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# **Animal Nutrition**



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Original Research Article

# Performances of local poultry breed fed black soldier fly larvae reared on horse manure



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#### ARTICLE INFO

# Article history: Received 9 July 2017 Received in revised form 2 October 2017 Accepted 9 October 2017 Available online 26 October 2017

Keywords: Insect farming Chicken Local breed Growth performance Meat quality Fatty acid profile

#### ABSTRACT

In poultry, feed based on maggots, like larvae of black soldier fly (Hermetia illucens) is an attractive option to substitute current ingredients which are expensive and often in direct or indirect competition with human food. Little information is currently available on the utility of these larvae in poultry feed, so goals of this study were to determine whether larvae could be reared on horse manure under traditional farming conditions and to evaluate the growth performances of a local poultry fed these larvae and the fatty acids profiles of their meat. After freezing and thawing, larvae were introduced in the feed of Ardennaise chickens between 30 and 80 days of age. Birds in the control group received a commercial standard feed, while those in the treatment group received the same commercial feed in which 8% was substituted with whole fresh larvae corresponding to 2% on a dry matter basis. Means ± standard errors of larval length and weight were 20.67  $\pm$  2.21 mm and 0.14  $\pm$  0.02 g, respectively. Mean larval percentages of dry matter and of substances extractable in diethyl ether were 24.6% and 23.1%, respectively. Larval fatty acids profiles were predominantly composed of lauric acid (28.1%) and palmitic acid (22.0%). Least squares means of weekly weights of chicken, adjusted for the effects of sex, replication and initial weights, were significantly higher (P < 0.05) by 77.03  $\pm$  53.37 g in larvae-fed than in control chickens. All other measurements were not statistically different between larvae-fed and control chicken, including fatty acid profiles, protein content and  $\omega 6/\omega 3$  ratio.

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#### 1. Introduction

Amount of horse manure generated worldwide is impressive. Consider, for example, that a horse produces daily about 51 kg manure/1,000 kg live animal mass (ASAE Standards, 2003) and that

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Peer review under responsibility of  ${\stackrel{\circ}{\text{Chinese}}}$  Association of Animal Science and Veterinary Medicine.



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1,655,383 horses live in Ethiopia, the country with the largest horse population in Africa (World Horse Population, 2006). Manure has traditionally been valued as organic fertilizers and soil improvers (http://edepot.wur.nl/362491) but it is often underutilized or misused. For example, in South Africa, only 25% of the estimated  $3 \times 10^6$  t of animal manure is used as fertilizers and a negligible percentage is used as energy in heating (Okorogbona and Adebisi, 2012). In the study of Vu et al. (2007) in Northern Vietnam, 19% of the total manure is discharged into public sewage systems, rivers and lakes. Farming insects on manure could increase its valuableness and possibly its exploitation. Indeed, a wide diversity of insects can be farmed on manure and be used to feed livestock (Sheppard and Larry Newton, 1994; Diener et al., 2009; Caparros Megido et al., 2015). Of these, black soldier fly (BSF) or Hermetia illucens (L. 1758)

(Diptera: Stratiomyidae) appears to be a particularly useful feed for pigs, chickens or fish (Diener et al., 2009; van Huis, 2013). Black soldier fly larvae can also convert manure from cattle, chickens or pigs into a product containing approximately 40% of protein and 35% of fat in dry matter; the amount of manure is reduced by at least 50% and the resulting product contains nutrients such as phosphorus (60% to 70%) and nitrogen (30% to 50%) (Sheppard and Larry Newton, 1994: Newton et al., 2005: Myers et al., 2008: van Huis, 2013). For example, BSF larvae farmed on cattle manure contain 21.5% lipids (on a dry matter basis) including lauric acid (21%), palmitic acid (16%), oleic acid (32%) and  $\omega$ 3 fatty acids (FA; 0.2%) (Gnaedinger et al., 2015; Makkar et al., 2014). If 10% of fish offal is added to cow manure, total lipid percentage increases by approximately 40%, while proportion of  $\omega 3$  increases from 0.2% to 3% (St-Hilaire et al., 2007). Proteins produced by BSF larvae contain threonine (0.6% to 1.41%), valine (2.23% to 3.4%), isoleucine (1.51% to 2%), leucine (2.61% to 3.5%) and lysine (2.21% to 3.4%) (Newton et al., 2005).

