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Inclusion of *Hermetia Illucens* larvae or prepupae in an experimental extruded feed: process optimisation and impact on *in vitro* digestibility

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ABSTRACT

This study investigated the effect of extrusion on digestibility of different blends containing *Hermetia illucens* (HI) larvae or prepupae. Five blends of HI larvae or prepupae and wheat flour, in a ratio of 25:75, with or without sunflower oil addition, have been formulated as follows: prepupae + wheat (no oil); prepupae + wheat (low oil); prepupae + wheat (medium oil); prepupae + wheat (high oil); larvae + wheat (no oil). Ether extract (EE) content in different blend was 31.5, 38.9, 46.3, 53.7 and 46.27 g kg⁻¹ on wet basis (wb), respectively. Blends were homogeneous for moisture (238.9 g kg⁻¹) and crude protein (112.6 g kg⁻¹ wb). Feed blends were extruded by a co-rotating, conical twin-screw mini extruder and net torque value (NTV) was recorded as indicator of extrudability. The best performing blend was furtherly tested at four barrel temperatures (60, 70, 80 and 90 °C). *In vitro* organic matter digestibility (OMD) and *in vitro* crude protein digestibility (CPD) were measured to evaluate the effect of extrusion process on nutritional value. Increasing the blend EE content up to 53.74 g kg⁻¹ wb, NTV was reduced by four times (<100 Ncm) compared to 31.5 and 38.9 g kg⁻¹ wb EE blends. The best performing mixture was larvae + wheat (no oil). Extrusion process increased OMD but not CPD compared to unextruded control, while different extrusion temperature did not affect OMD nor CPD. Concluding, extrusion can contribute to increase OMD in insect containing feed blends. EE content in the blends is a key variable that should be defined in the process.

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Introduction

There is an increasing pressure on the livestock sector to meet the growing demand for high-value animal protein (FAO 2009; Pinotti et al. 2014, 2016). This implies several feed supply problems, in general, and feed protein supply issues, in particular. In this context, insects have been proposed as an alternative protein source (Sánchez-Muros et al. 2014; Henry et al. 2015). In this respect, recently, the European Commission (2017) authorised for aquaculture animals the use of processed animal protein derived from farmed insects, and compound feed containing such protein. Both published and ongoing research on insects as novel feed are focussed on, type of feed substrate used for rearing insects, nutritional values and feed safety of the insects produced and the performance of animals that are fed with the insects (Ottoboni et al. 2017;

van Raamsdonk et al. 2017). Several insect species are able to convert organic waste into edible biomass, of which the composition may depend on the substrate. This latter has been deeply addressed by Spranghers et al. (2017), who reported that protein content and quality were high and comparable for *Hermetia illucens* (HI) prepupae reared on different organic waste-based substrates. However, fat and ash contents appear to be dependent on the rearing substrate. Furthermore, the same authors observed also that the total biomass of the harvested prepupae differed substantially in function of type of rearing substrate.

Although the growing substrate is determinant in defining the insect's composition, a further source of variation is the insect species considered, as well as their life stage (larvae vs. (pre)pupae vs. adult form). In general, larvae are fatter than prepupae. This last

