

Insect larvae (*Hermetia illucens*) as an alternative feed source for laying hens



Victoria Linder

Insect larvae (*Hermetia illucens*) as an alternative feed source for laying hens

Insektslarver (*Hermetia illucens*) som ett alternativt fodermedel för värphöns

Victoria Linder

Supervisor: Carlos E. Hernandez, SLU, Department of Animal Nutrition and Management

Examiner: Helena Wall, SLU, Department of Animal Nutrition and Management

Extent: 15 hp

Course title: Bachelor project in Animal Science

Course code: EX0553

Programme: Agriculture programme – Animal Science

Level: Basic G2E

Place of publication: Uppsala

Year of publication: 2018

Series name, part no: Examensarbete / Sveriges lantbruksuniversitet, Institutionen för husdjurens utfodring och vård, 631

On-line published: <http://epsilon.slu.se>

Omslagsbild: Dennis Kress

Nyckelord: Fjäderfä, *Hermetia illucens*, djurfoder, insektsmjöl

Key words: Poultry, *Hermetia illucens*, livestock feed, insect meal

Abstract

The European egg industry is dependent on imported soy which contributes to climate change, partly because of deforestation and transport. Therefore, alternative feed sources are needed. The levels of soy used in poultry feed in Sweden are lower than the general levels in Europe. Also, the soy used in poultry feed in Sweden is certified to ensure sustainable production. Insects like *H. illucens* can be produced locally using local byproducts or food waste and have a similar content of crude protein and composition of amino acids as soybean, which could make them a suitable alternative. This review compiles available information about limiting factors of replacing soybean with *H. illucens* in the diet of laying hens and its effects on feed intake, feed conversion rate, egg production and egg quality. Levels of inclusion are also evaluated. Main findings are that *H. illucens* contain a high amount of crude protein and adequate amounts of most nutrients for laying hens. Egg quality appears to be improved when the inclusion level of *H. illucens* is 17% but effects on other parameters are less clear and sometimes contradictory. Inclusion level of 12% has shown no adverse effects on feed intake, feed conversion rate, egg production and egg weight. Furthermore, a prebiotic effect of *H. illucens* in the diet of laying hens has been suggested where chitin may play a key role. In conclusion, an inclusion of 17% of *H. illucens* gives inconsistent results regarding production parameters but 12% inclusion entails no deleterious effects on production. To determine the optimal level of inclusion further research is required.

Sammanfattning

Den europeiska äggindustrin är beroende av importerad soja som bidrar till klimatförändring, delvis på grund av avskogning och transporter. Därför behövs alternativa foderkällor. I Sverige är sojanivåerna i hönsfoder lägre än de generella nivåerna i Europa. Sojan som används i hönsfoder i Sverige är också hållbarhetscertifierad. Insekter som *H. illucens* kan produceras lokalt med hjälp av lokala biprodukter eller matavfall och har en liknande råproteinhalt och aminosyrasammansättning som soja, vilket skulle kunna göra dem till ett lämpligt alternativ. Denna litteraturoversikt sammanställer tillgänglig information om begränsande faktorer för ersättning av sojamjöl med *H. illucens* i värphönsfoder och dess effekter på foderintag, foderomvandlingsförmåga, äggproduktion och äggkvalité. Även inklusionsnivåer av *H. illucens* utvärderas. De viktigaste resultaten är att *H. illucens* har hög halt råprotein och innehåller tillräckliga mängder av de flesta näringsämnen för värphöns. Äggkvalitén verkar förbättras när inklusionsnivån av *H. illucens* är 17%, men effekterna på andra parametrar är inte lika tydliga eller motsägande. Inklusionsnivå på 12% har inte visat några negativa effekter på foderintag, foderomvandlingsförmåga, äggproduktion och äggvikt. Vidare har en prebiotisk effekt av *H. illucens* i hönsfoder föreslagits där chitin kan spela en nyckelroll. Sammanfattningsvis ger en inklusionsnivå av *H. illucens* på 17% inkonsekventa resultat gällande produktionsparametrar medan en inklusionsnivå på 12% inte leder till några negativa effekter gällande produktionen. Ytterligare forskning krävs för att kunna bestämma den optimala inklusionsnivån.