Thanks to handy BSF farming devices, feeding chicken with BSF larvae is particularly well suited to the traditional system of poultry production which is the most common poultry production system in most developing countries. Especially that increasing cost and decreasing supply of traditional feedstuffs are expected to constrain the future expansion of this production system. However, there are only a few studies on effects of BSF larvae fed to chicken on their performances. In the study of Elwert et al. (2010), weight of chicken (Ross 308) farmed with diets containing 4.7% to 6.6% BSF larvae was comparable to weight of chicken fed a diet containing fish meal (3%) as a source of animal protein. In the study of Hale (1973), growth of broiler chicks fed corn mixed with 35% dried BSF larvae was similar to that of chicks receiving a corn-based diet mixed with 35% soybean.

Note BSF larvae used in the aforementioned studies were fed in a dehydrated, ground and/or partially defatted form. However, dehydration and grinding can influence both the nutritional and organoleptic properties of larvae. For example, it has been shown that drying larvae of *Musca domestica* L. 1758 (Diptera: Muscidae) flies leads to different degrees of defatting. Grinding destroys the physical form of the larvae and could influence palatability as chickens are sensitive to the structure of their feed (Briggs et al., 1999). Dehydration and grinding may also affect the rancidity of the product. Moreover, considering the poultry production practices and constraints of the traditional system of poultry production (Mengesha et al., 2011), feeding life insects may be more adequate than after processing.

Another feature of the few studies on insect fed to poultry is their use in fast-growing commercial breeds. These birds are often less adapted to harsh environments than local poultry breeds (Besbes, 2009) and require skilled stockpersons to manage them. Other breeds, such as the Ardennaise chicken, a Belgian local light breed, are more similar to indigenous village chickens in their behavior and management. These birds are kept in family units, they are rustic and love to rummage in open spaces and fly readily (Moula et al., 2009, 2012).

Therefore, we aimed to evaluate: 1) whether BSF larvae could be farmed on horse manure and collected under traditional husbandry conditions, knowing that horse manure as a larval rearing substrate has never been studied; and 2) the impact of the ingestion of these larvae on the growth of Ardennaise chickens and the fatty acid profile of their meat.

# 2. Material and methods

## 2.1. Micro-rearing of black soldier flies

Initially, 300 BSF larvae were obtained from a colony in operation at Ynsect (Paris, France). They were provided with fresh horse

manure originating from a family farm. Insects rearing conditions aimed to be simple: manure was kept humid through a daily watering and ambient temperature was between 20 and 30 °C (small heating appliance). Adult insects were transferred to a cage (232.5 cm  $\times$  46.5 cm  $\times$  46.5 cm) placed in natural light for mating and egg-laying on horse manure until hatching of larvae of the future generations. The same cycle was repeated over generations. A portion of larvae was manually collected at pre-pupal stage and frozen at  $-20~^{\circ}\text{C}$ , and another was allowed to metamorphose in adults.

## 2.2. Larvae characteristics

To obtain dry matter (DM) content of larvae, weight and length of BSF larvae were measured as fresh, after 3 days pre-drying at 60 °C, and after 8 h drying at 105 °C. Ash content of larvae was determined after their calcination at 600 °C for 8 h. Larvae insoluble ash content was measured by treating ash with hydrochloric acid (1 mol/L) and by distilling the substrate through a quantitative filter. Ether extract content was obtained by Soxhlet extraction and mineral contents by the Atomic Absorption Spectrometry method (García and Baez, 2012).