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aspect could impact not only on feed formulation, but also on feed production. Indeed, the fat content in the feed blends is a key variable that should be defined in the feed processing (e.g. extrusion). The nutritional features of HI larvae, housefly maggots (*Musca domestica*), mealworm (*Tenebrio molitor*), locusts-grasshoppers-cricket (several species) and silkworm meal (*Bombyx mori*) and their use as a substitute for soy-meal and fishmeal in farm animal diets are well documented (Veldkamp and Bosch 2015). The crude protein content of these insects ranges from 420 to 630 g kg⁻¹ on dry matter (DM), while the lipid content may reach 360 g kg⁻¹ DM (Barroso et al. 2014; Makkar et al. 2014; Veldkamp and Bosch 2015). Insect meals obtained from several insect species, such as HI and *Tenebrio molitor*, are also characterised by a high *in vitro* crude protein digestibility coefficient (Marono et al. 2015), proposing them as good protein source for most of the farm species. The lipid content has also a great potential, since fats be extracted and used in different fields including biodiesel production (Li et al. 2011). A further step in the insect meals use was their inclusion in complete food-producing animal diets. Complete diets for poultry (Bovera et al. 2015, 2016; Schiavone et al. 2017), swine (Ji et al. 2016) and commercial fish species (Newton et al. 2005; St-Hilaire et al. 2007; Gasco et al. 2016; Iaconisi et al. 2017; Magalhães et al. 2017; Piccolo et al. 2017), have been tested and proposed over last few years. Considering findings rising from these studies, it can be concluded that, insect protein meals are able to support good growth and, therefore, can be a suitable protein source for animal feed. Moreover, although insects have been proposed as an alternative protein source, they are also high in fat (Barroso et al. 2014; Makkar et al. 2014; Veldkamp and Bosch 2015), which elevates their energetic potential. On the other hand, in terms of fatty acids profile, insect meals deriving from several insect species are characterised by low polyunsaturated fatty acids levels. This last aspect, as reported elsewhere (Henry et al. 2015), could limit their use in aquafeed as replacements of fish oil. In this sense, defatted insect meal, has been also proposed for aquafeed formulation.

Furthermore, certain insect materials (e.g. larvae and prepupae) may contain large amounts of moisture (Veldkamp and Bosch 2015; Spranghers et al. 2017). This implies that such insect material, before being used as ingredient in a compound feed, needs to be processed in order to reduce its moisture content. These kind of processes are both time- and energy-

consuming and therefore limiting in several cases the use of wet ingredients in compound feed formulation. Of note, proper and efficient drying of the feed is one of the key elements in producing profitable feed (Hamm 2017). In general, the optimum moisture content for compound feed production ranges from 12% to 18%. Moving above or under these reference values, traditional technological processes for feed production, such as pelleting, can generate several problems in a feed plant (Thomas et al. 1998). By contrast, adopting extrusion technology makes possible the inclusion of high proportions (up to 35%) of wet ingredients (e.g. fresh meat) (Guy 2001). In particular, twin-screw extruders can handle viscous, oily, sticky or wet material, including products with more than 25% of internal fat (Riaz 2000; Guy 2001). Additionally, the extrusion process can control the density of a product, resulting in high digestibility and improved physical characteristics (Guy 2001). When the mash is extruded through the die, the release of pressure and heat causes starch expansion and gelatinisation (Shankar and Bandyopadhyay 2005). Both gelatinisation and expansion of the starch also increase its nutritional value by increasing its digestibility (Bergot and Breque 1983; Jeong et al. 1991; Glencross 2011). Another benefit of the extrusion process is its denaturing effect on some of the antinutritional factors in raw materials such as protease inhibitors and lectins, which are affected by the heat treatment (Refstie et al. 1998; Francis et al. 2001). With regard to the effects of feed formulas on extrusion performance, several authors (Madeleine 1979; Schweizer et al. 1986) reported that adding lipids during extrusion generally retards and modulates starch gelatinisation and affects dough rheology in the barrel (Hsieh et al. 1991). In the light of these considerations, it can be speculated that extrusion may represent a suitable way for processing raw insect materials with limited pre-processing, such as drying and defatting. Accordingly, the purposes of this research were (i) to examine the effect of extrusion process on different HI larvae or prepupae blends with or without oil addition and (ii) to evaluate the impact of extrusion temperature on the *in vitro* organic matter digestibility (OMD) and *in vitro* crude protein digestibility (CPD) in basic cereal insect blends.

Materials and methods

Mixture formulation

Wheat baking flour, *Hermetia illucens* (HI) larvae and prepupae, and vegetable oil were used as ingredients in this study. Wheat baking flour obtained from the

Table 1. Chemical composition of *Hermetia illucens* (HI) prepupae and larvae; chemical composition and oil inclusion rate in mixtures used in experiment 1.