Introduction

There is a demand for an alternative source of protein for poultry diets to minimize today's reliance on imported feedstuffs such as soybean meal. Diets of laying hens consist of 26% soy in dry matter (DM) (Cutrignelli *et al.*, 2017; Marono *et al.*, 2017; Secci *et al.*, 2018) but this figure does not account for Sweden which in general have levels of 0-17% soy in DM¹. The soy used in poultry feed in Sweden is certified to ensure sustainable production from a social, environmental and climate point of view¹. European egg production uses 32 grams of soybean meal per egg produced (van Gelder *et al.*, 2008). This is not optimal as deforestation and transport contribute to climate change (da Silva *et al.*, 2010; Castanheira & Freire, 2013). Soy can also be used in food for humans (Mariotti *et al.*, 1999; Friedman & Brandon, 2001), which implies competition between animals and humans regarding soy as a nutrient source. For these reasons, there is a growing interest in the use of alternative food sources to feed animals, in particular insects because they have high nutritive value. For example, content of crude protein (CP) and composition of essential amino acids of *Hermetia illucens*, also known as black soldier fly, is similar or superior to that of soybean meal (Veldkamp *et al.*, 2012; Bosch *et al.*, 2014) and would therefore be a suitable replacement ingredient in the diet of laying hens. *H. illucens* originates from the Neotropic, which includes South America, Central America and the Caribbean, but is established nearly worldwide (Brammer & von Dohlen, 2007). Wild *H. illucens* larvae occur in abundant quantities in southeastern United States at farms with caged laying hens (Sheppard *et al.*, 1994). The life cycle of *H. illucens* is comprised of egg, larva, pupa and adult (Liu *et al.*, 2017) with three generations per year (Sheppard *et al.*, 1994). Weight of wild prepupa (last stage of larva before pupation) is significantly higher than *H. illucens* reared on artificial diets (Tomberlin *et al.*, 2002). The larvae can utilize nutrients from organic waste such as animal manure (Li *et al.*, 2011; Rehman *et al.*, 2017) and vegetable waste from industry, restaurants and biogas fermentation (Spranghers *et al.*, 2017). Fat is accumulated during the larval stage of *H. illucens* (Liu *et al.*, 2017). Adults only consume water (Paulk & Gilbert, 2006) which means that it is neither a pest or disease vector. A dense population of *H. illucens* larvae prevents houseflies from reproduction during May-January (Sheppard *et al.*, 1994). All the above-mentioned reasons indicate that *H. illucens* is an ideal insect candidate to be used in poultry diets. The aim of this literature review is to evaluate limiting factors of replacing soybean meal by *H. illucens* in the diet of laying hens, and its effect on feed intake, feed conversion rate (FCR), egg production and egg quality as well as the optimal level of inclusion.

Nutrient composition of *H. illucens*

Crude protein

The content of nutrients varies with different life stages of *H. illucens* (Liu *et al.*, 2017) and is

¹ Åsa Carlsson Product Manager Poultry, Svenska Foder, 2018-05-18 & Sven Hellberg Product Manager Poultry, Lantmännen, 2018-05-18

also dependent on diet (Jucker *et al.*, 2017). One day old larvae contain the highest amount of CP, then it decreases and is the lowest when the larvae are 12 days old, after which the amount increases again (Liu *et al.*, 2017). The larva stage lasts for 14 days after which it is referred to as early prepupa (Liu *et al.*, 2017). Content of CP of prepupae differs significantly between the rearing substrates fruit, vegetables, and fruit and vegetables (Jucker *et al.*, 2017) (Table 2). Though, no significant differences in CP content are shown in prepupae reared on different vegetable waste; industry, in this case a fruit and vegetable processing company (peas, celery, salsify and carrots), restaurant (rice, potatoes, vegetables and pasta) and digestate (the solid fraction resulting from biogas fermentation), or chicken feed (Spranghers *et al.*, 2017). CP level of larvae is mostly within the interval 39-56% (Table 1). Processed, defatted, larvae of *H. illucens* consists of 55-66% CP. The level of CP of prepupae varies between 28% and 60%.

Amino acids

Age (Liu *et al.*, 2017) and diet of *H. illucens* affects its composition of amino acids (Table 1 & Table 2). Larvae reared on broiler starter diet (Bosch *et al.*, 2014) contain higher levels of isoleucine, leucine, lysine, threonine and valine in comparison to larvae fed broiler chicken feed (Liu *et al.*, 2017) which in turn contain higher levels of histidine, methionine and phenylalanine. Age, and nutritional content of broiler starter diet, is not specified in Bosch *et al.* (2014), therefore it cannot be stated if the differences in amino acid composition of larvae in comparison to the larvae in the study of Liu *et al.* (2017) are because of differences in age or diet. Lysine, valine and arginine are the most common essential amino acids in prepupae of *H. illucens* according to Spranghers *et al.* (2017), their prevalence is 2-3% of DM. The content of arginine, histidine, methionine and threonine are higher in early prepupae reared on broiler chicken feed (Liu *et al.*, 2017) than prepupae fed chicken feed (Spranghers *et al.*, 2017), vegetable waste (Spranghers *et al.*, 2017) and restaurant waste (Spranghers *et al.*, 2017) (Table 2). The specified ages of prepupae are in stage of prepupae and days respectively, thus it cannot be stated if the differences in amino acid composition is because of difference in age. Small differences have been observed in amino acid content of prepupae reared on several substrates with varying composition of amino acids (Spranghers *et al.*, 2017).