Fatty acids profiles of fresh larvae and manure were obtained according to the method of Douny et al. (2015). Briefly, 50 mg of fat extracted from samples were mixed with 5 mL hexane and 10 µL were used for the saponification/methylation of the FA. Internal standard nonadecanoic acid (C19:0) was then added and hexane was evaporated to dryness under a stream of nitrogen. Toluene and sulfuric acid 2% (vol/vol. in methanol) were added and the capped tube was heated in a water bath at 100 °C for 1 h, with vigorous agitation by a magnetic stirrer. Then, NaCl 5% was added and the fatty acids methyl esters (FAME) were extracted 2 times with hexane. The extract was washed with K2CO3 2% (wt/vol) and Na<sub>2</sub>SO<sub>4</sub> was added to a part of the extract. The extract was then evaporated to dryness in order to eliminate the toluene. Three hundred and fifty five microliters hexane were added and the tube was vortexed. Finally, 80 µL were transferred into an injection vial. The FAME in the extract were separated on a Focus GC gas chromatographer (Thermo Fisher Scientific) using a CP-Sil88 column for FAME (100 m  $\times$  0.25 mm, 0.2  $\mu$ m) (Varian, Agilent Technologies, Santa Clara, California, USA) and analyzed with an ion trap PolarisQ mass spectrometer (MS) (Thermo Fisher Scientific). The GC conditions were: inlet 250 °C; splitless injection; helium as the carrier gas at 1.5 mL/min; temperature program: 55 °C for 1 min, followed by an increase of 5 °C/min to 180 °C, then 10 °C/min to 200 °C for 15 min, then an increase of 10 °C/min to 225 °C for 14 min; total run time was 59.50 min. Injection volume was 1 µL. The peaks were identified by comparing their mass spectrum and retention times with those of the corresponding standards. The MS conditions were: transfer line 250 °C, ion source 220 °C, collision energy 35 eV (1 eV =  $1.6 \times 10^{-19}$  J), positive ionization mode. The FAME were detected using selected ion monitoring mode in 5 segment windows. In each chromatographic run, different ions were monitored for each FA analyzed, which allowed to perform detection and quantitative analysis: m/z 101 + 143 for saturated, 79 + 91 for mono and polyunsaturated fatty acids.

# 2.3. Birds and management

In total, 22 thirty-day-old Ardennaise chicks were obtained from a small poultry breeding unit, called "Elevage du Sart Tilman", in which animals are kept under familial management. Birds (15 males and 7 females) of similar weight were randomly distributed to a control and experimental groups (2 replicates per group). Males were more numerous than females because they were kept

for other experiments. Numbers of animals and samples were ruled by the available resources and ethical considerations. Power analyses were performed with  $G^*$ Power (Faul et al., 2007), using data from literature (Moula et al., 2009): considering a standard deviation of 10 g and a mean weight of 500 g, our sample size would be sufficient to detect a difference of 15 g between both diets (type I error = 5%, type II error = 20%).

The control group received a commercial feed and the experimental group (BSF group) received the same commercial feed with 8% replaced by de-frozen BSF larvae corresponding to 2% on a DM basis. In the BSF group, 4 females and 8 males were randomly allocated to both replicates. In the control group, 3 females and 7 males were randomly allocated to both replicates. Birds were identified individually by a numbered metallic ring and housed under identical conditions: same building, photoperiod (16 h of light and 8 h of dark), ventilation system and sawdust floorbedding. The experiment took place over a period of 50 days at the experimental farm of the Faculty of Veterinary Medicine of the University of Liège. Age of chickens at the start of experiment was 30 days. Feed was supplied *ad libitum* throughout the experiment. Composition of the feed is given in Table 1.

#### 2.4. Data collection

Weekly body weights and feed intake were recorded on each chicken. At the end of the 50-day period, each bird was slaughtered (after 12 h of feed deprivation), weighted and eviscerated. All organs were examined for apparent anatomical lesions. Internal organs, wings, legs and drumsticks were weighed after skinning. Differences between final and initial weights were used to compute average daily gains (ADG) between 30 and 80 days. Feed conversion ratio (FCR) was defined as the ratio of the amount of feed ingested throughout the rearing period to the body weight gained during that period. Dressing-out percentages were computed as the ratio between warm carcass and live weights at slaughter. Left tibias were sectioned at the tibiotarsus—metatarsus joint, cleaned of all

**Table 1**Percentages of each feed ingredient given to the control and experimental groups of Ardennaise chicken (on a dry matter basis).