	Oil inclusion, g kg ⁻¹ wb	Moisture, g kg ⁻¹	CP, g kg ⁻¹ wb	EE, g kg ⁻¹ wb	Ash, g kg ⁻¹ wb
<i>Hermetia illucens</i> insects					
HI Prepupae	–	647.20	129.83	96.14	53.41
HI Larvae	–	627.50	108.14	154.77	26.63
Mixtures					
Prepupae + wheat 25:75 (NO oil)	–	242.1	114.8	31.5	23.4
Prepupae + wheat 25:75 (Low oil)	7.5	240.2	113.9	38.9	23.2
Prepupae + wheat 25:75 (Medium oil)	15.0	238.4	113.0	46.3	23.0
Prepupae + wheat 25:75 (High oil)	22.5	236.5	112.1	53.7	22.9
Larvae + wheat 25:75 (NO oil)	–	237.1	109.3	46.2	16.7

CP: crude protein; EE: ether extract; wb: on wet basis.

experimental baking laboratory of the Department of Applied Bioscience of Ghent University. Larvae were fully grown and sourced from a private company producing HI for experimental use (Millibeter BVBA, Antwerp, BE), while the prepupae were supplied by the Department of Crop Protection of the Faculty of Bioscience Engineering at the University of Ghent, as described in Spranghers et al. (2017). Sunflower oil was obtained from a local supermarket. Five blends of HI larvae or prepupae (wet material) and wheat flour in a ratio of 25:75, with or without sunflower oil addition, were formulated as follows: prepupae + wheat (no oil); prepupae + wheat (low oil); prepupae + wheat (medium oil); prepupae + wheat (high oil); larvae + wheat (no oil). These premixes of flour and raw insects were prepared in a household blender. Sunflower oil was added to the prepupae premix in order to obtain mixtures ranging from 31.5 to 53.7 g kg⁻¹ EE on wet basis (wb). The details of these blends are reported in Table 1. All blend samples have been analysed for moisture, crude protein (CP), ether extract (EE) and ash. Specifically, the moisture content of the samples was determined by an oven-drying method, at 130 °C for 12 h, as proposed by the European Commission (Commission Regulation No. 152/2009); crude protein (CP) content has been measured according to the Kjeldahl method (proc.2001.11; AOAC 2005). Ether extract was determined by the Soxhlet method, with prior hydrolysis, as proposed by the European Commission (Commission Regulation No. 152/2009). Ash was also measured by using a muffle furnace at 550 °C (proc. 942.05; AOAC 2005).

Extrusion

Extrusion was performed on a co-rotating, conical twin-screw mini extruder (HAAKE™ MiniLab II). The barrel was composed of a single controlled temperature zone and heated by an electric cartridge heating system (air-cooled). The barrel can be split horizontally and opened to enable rapid removal and cleaning of

the barrel and the screws. The mini extruder was manually fed using a laboratory spoon, and a pestle was used to force the raw materials into the extruder. The end of the extruder was equipped with a single circular die opening of 2 mm in diameter. Extruded material was collected when both, die flow and torque value were stable for at least 2 min, then cooled at room temperature and packed in plastic bags. The variables studied were: the level of fat used in the premixes, net torque value and barrel temperature.

The extrusion test was split in two Experiments:

Experiment 1

The experimental design consisted of four EE levels (31.5, 38.9, 46.3 and 53.7 g kg⁻¹ wb), a single-barrel temperature (60 °C) and a single screw speed (60 rpm). Premix containing larvae material without added oil was compared to premix containing prepupae material, in the same flour to insect ratio, with increasing amounts of oil (EE 31.5–53.7 g kg⁻¹ wb).

The torque value was recorded for each tested mixture; mixtures with net torque value (NTV) < 100 Ncm were considered extrudable and a decrease in this value in a mixture indicates higher extrudability.

