefly larvae reared on unsterilised (E) and sterilised (sE) chicken excreta substrates with or without starch (star) or fructo-oligosaccharides (FOS) addition.[1]

Parameter	Substrate					
	E	E + star	E + FOS	sE	sE + star	sE + FOS
Count[2]	898 (836-965)	356 (281-451)	106 (80-141)	886 (802-978)	736 (669-807)	100 (80-125)
Survival (%)[3]	70±3[a]	34±5[c]	9±4[d]	70±5[a]	57±8[b]	8±9[d]
Individual wet weight (mg)[4]	8.1±1.5[b]	7.5±4.9[b]	2.9±2.4[c]	6.7±1.8[b]	13.2±3.5[a]	1.7±1.0[d]
Total larval wet weight (g)	7.2±0.2[b]	3.3±0.5[c]	0.3±0.2[d]	5.9±0.9[b]	9.7±2.1[a]	0.2±0.2[d]
Dry matter (g/kg)	144.1±7.4[b]	186.1±12.3[a]	193.7±38.5[a]	140.7±4.5[b]	209.8±6.6[a]	256.9±29[a].3[a]
C/N	4.4±0.1[c]	7.1±0.4[b]	6.1±0.7[b]	4.5±0.1[c]	8.1±0.2[a]	6.8±0.1[b]

[1] Data presented as mean ± SD and based on n=6 unless otherwise indicated. Means with different superscript letters within row differ with $P \leq 0.05$.
[2] Data presented as mean and 95% confidence interval derived from a gamma distribution.
[3] Mean and SD are based on estimated mean number of larvae divided by estimated number eggs used for inoculation.
[4] Mean and SD are based on 25-30 larvae per substrate replicate (n=6).

E, sE and sE + star, respectively, with lower conversion for E + star (1.0%) and minimal for E + FOS and sE + FOS (both 0.1%). Nitrogen conversion was highest for sE + star (9.9%), followed by E (9.3%), sE (6.6%), E + star (4.0%) while E + FOS and sE + FOS had conversions close to 0. Carbon conversion was highest for E and sE + star (4.1 and 3.7%, respectively), lower for sE (3.1%) and E + star (1.0) with E + FOS and sE + FOS showing conversions close to 0.

Uric acid, ammonia, pH and C/N ratio

Substrate residue uric acid and ammonia concentrations, pH and C/N differed between the six substrates (Table 4). Compared to the fresh substrates (Table 1), a decrease in uric acid content was observed in substrate residues of E, E + star and sE (92, 27 and 45%, respectively) while E + FOS, sE + star and sE + FOS showed an increase in uric

Table 3. Mass of dry matter, nitrogen and carbon of housefly larvae reared on unsterilised (E) and sterilised (sE) chicken excreta substrates with or without starch (star) or fructo-oligosaccharides (FOS) addition and their bioconversion efficiency (BE).[1]

Parameter	Substrate					
	E	E + star	E + FOS	sE	sE + star[2]	sE + FOS[3]
Dry matter						
Substrate (g)	29.7±0.1	60.2±0.1	60.2±0.1	32.4±0.1	62.9±0.1	63.0±0.1
Larvae (g)	1.0±0.1	0.6±0.1	0.1±0.1	0.8±0.1	1.9±0.4	0.1±0.1
BE (%)	3.5±0.3[a]	1.0±0.2[c]	0.1±0.1[d]	2.6±0.4[b]	3.1±0.6[ab]	0.1±0.1[d]
Nitrogen						
Substrate (g)	1.2±0.0	1.2±0.0	1.2±0.0	1.4±0.0	1.4±0.0	1.4±0.0
Larvae (g)	0.1±0.0	0.1±0.0	0.0±0.0	0.1±0.0	0.1±0.0	0.0±0.0
BE (%)	9.3±0.6[a]	4.0±0.6[c]	0.5±0.3[d]	6.6±1.1[b]	9.9±2.0[a]	0.5±0.3[d]
Carbon						
Substrate (g)	11.3±0.0	25.5±0.0	24.1±0.0	12.1±0.0	26.4±0.0	25.0±0.0
Larvae (g)	0.5±0.0	0.3±0.1	0.0±0.0	0.4±0.1	1.0±0.2	0.0±0.0
BE (%)	4.1±0.3[a]	1.0±0.5[b]	0.1±0.1[c]	3.0±0.5[d]	3.7±0.8[ad]	0.2±0.1[c]