Item	Control group	Experimental group	
Ingredients, %			
Larvae of black soldier fly	0.0	2.0	
Corn	34.0	33.3	
Soyabean oil cake	29.1	28.5	
Wheat	25.0	24.5	
Bran meal	5.0	4.9	
Soyabean oil	2.5	2.4	
Calcium phosphate	1.4	1.4	
Calcium carbonate	1.3	1.3	
Minerals (vitamins, micronutrients) <sup>1</sup>	1.1	1.1	
Salt (NaCl)	0.3	0.3	
Methionine	0.2	0.2	
Essential oils	0.1	0.1	
Analytical composition, g/kg			
Metabolizable energy, kcal/kg	3,060.40		
Dry matter	561.26		
Fat content	54.53		
Crude protein	189.00		
Lysine	11.28		
Methionine	4.36		
Calcium	10		
Phosphorus	5.68		

<sup>&</sup>lt;sup>1</sup> Vitamin A 13,500 IU/kg, vitamin D<sub>3</sub> 3,000 IU/kg, vitamin E 25 mg/kg, copper sulfate 15 mg/kg, manganese (E5) 79.92 mg/kg, zinc [E6, zinc oxide (II)] 70 mg/kg, iron (E1, ferrous sulfate) 41 mg/kg, copper [E4, copper sulfate (II)] 15 mg/kg, iodine (E2, calcium iodate) 1 mg/kg, selenium (E8, sodium selenite) 0.4 mg/kg.

tissues (including cartilage caps), dried, weighed, incinerated at 560 °C for 24 h, and weighed for ash content determination.

#### 2.5. Meat quality

Left pectoral muscles (pectoralis major and pectoralis profondus) were sampled and packed in plastic bags for conservation at -80 °C before analysis. The Kjeldahl method (Norma ISO 937) was used to determine protein content. Fatty acid profiles were established after lyophilization for 48 h and lipid extraction by the method described in Folch et al. (1957). After saponification of the fat and methylation of the FA, fatty acid methyl esters were assayed by GC—MS according to the method described above for BSF samples (Douny et al., 2015). Water content was estimated as the difference between fresh and dry weights after lyophilization.

#### 2.6. Statistical analyses

All analyses were performed on SAS (version 9.3) and significance levels were set at 5%. All variables were checked for normality assumption. Individual body weights were analyzed with a mixed model for repeated measurements with replicates (n = 2), group (BSF or control), sex (female or male), age (in weeks) and all their interactions as categorical fixed effects, weights at the first day of measurement as covariate, and animal and time as random effects. From this analysis, weight least-squares means in the BSF group were compared with those in the control group. Next. analysis of variance were used to determine whether means of ADG. FCR and tibia ash percentages were different between groups. after adjusting for the effects of sex and replicates. Finally, truncated regression models were used to test whether addition of BSF larvae add an effect on FA profiles because some values were outside the limits of detection. In the results section, all means are given with their standard errors.

## 3. Results

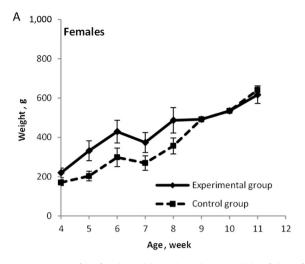
## 3.1. Characteristics of black soldier fly larvae and horse manure

Average length and weight of fresh BSF larvae were  $20.7 \pm 2.21$  mm and  $0.14 \pm 0.02$  g, respectively. Larvae DM and ash contents were  $(24.6 \pm 0.01)\%$  and  $(14.42 \pm 6.71)\%$ , respectively. Level of insoluble ash was below the balance detection limit indicating a lack in silica and other non-digestible minerals. On DM basis, lipid fraction was 23.15%. Most FA found in BSF larvae (65.5% of total FA) and horse manure (75.8% of total FA) was saturated fatty acids. Oleic acid (C18:1  $\omega$ 9) was the predominant unsaturated fatty acid (24.2% of total FA in horse manure and 22.9% of total FA in BSF larvae). Proportions of total FA were 8.3% and 28.1% for lauric acid (C12:0), 13.3% and 6.7% for myristic acid (C14:0), 33.6% and 22.0% for palmitic acid (C16:0), and 20.6% and 5.1% for stearic acid (C18:0), in horse manure and BSF larvae, respectively.

Mineral contents were 4.43% for calcium, 0.92% for phosphorus, 1.56% of potassium, 0.3% of sodium and 0.41% of magnesium (% of DM). Total nitrogenous content was 106.33  $\pm$  2.84 g/kg of fresh larvae.