Experiment 2

According to the extrudability test (based on NTV value < 100), the best performing blend has been used to evaluate the effect of barrel temperatures on digestibility. This experiment used a single screw speed (100 rpm) and four different barrel-temperatures (60, 70, 80 and 90 °C).

In vitro digestibility test

In vitro digestibility assays were performed on extruded experimental feed, as is, according to the protocol described in Dierick (1991) with minor adaptations. The protocol included two different *in vitro*

Table 2. *In vitro* digestion protocol (modified from Dierick et al. 1991).

	<i>In-vitro</i> OMD	<i>In-vitro</i> CPD
Step 1	2 g sample Incubation with pepsin 4h 37° C	150 mg protein Incubation with pepsin 4h 37° C
Step 2	Incubation with pancreatin 4h 37° C	Incubation with pancreatin 4h 37° C
Step 3	Centrifugation residue: undigested organic matter	+ Phosphotugstenic acid (PTA) Centrifugation residue: undigested crude protein Supernatant: α amino-group det.

OMD: organic matter digestibility; CPD: crude protein digestibility.

assays for (i) organic matter digestibility and (ii) crude protein digestibility, respectively (Table 2).

Both organic matter digestibility and crude protein digestibility assays were serially performed on each extruded experimental mixture in triplicate. The results were used for the calculation of average values per sample.

***In vitro* organic matter digestibility**

For every replicate, 2 g of sample was finely ground (<1 mm), weighed to an accuracy of ± 0.1 mg and placed in a 100-mL incubation flask with 40 mL of 0.075 N hydrochloric acid-pepsin solution containing 2 mg/mL of pepsin (P7000 Sigma-Aldrich, St. Louis, MO). In order to minimise bacterial fermentations during digestion, one drop of thimerosal solution (50 mg/mL ethanol) was added to the mixture. The incubation flasks were placed in a shaking water bath at 37 °C for 4 h (simulation of the gastric phase). Subsequently, samples were cooled in an ice bath and the pH was adjusted to 7.5 with 1 N NaOH. Forty millilitres 0.2 N phosphate buffer containing 10 mg/mL of porcine pancreatine (P3292, Sigma-Aldrich) were added to the mixture. The bottles were placed in a shaking water bath at 37 °C for 4 h (simulation of the small intestinal phase). The digestion was stopped by placing the incubation flasks in an ice bath. The undigested residue was separated from the supernatant by centrifugation. Specifically, three consecutive centrifugations and washings with 2×20 mL distilled water were performed for each sample, and undigested residues were oven-dried, at 130 °C for 2 h, as proposed by the European Commission (Commission Regulation N° 152/2009). Dry matter content of undigested residue was recorded for each sample. Following, the residue was incinerated in a muffle oven (proc. 942.05; AOAC 2005), and *in vitro* organic matter digestibility coefficients were calculated as follows:

$$\text{OMD} = (\text{OMs} - \text{OMr}) / \text{OMs}$$

where

OMD is organic matter digestibility;

OMs is the organic matter of samples;

OMr is the organic matter content of residual material after digestion.

***In vitro* crude protein digestibility**

For *in vitro* crude protein digestibility determination, 1.35 g of sample corresponding to 150 mg of protein was used. Each sample was finely ground (<1 mm), weighed to an accuracy of ± 0.1 mg and placed in a 100-mL incubation flask with 20 ml of 0.075 N hydrochloric acid-pepsin solution containing 2 mg/mL of pepsin (P7000 Sigma-Aldrich). In order to minimise bacterial fermentations during digestion, one drop of thimerosal solution (50 mg/mL ethanol) was added to the mixture. The incubation flasks were placed in a shaking water bath at 37 °C for 4 h (simulation of the gastric phase).