[1] Data presented as mean ± SD based on n=6 unless otherwise indicated. Means with different superscript letters within row differ with $P \leq 0.05$.
[2] Based on n=5.
[3] Based on n=3.

acid (24, 47 and 53%, respectively). The highest free NH_3 concentrations were found in E and sE (0.4% of DM) with E + FOS, sE + FOS, sE + star having lower and similar content (0.1%) and no ammonia was found in E + star. The substrate residues were alkaline for E (8.2) and sE (8.5) and acidic for the others, pH decreasing from E + star (5.3), sE + FOS (4.8), sE + star (4.6) to E + FOS (4.4). When C/N of substrate residues is compared to fresh substrates (Table 1), E and sE showed an increase from 9 to 16 and 12, respectively, whereas E + star and sE + star showed a large decrease to 14 and 15. E + FOS showed a minor decrease to 18 and sE + FOS residues showed no change in C/N.

4. Discussion

The chicken excreta was autoclaved for 30 min at 121 °C and 103 kPA, parameters that are commonly effective to inactivate the vast majority of microbiota including spores in various food and feed products. Sterilisation led to slight increases in DM and N content and a decrease in ammonia content. Though not statistically significant, larvae grown in sE were lighter and total larval mass lower than that of E. This difference could be caused by destruction of heat sensitive compounds essential to larvae, such as B vitamins. Differences in temperature profile of the inoculated substrates support lower microbial metabolism in the sterilised substrates (Figure S2). The temperature profile of E showed an initial rise of temperature during the first 24 to 48 h, similar to that of aerobic composting of fresh chicken manure (Hwang *et al.*, 2020; Kajiya *et al.*, 2015), whereas sE showed a later more distinct peak. In contrast, sE + star heated up faster than E + star, but the latter attained a higher temperature for a longer time. However, the difference in temperature profile could also be due to heat production of the larvae themselves, that had a considerably larger mass in sE + star. No reports of heat production by housefly larvae were found in the literature, but larval aggregations of calliphorid species that are taxonomically related with and ecologically similar to the muscid flies were found to be much warmer than ambient temperature (Anderson and Van Laerhoven, 1996). The pH of E + star and E + FOS was higher than their sterilised counterparts, which could indicate differential microbial activity as a result of sterilisation, the effect not being confounded for FOS due to the low number of larvae present. To distinguish the effects of sterilisation from those of larvae, it is of interest to include control substrates without larvae in future larval nutrition studies that involve substrates with a high microbial load.

Larval performance and bioconversion were much lower for FOS compared to the other substrates. Though water content was equivalent to the other substrates, adding FOS resulted in a much more viscous consistency compared to starch. The FOS substrates appeared dry during mixing, but turned to a more viscous consistency after several hours. The high viscosity appeared to prevent larvae from entering the substrates, as larvae were only observed on the surface, where for the other substrates larvae were observed throughout the material after 5 days of larval growth. The viscosity possibly limited the availability of oxygen, which not only limited the larvae burrowing but is also the probable cause for the absence of heat production of the FOS substrates (Figure S2). The intended effect of FOS addition was to provide carbohydrates, indigestible by endogenous enzymes of larvae but digestible by microorganisms such as *Bifidobacterium* sp. (Kelly, 2008). The FOS-metabolising microorganisms could subsequently be digested by the larvae, enhancing larval performance compared to the control (sE + FOS). The current study design (high dose in a moist environment) resulted in a negative effect of the FOS treatments. To investigate the effects of manipulation of microbiota by addition of FOS, future studies should not only control moisture levels but also control substrate consistency.