#### 3.2. Growth parameters and chicken carcass yields

Growth profiles are given in Fig. 1. Initial weights, age, diet, interaction between age and sex influenced significantly weekly weights. At 50 days, overall mean weight was  $202.73 \pm 10.33$  g and reached  $843.23 \pm 39.06$  g at 80 days. Given these observations, it was possible to determine post-hoc that the inclusion of more individuals in the experiment was not necessary. In the sequential



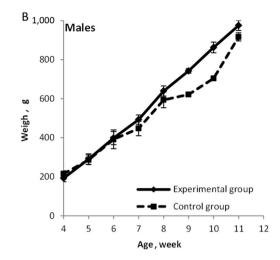


Fig. 1. Means weight of (A) females and (B) males Ardennaise chicken fed 8% of fresh larvae of black soldier fly (experimental group) or not (control group).

probability ratio test, the sum of observations (6,425) was above the right test boundary (6,117) with a sample size of 10 individuals in the BSF and a difference of 70 g between diets.

Results of the analysis of variance showed all effects in the model influenced significantly weights, with the exception of the effect of the replicates and of their interactions with group, sex and time. Weight least squares means in the BSF group were significantly higher (P < 0.05) than in the control group by  $77.03 \pm 53.37$  g. Weight least squares means were  $558.31 \pm 12.91$  g and  $321.12 \pm 14.44$  g in males and females, respectively. Weights increased by  $0.84 \pm 0.22$  g for each gram increase in the initial weight. Least squares means of ADG were similar in both groups, with  $11.77 \pm 0.62$  g and  $11.07 \pm 0.65$  g in the BSF and control group, respectively.

No significant difference between diets was found in means of FCR: means were 4.01 and 4.04 kg feed intake per kg gain for the BSF and control groups respectively. Weights of internal organs are given in Table 2. Mean weights of almost all organs were higher in males than females but not statistically different between groups.

# 3.3. Fatty acid profiles and protein content

Proportions of saturated and mono-unsaturated fatty acids were not significantly different between groups (Table 3) with the anecdotic exception of palmitoleic acid (C16:1 $\omega$ 7). Overall proportions of polyunsaturated fatty acids were slightly higher (P < 0.05) in the BSF [(36.7  $\pm$  0.69)% of total FA] than control [(33.8  $\pm$  0.39)% of total FA] groups, especially for the proportion of arachidonic acid (C20:4 $\omega$ 6). Mean  $\omega$ 6/ $\omega$ 3 ratio was similar in both groups. Mean protein content of chicken meat was also similar between groups: 24% in control and 26% in BSF groups.

#### 4. Discussion

This study focuses on BSF larvae fed to a local poultry breed after being grown on horse manure. Although the rearing system was very simple (see material and methods), it was successful to obtain healthy generations of BSF larvae and could be easily adapted for African situation and implemented at the farm and community levels, as suggested by Kenis et al. (2014). Results on characteristics of BSF larvae are consistent with those in the literature for mean length and weight (Gnaedinger et al., 2015) and for lipid fraction which was between 21% and 30% depending on the composition of the substrate on which the larvae were raised (Gnaedinger et al.,

**Table 2**Carcass traits of 80-day-old Ardennaise chickens fed a diet supplemented with 8% fresh larvae of black soldier fly (experimental group) or not (control group).