Subsequently, samples were cooled in an ice bath and pH was adjusted at 7.5 with 0.2 N NaOH. Twenty millilitres 0.2 M phosphate buffer containing 1.5 mg/mL of porcine pancreatine (P3292, Sigma-Aldrich, St. Louis, MO) were added to the mixture. The bottle was placed in a shaking water bath at 37 °C for 4 h (simulation of the small intestinal phase). The digestion was stopped by placing the incubation flasks in an ice bath. After cooling, 10 mL of phosphotugstenic acid (PTA) 0.2 M were added to each bottle and the bottles were kept at room temperature for 5 min to facilitate undigested soluble protein precipitation. The undigested residue was separated from the supernatant by centrifugation. Subsequently, each sample was rewashed with 5 mL of PTA +20 mL of distilled water, centrifuged and the supernatant was removed. Supernatants resulting from the two consequent centrifugations of each sample were collected for further analysis. The undigested residue was transferred to Kjeldahl tubes and undigested crude protein was measured (proc.2001.11; AOAC 2005).

The *in vitro* crude protein digestibility coefficients were calculated as follows:

$$\text{CPD} = (\text{CPs} - \text{CPr}) / \text{CPs}$$

where

CPD is crude protein digestibility;

CPs is the crude protein content of samples;

Table 3. Effects of fat on extrusion performances of tested mixtures.

Mixture	Ratio	EE (g kg ⁻¹ wb)	NTV (Ncm)	Extrudability
Prepupae + wheat	25:75 (NO oil)	31.5	200–400	—
Prepupae + wheat	25:75 (low oil)	38.9	>400	—
Prepupae + wheat	25:75 (medium oil)	46.3	100–130	+
Prepupae + wheat	25:75 (high oil)	53.7	50–100	++
Larvae + wheat	25:75 (NO oil)	46.2	80–120	+++

EE: ether extract; NTV: net torque value; wb: on wet basis; —: not extrudable; +: fair extrudability; ++: acceptable extrudability; +++: good extrudability.

CPr is the crude protein content of residual material after digestion.

Determination of free and total digestible amino acids

Supernatants resulting from the crude protein digestibility assay were filtered with filter paper and free and total amino acids concentrations were determined. Specifically, free amino acid (AA) content in the supernatants was determined according to the α amino-group method, described by Oddy (1974), which measures α -amino nitrogen (α NH₂-N) content in the sample.

To determine the total amino acids (including, free AAs, and di-, tri- and tetra peptides), Dierick's (1991) protocol has been adopted: 1 mL of supernatant of each sample was hydrolysed with 1 mL of HCl 12 M, 24 h at 100 °C. Samples were successively cooled at room temperature, and 2 mL of NaOH 6 M were added to neutralise the solution. The total amount of AAs was determined after 20x dilution with distilled water of the hydrolysed supernatant.

Statistical analysis

Water removal, OMD, CPD and total AA values for the technological treatments (CTR 60, 70, 80 or 90 °C) were analysed using one-way analysis of variance (one-way ANOVA) in order to compare means of the four extrusion temperatures +1 control (no extrusion) (IBM SPSS Statistics 22). The analysis has been performed using the following model:

$$y_{ij} = \mu_j + \varepsilon_{ij}$$

where y_{ij} are the observations (values), μ_j is the mean of the observations for the j th group (technological treatment) and ε_{ij} represents the within- technological treatment random variability. Differences with p values <.05 were considered significant.

The coefficients of correlation between the extrusion temperature and water removal, total AAs, and free AAs were estimated using a PROC CORR procedure (SAS, 9.4, SAS Inst. Inc., Cary, NC).

Results

Extrusion

Experiment 1

In the first experiment, extrudability of different blends with added oil or not have been tested. Results obtained are reported in Table 3. The two lowest fat mixtures were considered not extrudable (NTV >100 Ncm). By increasing the fat content from 39 to 46 g kg⁻¹ wb, the NTV decreased substantially (up to four times) to an acceptable level for extrusion (Table 3). The best extrusion performance (NTV 50–100 Ncm) were obtained with the highest fat mixtures, namely prepupae + wheat 25:75 (high oil), and larvae + wheat 25:75 (NO oil). Both blends were characterised by a fat content in the range of 46.2–53.7 g kg⁻¹ wb. Among these two blends, larvae + wheat 25:75 (NO oil) blend with 46 g kg⁻¹ wb of EE has been selected, since no oil addition, at comparable NTV, was required. This implies a simplest feed preparation, which is always appreciated in the feed manufacture.