Sterilisation improved larval performance of the chicken excreta with starch. Average wet yield increased (3.3 vs 9.7 g) as did the average larval weight (7.5 vs 13.2 mg). These results support the hypothesis that larvae and microbiota

Table 4. Uric acid and ammonia concentration (% of DM), pH and C/N in the residue of unsterilised (E) and sterilised (sE) chicken excreta substrates with or without starch (star) or fructo-oligosaccharides (FOS) addition.[1]

Parameter	Substrate					
	E	E + star	E + FOS	sE	sE + star[2]	sE + FOS
Uric acid	0.4±0.2^{a}	1.9±0.7^{b}	3.1±0.6^{c}	1.6±0.5^{b}	2.2±0.3^{bcd}	2.3±0.1^{c}
Ammonia	0.4±0.1^{a}	0.0±0.0^{b}	0.1±0.0^{c}	0.4±0.1^{a}	0.1±0.0^{d}	0.1±0.0^{c}
pH	8.2±0.1^{a}	5.3±0.4^{c}	4.4±0.0^{d}	8.5±0.2^{b}	4.6±0.1^{ab}	4.8±0.1^{d}
C/N	16.0±1.1^{a}	14.2±0.8^{ab}	17.8±1.9^{ac}	12.4±1.1^{bd}	15.3±1.6^{ab}	18.3±0.8^{c}

[1] Data presented as mean ± SD based on n=6 unless otherwise indicated. Means with different superscript letters within row differ with $P \leq 0.05$.
[2] Based on n=5.

compete for carbohydrates. The contrast between E + star, E and sE also supports the study hypothesis. The daily temperature profile (Figure S2) shows that E + star reached a higher temperature and produced heat later and longer than the other substrates. Aerobic decomposition is known for its phases of heat production by microorganisms in relation to the availability of metabolisable nitrogen and carbon (Kutzner, 2008). If enough metabolisable carbon is present, microbiota (i.e. *Bacillus* sp., *Pseudomonas* sp., *Bifidobacterium* sp., *Candida* sp.) generate heat by oxidising the carbon while incorporating N into microbial biomass. This process could very well have a negative impact on larval nutrition if larvae are dependent on available free protein or on specific nutrients provided by microbiota associated with low C/N substrates, that are depleted when easily digestible carbohydrates are abundant.

Leaving the larval survival of <10% in substrates with FOS out of consideration, survival was high (70%) in E and sE, lower in sE + star (57%) and lowest in E + star (34%). These survival rates are comparable to those found in literature, 40 to 60% for fresh pig faeces with variable amounts of sawdust and 40 to 85% for pure chicken excreta at larval densities of 3 to 6 larvae per g substrate (Barnard *et al.*, 1998; Calvert *et al.*, 1970; Cickova *et al.*, 2012). However, studies are difficult to compare as studies differ in terms of manure quantities, larval densities and definitions of survival (i.e. eggs to pupae, eggs to adults, larvae to pupae). It is not known which age group was most affected by mortality but, when not related to differences in mechanical properties of the substrates, the differential survival found might also indicate a nutritional dependency of larvae for free protein or microbiota associated with high protein (low C/N) substrates.

Individual larval mass distribution differed between substrates (Figure S1), with E, sE and sE + star showing an approximately normal or symmetric distribution, while the distributions in E + star and the FOS substrates were left skewed (relatively more small individuals). This might also be an indication of larval competition for nutrients or microclimates as (animal) body size tends to be inversely related to competition strength with relatively few large individuals suppressing nutrient intake of relatively numerous small ones (see for example Ward *et al.*, 2006). But this relation in (housefly) larvae could be complicated by the existence of Allee effects, high densities of larvae feeding more efficiently than low densities, as found for *Drosophila*, or cannibalism (Lam *et al.*, 2007; Wertheim *et al.*, 2002). Additional research using different densities of larvae is needed to investigate the relation between individual larva weight, competition for food and total larvae mass.

The DM values for larvae grown on E and sE are low compared to the values found for larvae reported in literature of 240 to 250 g/kg of larvae grown in a mixture of layer manure and food waste (Van Zanten *et al.*, 2015) and 300 g/kg of larvae grown in fresh dairy cattle faeces (Hussein *et al.*, 2017). Larvae reared in E and sE had a C/N of 4.5 while larvae from E + star and sE + star had ratios of 7.1 and 8.1, respectively and larvae from E + FOS and sE + FOS ratios of 6.1 and 6.8, respectively. Adding starch clearly increased the C/N, potentially due to larvae being fatter or the composition of the gut content.