Item	Sex	Control group	Experimental group
Carcass, g	Female	622.60 ± 51.21	621.45 ± 41.81
	Male	930.36 ± 29.57	935.54 ± 32.39
Carcass yield, %	Female	$65.27 \pm 1.66$	$63.46 \pm 1.36$
•	Male	$66.29 \pm 0.96$	$64.99 \pm 1.05$
Head weight, g	Female	$34.31 \pm 4.14$	$37.85 \pm 3.38$
	Male	$48.79 \pm 2.39$	$47.65 \pm 2.62$
Legs weight, g	Female	$26.02 \pm 3.57$	$28.41 \pm 2.92$
	Male	$39.51 \pm 2.06$	$34.45 \pm 2.26$
Gizzard weight, g	Female	$19.66 \pm 2.69$	$20.18 \pm 2.20$
	Male	$24.38 \pm 1.55$	$24.94 \pm 1.70$
Proventriculus weight, g	Female	$4.27 \pm 0.51$	$3.95 \pm 0.42$
	Male	$4.90 \pm 0.29$	$5.25 \pm 0.32$
Heart weight, g	Female	$3.25 \pm 0.44$	$4.24 \pm 0.36$
	Male	$4.82 \pm 0.25$	$5.02 \pm 0.28$
Liver weight, g	Female	$16.53 \pm 1.88$	$15.39 \pm 1.53$
	Male	$22.27 \pm 1.08$	$20.94 \pm 1.19$
Pectoral muscle weight, g	Female	$60.42 \pm 10.79$	$64.16 \pm 8.81$
	Male	$101.24 \pm 6.23$	$100.35 \pm 6.82$
Drumsticks and thighs weight, g	Female	$122.01 \pm 14.43$	$131.82 \pm 11.79$
	Male	$192.06 \pm 8.33$	$200.36 \pm 9.13$
Wings weight, g	Female	$62.24 \pm 5.07$	$59.94 \pm 4.14$
	Male	$84.74 \pm 2.93$	$86.98 \pm 3.21$
Spleen weight, g	Female	$1.21 \pm 0.19$	$1.21 \pm 0.16$
	Male	$1.55 \pm 0.11$	$1.59 \pm 0.12$
Tibia ash, g	Female	$20.01 \pm 1.32$	$23.44 \pm 1.62$
	Male	$21.90 \pm 1.14$	$21.87 \pm 1.02$

2015). Makkar et al. (2014) reported lipid fraction ranging from 15% to 25% in BSF larvae raised on chicken manure, 28% on pork manure, 35% on cattle manure, and 42% to 49% on an oil-rich substrate. In Table 4, comparison of FA profiles of BSF larvae raised on ours and other substrates confirms results of Makkar et al. (2014) that FA composition depends upon substrate components. Percentage of lauric acid is higher in fresh larvae (28.1% of total FA) than in manure (8.3% of total FA). This is interesting because lauric acid is known to be active against lipid coated viruses, clostridium, and many pathogenic protozoa (Lieberman et al., 2006). Mineral content are similar to those found in dried larvae raised on poultry and swine manure (Makkar et al., 2014), with high content of calcium and phosphorus.

As detailed in the result section, an important finding is that inclusion of 8% fresh BSF larvae improved chicken growth pattern during their rearing periods. Besides this effect, inclusion of BSF larvae had no negative influence on the other measured parameters

**Table 3**Fatty acid composition (expressed as a percentage of total fatty acids) of left pectoral muscles of 80-day-old Ardennaise chicken fed a diet supplemented with 8% fresh larvae of black soldier fly (experimental group) or not (control group).

Fatty acids	Control group	Experimental group
Myristic acids (C14:0)	0.72 ± 0.39	<loq<sup>1</loq<sup>
Palmitic acids (C16:0)	$26.07 \pm 0.87$	$25.48 \pm 0.09$
Heptadecanoic acids (C17:0)	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
Stearic acids (C18:0)	$11.93 \pm 0.72$	$12.13 \pm 0.55$
Palmitoleic acids (C16:1 ω7)	$1.95 \pm 0.11$	<loq< td=""></loq<>
Heptadecanoic acids (C22:5)	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
Oleic acids (C18:1 ω9)	$25.53 \pm 1.82$	$25.68 \pm 1.16$
Linoleic acids (C18:2 ω6)	$20.23 \pm 0.86$	$20.92 \pm 0.39$
Linolenic acids (C18:3 ω6)	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
Eicosadienoic acids (C20:5 ω3)	$0.33 \pm 0.18$	<loq< td=""></loq<>
Arachidonic acids (C20:4)	$6.95 \pm 1.04$	$8.83 \pm 1.24$
Alpha linolenic acids (C18:3)	$1.35 \pm 0.75$	$1.79 \pm 0.28$
Docosapentaenoic acids	3.64 (0.45)	3.85 (0.01)
Docosahexaenoic acids (C22:6)	1.28 (0.26)	1.31 (0.11)

<sup>&</sup>lt;sup>1</sup> <LOQ: inferior to the lower limit of quantification (0.5% of total fatty acids).