Experiment 2

In the second experiment, the selected blend (larvae + wheat 25:75 NO oil) has been tested for different barrel temperatures, that did not affect NTV (data not presented). By contrast, as reported in Table 4, different barrel temperatures (from 60 °C to 90 °C) did affect water removal in the tested material. The highest value for water removal was recorded after extrusion at 60 °C. At the highest extrusion temperature (90 °C), water removal was 26% lower compared to that recorded at 60 °C. This result is also supported by the negative correlation between water removal and extrusion temperature (Pearson's $r = -0.97$; $p < .001$).

In vitro digestibility test

Results obtained for *in vitro* organic matter digestibility are reported in Table 4. Organic matter digestibility was higher (+16.8%; $p < .05$) in extruded mixtures compared to the control mixture, indicating an increase of digestibility due to the extrusion treatment. On the other hand, no differences have been observed

Table 4. Effect of barrel temperature on water removal per kg of product (g), *in vitro* organic matter digestibility coefficient, and *in vitro* crude protein digestibility coefficient in extruded mixtures and relative standard deviation (\pm SD).

Extr. T°	Water removal g kg ⁻¹ of mixture extruded	OMD		CPD	
Control	–	0.81a	(\pm 0.02)	0.93	(\pm 0.01)
Unextruded					
60	50.1a (\pm 1.86)	0.96b	(\pm 0.01)	0.94	(\pm 0.01)
70	44.7b (\pm 0.22)	0.94b	(\pm 0.01)	0.94	(\pm 0.01)
80	42.9b (\pm 0.29)	0.95b	(\pm 0.01)	0.94	(\pm 0.01)
90	37.3c (\pm 0.92)	0.95b	(\pm 0.01)	0.94	(\pm 0.01)

CPD: *in vitro* crude protein digestibility; OMD: *in vitro* organic matter digestibility.

Different letters (a, b, c) indicate statistical difference ($p < .05$) within the same column.

Table 5. Alpha-amino group determinations in supernatant on control (unextruded) and extruded material and relative standard deviation (\pm SD).

	mmol L ⁻¹			
	Total AAs		Free AAs	
Control unextruded	7.38	(\pm 0.65)	1.77a	(\pm 0.28)
60	7.59	(\pm 0.50)	2.58b	(\pm 0.04)
70	7.82	(\pm 0.54)	2.40b	(\pm 0.37)
80	7.34	(\pm 0.52)	2.21ab	(\pm 0.03)
90	7.63	(\pm 0.14)	1.61ac	(\pm 0.18)

AAs: amino acids.

Different letters (a, b, c) indicate statistical difference ($p < .05$) within the same column.

for OMD values among four extrusion temperatures (average OMD 0.95).

Results obtained for CPD are reported in Table 4. All mixtures analysed, including control mixture (unextruded), showed high coefficients of CPD with an average value of 0.94. Both extrusion and different temperatures tested (60, 70, 80 and 90 °C), did not affect CPD.

With regard to the total AAs in supernatants (Table 5), no differences have been observed between the control and the extruded material nor among material extruded at four tested barrel temperatures. This is also supported by the absence of any correlation (data not presented) between total AAs in supernatants and extrusion temperature. By contrast, free AA values were higher in 60 °C extruded mixtures than in controls and significantly decreased (Spearman's Rho, ρ_s -0.83 ; $p < .01$) as the barrel temperature was increased from 60° to 90 °C (Table 5).