In this study, larval bioconversion of DM, N and C was calculated. Bioconversion of the DM and N fraction are important determinants for the potential of bioconversion with insects in order to minimise waste and maximise N deposition while preventing N emission. Excluding the FOS substrates, the highest bioconversion of DM was found for E (3.5%), followed by sE + star (3.1%), sE (2.6%) and E + star (1.0%). The observed DM bioconversions of E and sE are low in the range found in literature for poultry manure (1.1-10.2%) (Barnard *et al.*, 1998; Calvert *et al.*, 1970; Zizhe *et al.*, 2017). The difficulty in comparing results with data in the literature is that other studies were conducted with different densities of larvae and different amounts of manure. The study closest in terms of density to the present study is the one of Barnard *et al.* (1998) who used 3 larvae per g in 100 g fresh to 2 week old layer manure and found a DM bioconversion of 2.4%. The observed DM conversion of E is similar to that of black soldier fly larvae (*Hermetia illucens*) reared on chicken manure, with values found of 3.4% by Oonincx *et al.* (2015) but lower than the 9.8% found by Rehman *et al.* (2017).

Data on N bioconversion by housefly larvae are not available in the literature, this study being the first to report N bioconversion. The highest N bioconversion was found for sE + star and E (9.9 and 9.3%), lower for sE (6.6%) and E + star (4%) and only 0.5% for the FOS substrates. Interestingly, while sE + star (9.9%) proved to be the best of the substrates for N deposition into larvae, N bioconversion was also high for pure E (9.3%). During the larval growth period, the E + star and sE + star treatments were observed to have little to no NH_3 odour while both E and sE substrates emitted a strong NH_3 odour during the larval growth period, indicating NH_3 emission. The high NH_3 emission compared to sE + star did not seem to affect larval N deposition. This high N bioconversion of pure excreta might again be an indication of larval specialisation to a protein diet or interaction with specific microorganisms that play a role in larval N utilisation. The observed low N bioconversion of E + star could be caused not only by fixation of N into microbial biomass but also by changing the microbial species composition rendering the substrate unfit for (early stage) larval growth. The N bioconversion for excreta found in this study is much higher than the 4.6% reported for black soldier flies by Oonincx *et al.* (2015). This indicates that housefly larvae have bioconversion potential for nitrogenous material and efficient upcycling of N.

5. Conclusions

This study supports the hypothesis that housefly larvae compete with microorganisms for an easily digestible carbohydrate (starch) in fresh chicken excreta. Heat sterilised chicken excreta alone led to lighter larvae and lower total larval mass compared to unsterilised chicken excreta, whereas sterilised chicken excreta with added starch (50% of substrate DM) led to heavier larvae and a much higher total larval mass while showing a lower survival compared to unsterilised chicken excreta with added starch. Addition of fructo-oligosaccharides (50% of substrate DM) to both sterilised and unsterilised excreta resulted in minimal growth and total biomass of larvae, attributable to the high viscosity of the substrate. Different substrates showed different temperature profiles during larval growth but effects of microorganisms and larvae on temperature cannot be separated with the used experimental design. The size distribution of individual larvae differed markedly between experimental substrates, which might be an indication of nutritional status. The found bioconversion efficiencies for DM are on the low side of the range found in literature, while the bioconversion efficiency of N is reported for the first time in this study. N bioconversion efficiency is relatively high, around 9%, for both unsterilised excreta and sterilised excreta with starch.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2021.0161

Figure S1. Violin plot of individual larva weight (n=944) grown on unsterilised (E) and sterilised (sE) chicken excreta substrates with or without starch (star) or fructo-oligosaccharides (FOS) addition. Group means are represented by diamond shapes.

Figure S2. Substrate temperature per day for unsterilised (E) and sterilised (sE) chicken excreta substrates with or without starch (star) or fructo-oligosaccharides (FOS) addition. Solid lines are day means with the grey scale indicating the 95% confidence interval.

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Conflict of interest

The authors declare no conflict of interest.

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