**Table 4**Fatty acid composition (expressed as a percentage of total fatty acids) of larvae of black soldier fly (BSF) reared on cow manure, 50% cow manure and 50% fish waste, pig manure and horse manure.<sup>1</sup>

Fatty acids	Origin of BSF rearing manure			
	Cow	Cow and fish	Pig	Horse
Capric acids (C10:0)				2.8
Lauric acids (C12:0)	21.4	49.3	42.6	28.1
Myristic acids (C14:0)	2.9	6.8	6.9	6.7
Palmitic acids (C16:0)	16.1	10.5	11.1	22.0
Heptadecanoic acids (C17:0)				0.8
Stearic acids (C18:0)	5.7	2.8	1.3	5.1
Palmitoleic acids (C16:1 ω7)		3.5		8.2
Heptadecanoic acids (C22:5)				1.4
Oleic acids (C18:1 ω9)	32.1	11.8	12.3	22.9
Linoleic acids (C18:2 ω6)	4.5	3.7	3.6	2.1
Linolenic acids (C18:3 ω6)	0.2	0.1	0.7	<loq<sup>2</loq<sup>
Eicosapentaenoic acids (C20:5 ω3)	_	_	1.7	<loq< td=""></loq<>
Docosahexaenoic acids (C22:6)	-	_	0.6	<loq< td=""></loq<>

 $<sup>^{1}</sup>$  With the exception of horse manure, data are from Makkar et al. (2014) (Table 35).

(weights of internal organs, ash percentages, meat fatty acid profiles and  $\omega 6/\omega 3$  ratio). Performances of the Ardennaise breed are consistent with previous findings observed over a period from hatch to 84 days (Moula et al., 2009). ADG means were similar to the previously reported value of 12.4 g, and FCR values were only slightly lower than the value of 4.4 recorded at an age of 12 weeks. Means of carcass yields of both groups (66.9%) were close to those reported by Sauveur (1997) as the standard (66.1%) but slightly lower than those reported by Moula et al. (2013) in the Ardennaise silver (67.3%) and golden (68.6%) breeds. Fatty acid profiles were also consistent with those already reported in the literature for chicken meat (Table 4), i.e., high proportions of C16 and C18 FA (saturated,  $\omega$ 9 or  $\omega$ 6), even though feed can deeply alter characteristics of meat (Brunel et al., 2006).

#### 5. Conclusions

Feeding chicken with BSF larvae is particularly well suited to traditional systems of poultry production but only a few studies reported on the effects of BSF larvae on chicken growth rates and on the fatty acid profiles of BSF larvae and chicken meat. Results of this study showed that BSF larvae can grow successfully on horse manure under simple rearing conditions. Larval fatty acids profiles were predominantly composed of lauric acid (28.1%) and palmitic

acid (22.0%). Weekly weights of Ardennaise chickens fed a commercial standard feed in which 8% was substituted with whole defrozen larvae were slightly higher than those of control chickens. All the other measurements were not statistically different between larvae-fed and control chickens, including fatty acid profiles, protein content and  $\omega 6/\omega 3$  ratio. More research is needed to confirm the results on other slow growing chicken breeds and BSF larvae raised on the other substrate.

#### Conflict of interest

The authors declare that they have no conflict of interest.

# Acknowledgments

The authors thank Ynsect for the supply of the larvae used in this study and staff of the experimental farm of the faculty of veterinary medicine of the University of Liège for their help in providing accommodation.

#### References

ASAE Standards. http://www.manuremanagement.cornell.edu/Pages/General\_Docs/ Other/ASAE\_Manure\_Production\_Characteristics\_Standard.pdf; 2003 [accessed 10.06.17].

Besbes B. Genotype evaluation and breeding of poultry for performance under suboptimal village conditions. World's Poult Sci J 2009;65:260—71.

Briggs JL, Maier DE, Watkins BA, Behne KC. Effect of ingredients and processing parameters on pellet quality. Poult Sci 1999;78:1464–71.

Brunel V, Jehl N, Drouet L, Portheau MC. Viande de volailles: Sa valeur nutritionnelle présente bien des atouts. Viandes Produits Carnés 2006;25:18—22.

Caparros Megido R, Alabi T, Nieus C, Blecker C, Danthine S, Bogaert J, et al. Optimization of a cheap and residential small-scale production of edible crickets with local by-products as an alternative protein-rich human food source in Ratanakiri Province (Cambodia). J Sci Food Agric 2015;96:627–32.