Discussion

Extrusion

In the first experiment of this study, extrudability of different blends with or without oil addition has been tested. Results obtained evidenced that increasing the mixtures fat content, the NTV decreased substantially

indicating a better extrudability. According to literature, this result was expected: Lin et al. (1998) observed an improvement in extrudability when fat inclusion was increased from 0 to 75 g kg⁻¹ in experimental premixes intended for dry pet food production. The lubricating effect of fat inside the extruder probably reduced the friction between the dough and the screw elements and between the dough and the barrel, resulting in a decrease of torque value (Guy 2001). In the present study, however, the fat content in the mixtures ranged from 31.5 to 53.7 g kg⁻¹ wb, which impose some further considerations. Mixing prepupae, wheat flour and oil, that allow to reach a fat content higher than 5%, improved NTV values and consequently extrudability of the mixture. Comparable values, however, have been obtained for the larvae and wheat (no oil) blend. These results can be attributed to the fact that larvae are fatter compared to prepupae, and as consequence, no added oil is required to reach the target value of 46 g kg⁻¹ wb of fat, tested in the present experiment. Furthermore, as reported elsewhere (Singh et al. 2007), the relative low lipid content as well as its native nature in the larvae + wheat (NO oil added) blend, probably facilitate the lubricating effect, which in turn improved extrudability. In this respect, Singh et al. (2007) reported also that food ingredients with low-fat level (5–6%) are able to facilitate steady extrusion and improve the product texture. By contrast, high-fat ingredients are generally not advisable, especially for food (Singh et al. 2007). Levels of fat too high lead to a reduction of pressure during extrusion, resulting in poor expansion. However, this was not the case for mixtures extruded in the present experiment, which were below the upper limit proposed for feed/food. Actually, even though 5% of fat content resembles a quite common lipid content for non-ruminants farm animals diets (pig and poultry), extrusion is not a common processing for these species for the moment. Thus, it can be it can be speculated that extrusion may represent a suitable way for processing raw insect materials with limited pre-processing, such us drying and defatting, but its effectiveness for a target farm species and relatives' complete diets, need further investigation.

With regard to moisture content, it has been reported (Riaz 2000; Guy 2001) that in extruded low moisture mixtures, the initial physical interactions between ingredients cause functional and mechanical energy dissipation. This energy source serves to heat the dough mass. The heating rate is very high in low moisture systems, so that for recipes up to 25% moisture no external heating is required to reach a high operating temperature. Even though, in the present experiment, the internal temperatures were not

measured, the combination of the low moisture in the mixture (25% on w/w basis) and the heating at 60 °C adopted, probably affected the extrusion process.

Combining the reported results, larvae + wheat 25:75 (NO oil) blend with 46 g kg⁻¹ wb of EE has been selected instead of the prepupae mixture, since no oil addition, at comparable NTV, was required. This implies a simplest feed preparation, which is always appreciated in the feed manufacture. Accordingly, the larvae + wheat 25:75 (NO oil) blend has been selected for further investigations, as reported below.

In the second experiment, the selected blend (larvae + wheat 25:75 NO oil) has been tested for different barrel temperatures. Although, different barrel temperatures did not affect NTV (data not presented), increasing them from 60 °C to 90 °C, reduced linearly water removal in the tested material. Thus, the highest value for water removal was recorded for the lowest extrusion temperature. The reason for that is unknown. One hypothesis is linked to the texture of the extruded material. All food and feed products have basic structures that are formed by specific biopolymers of macronutrients (starch, proteins, fats, etc.) in the raw materials. The structure of an extruded product is different according to the processing procedure in the extrusion. In the present work, for instance, when barrel's temperature has been increased, the surface of the resulting products became smoother and brighter compared to the 60 °C. These changes can be attributed to a different degree of gelatinisation of starch. This is in line with the literature (Chiang and Johnson 1977; Kokić et al. 2013), which reported that extrusion is able to induce complete starch gelatinisation and increased *in vitro* OMD in food matrixes. Both temperature and moisture content are essentials in defying the results of the process. Therefore, it can be speculated that in the present experimental conditions (for temperature above 70 °C) the formation of an external layer probably prevented the water removal. By contrast, the material extruded at 60 °C presented a coarse and rough surface, which facilitates the water removal. However, further investigations are needed to confirm this hypothesis.