Diener S, Zurbrügg C, Tockner K. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Manag Res 2009;27:603–10.

Douny C, El Khoury R, Delmelle J, Brose F, Degand G, Moula N, et al. Effect of storage and cooking on the fatty acid profile of omega-3 enriched eggs and pork meat marketed in Belgium. Food Sci Nutr 2015;3:140—52.

Elwert C, Knips I, Katz P. A novel protein source: Maggot meal of the Black Soldier fly (*Hermetia illucens*) in broiler feed. In: Gierus M, Kluth H, Bulang M, Kluge H, editors. Tagung Schweine- und Geflügelernährung. Germany: Universität Halle-Wittenberg; 2010. p. 140–2.

Faul F, Erdfelder E, Lang A, Buchner A. G\*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res 2007;39:175–91.

Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. I Biol Chem 1957;226:497–509.

García R, Baez AP. Atomic absorption spectrometry (AAS). In: Farrukh MA, editor. Atomic absorption spectroscopy. Rijeka, Croatia: Intech; 2012. p. 1–12.

Gnaedinger C, Mellin C, Tran G. Black soldier fly larvae (Hermetia illucens). Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. 2015. http://feedipedia.org/node/16388 [accessed 05.02.17].

Hale OM. Dried *Hermetia illucens* larvae (Diptera: Stratiomyidae) as a feed additive for poultry. I Georgia Entomol Soc 1973;8:16–20.

Kenis M, Koné N, Chrysostome CAAM, Devic E, Koko GKD, Clottey VA, et al. Insects used for animal feed in West Africa. Entomologia 2014;2:107–14.

Lieberman S, Enig MG, Preuss HG. A review of monolaurin and lauric acid. Natural virucidal and bactericidal agents. Altern Complement Ther 2006;12:310–4.

Makkar HPS, Tran G, Heuze V, Ankers P, State-of-the-art on use of insects as animal feed. Anim Feed Sci Technol 2014;197:1–33.

Mengesha M, Tamir B, Dessie T. Village chicken constraints and traditional management practices in Jamma District, South Wollo, Ethiopia. Livestock Res Rural Dev 2011;23:37. http://www.lrrd.org/lrrd23/2/meng23037.htm [accessed 12 06 17]

Moula N, Antoine-Moussiaux N, Farnir F, Philippart M, Leroy P. Performances zootechniques de la poule Ardennaise, une race ancienne pour le futur. Ann Méd Vét 2009:153:66–75.

Moula N, Jacquet M, Verelst A, Antoine-Moussiaux N, Farnir F, Leroy P. Les races de poules belges. Ann Méd Vét 2012;155:37–65.

Moula N, Michaux C, Philippe F-X, Antoine-Moussiaux N, Leroy P. Egg and meat production performances of two varieties of the local Ardennaise poultry breed: silver black and golden black. Anim Genet Resour 2013;53:57–67.

Myers HM, Tomberlin JK, Lambert BD, Kattes D. Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. Environ Entomol 2008;37: 11–5.

 $<sup>^{2}\,</sup>$  <LOQ: inferior to the lower limit of quantification (0.5% of total fatty acids).

- Newton L, Sheppard C, Watson DW, Burtle G, Dove R. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. Development of environmentally superior technologies. 2005. p. 1–17.
- Okorogbona AOM, Adebisi LO. Animal manure for smallholder agriculture in South Africa. Farming for food and water security, vol. 10; 2012. p. 201–42.
- Sauveur B. Les critères et facteurs de la qualité des poulets Label Rouge. Prod Anim 1997;10:219—26.
- Sheppard CD, Larry Newton G. A value added manure management system using the black soldier fly. Bioresour Technol 1994;50:275–9.
- St-Hilaire S, Cranfill K, McGuire MA, Mosley EE, Tomberlin JK, Newton L, et al. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. J World Aquacult Soc 2007;38:309—13.
- van Huis A. Potential of insects as food and feed in assuring food security. Annu Rev Entomol 2013;58:563–83.
- Vu TKV, Tran MT, Dang TTS. A survey of manure management on pig farms in Northern Vietnam. Livest Sci 2007;112:288–97.
- World Horse Population. http://www.worldhorse.org/public/World-Horse-Populations-Gathering-Accurate-Information-2.cfm; 2006 [accessed 11.06.17].