In vitro digestibility test

A further result obtained in the present experiment was the evaluation of the effect of extrusion of insect-based experimental feed on OMD and CPD. Considering results obtained for OMD, it can be suggested that extrusion process, even though at a quite low temperature, was able to affect digestibility. This result is in line with other studies (Alonso et al. 2000; Dust

et al. 2004; Sun et al. 2006; Kokić et al. 2013) in which extrusion was effective in improving nutrient digestibility, measured *in vitro*. In most of these studies, however, temperatures reached by the mass in the extruder were above the barrel temperature adopted in the present experiments. Of note, the laboratory-scale extruder device herein used was not able to measure dough temperature during extrusion (i.e. at die level), thus extrusion temperatures (60, 70, 80 and 90 °C) are referred only to barrel temperature. Consequently, an increase of mass temperature as effect of the pressure within the barrel, due to a restriction at the die opening, can be expected. Nevertheless, the real mechanism of this effect needs to be addressed furtherly since in this study a very simple blend/mixture has been tested.

In the case of CPD, obtained results were expected according to the tested temperatures, that is from 60 to 90 °C, which were too limited for inducing a significant effect on CPD. This result disagrees with other studies reported in literature. Mild extrusion cooking conditions in general are able to improve digestibility in vegetable protein. This improvement is probably the upshot of several modifications such as protein modification and inactivation of antinutritional factors, operated by temperature and pressure, during the extrusion process (Singh et al. 2007). Furthermore, in the case of simple mixtures including animal proteins, for example wheat meal and fish, other authors (Bhattacharya et al. 1988) observed an increase in CPD related to the extrusion process. A different situation exist when a severe extrusion is applied to the feed receipts, in which Maillard's reaction cannot be excluded (Björck and Asp 1983). However, this seems not to be the case for the present experiment, where the CPD was already higher than 0.93 in unextruded material and did not change for extruding mixtures at any barrel temperature.

With regard to the determination of free AAs in the supernatants, results indicated that the extrusion was able to increase this value at low temperature (60 °C). However, increasing barrel temperature above this value, the availability of free AA linearly decreased. Boye et al. (1997) reported that at temperatures above 80 °C, there is a loss of almost all secondary and tertiary structures. In this sense, it can be speculated that the irreversible alteration of protein structures reduced the final breakdown of peptides in free AAs.

Conclusions

This study investigated the inclusion of *Hermetia illucens* larvae or prepupae in an experimental extruded

feed, and the impact of extrusion on the nutritional value and digestibility. By combining extrusion results, it can be suggested that: (i) the best performing mixture was larvae + wheat (no oil addition), in which the natural occurring fat, provided by the larvae, was enough for guarantee an adequate extrusion process; (ii) sixty degree celsius (60 °C) seems an adequate temperature for a mild extrusion of simple insect-containing feed formulas; (iii) increasing barrel temperatures from 60 °C to 90 °C, reduced linearly water removal in the tested material; (iv) different barrel temperatures did not affect OMD of insect-containing feed blends. However, present findings must be considered with caution, not only for the very simple blends considered (2 ingredients), but also because any safety and stability aspects have been addressed in the study. Technological treatments are essential in defining nutritional and safety features of any kind of raw material intended for animal nutrition (EFSA 2015), and in the present work, only the nutritional aspects have been considered. A further consideration emerging from this study is the role of fat (added or not) in determining the potential technological quality of the extruded feed: fat content in the mixture is a key variable that should be monitored in the process. Extremely low-fat content in the mixture seems to be a limiting factor for extrusion, whereas at least more than 45 g kg⁻¹ of fat (on wet basis) embedded in the matrix, is effective in terms of extrudability. Combining evidences obtained in the present study, it can be speculated that extrusion may represent a valuable technology for processing raw insect materials intended for feed production, limiting pre-processing steps, such as drying and defatting.

Disclosure statement

No potential conflict of interest was reported by the authors.

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