Crude fat

Content of crude fat of *H. illucens* varies with age (Liu *et al.*, 2017) and rearing substrate (Spranghers *et al.*, 2017). Crude fat increases from the first day of larva stage until early prepupa from where it declines (Liu *et al.*, 2017). Diet of prepupae have significant effect on the content of crude fat but there is no correlation between crude fat content of substrate and crude fat content of prepupae (Spranghers *et al.*, 2017). The level of crude fat in larvae of *H. illucens* is 13-45% while defatted larvae contain 5-18% crude fat (Table 3). Prepupae contain a varying amount of crude fat; 8-55%. Diets consisting of vegetables (Jucker *et al.*, 2017), and fruit and vegetables (Nguyen *et al.*, 2015) results in considerably low contents of crude fat of prepupae in comparison to substrates such as restaurant waste (Spranghers *et al.*, 2017), manure from laying hens (Sheppard *et al.*, 1994), fruit (Jucker *et al.*, 2017), and vegetable

waste (Spranghers *et al.*, 2017) (Table 3), but there may be a possible unknown age effect.

Fatty acids

Composition of fatty acids of *H. illucens* vary significantly during different life stages (Liu *et al.*, 2017) and is also reliant on diet (Table 3). *H. illucens* contains relatively high levels of the essential fatty acid linoleic acid (18:2, n-6) in all different life stages but varies significantly with age (Liu *et al.*, 2017). Fruit and vegetables (Jucker *et al.*, 2017) as rearing substrate results in prepupae with considerably higher content of linoleic acid in comparison with prepupae reared on other substrates (Table 3). The lowest content of linoleic acid is found in prepupae fed vegetables (Jucker *et al.*, 2017). The majority of the fatty acids of *H. illucens* are saturated, varying between 65% and 83% depending on feed source (Spranghers *et al.*, 2017). Prepupae fed digestate contain lower levels of saturated fatty acids compared to prepupae reared on chicken feed, vegetable restaurant waste and vegetable industry waste (Spranghers *et al.*, 2017). Both larvae and prepupae of *H. illucens* contain high amounts of C12:0 (Table 3) but different feed substrates result in varying content of C12:0 (Spranghers *et al.*, 2017).

Minerals and vitamins

Ash content is significantly affected by rearing substrate (Spranghers *et al.*, 2017). Larvae consist of between 4.5% (Maurer *et al.*, 2016) and 8.3% (Liu *et al.*, 2017) ash. Larvae reared on chicken feed (Liu *et al.*, 2017) contain more ash in comparison with larvae reared on chicken feed and vegetarian by-products (Maurer *et al.*, 2016). Partially defatted larvae fed chicken feed and vegetarian by-products contain 5.2% ash (Maurer *et al.*, 2016). The amount of ash in prepupae varies greatly; 2.7-19.7% (Jucker *et al.*, 2017; Liu *et al.*, 2017; Spranghers *et al.*, 2017). Prepupae reared on digestate contain the highest level of ash (Spranghers *et al.*, 2017) while prepupae fed broiler chicken feed (Liu *et al.*, 2017), fruit and vegetables (Jucker *et al.*, 2017), vegetable waste (Spranghers *et al.*, 2017) and chicken feed (Spranghers *et al.*, 2017) results in 8-10% ash. Prepupae of *H. illucens* fed a vegetable diet contain almost 14% ash (Jucker *et al.*, 2017). Restaurant waste (Spranghers *et al.*, 2017) and fruit (Jucker *et al.*, 2017) as rearing substrates result in the lowest amounts of ash; 2.7% and 5.3% respectively.

Mineral content varies with rearing substrate (Table 4). Larvae of unspecified age reared on an unspecified substrate (Cuttrignelli *et al.*, 2017) contain higher levels of calcium, phosphorus, and sodium in comparison to larvae fed chicken feed and vegetarian by-products (Maurer *et al.*, 2016), and broiler chicken feed (Liu *et al.*, 2017) (Table 4). Age of larvae fed the two latter diets are similar (Table 4). Prepupae fed digestate (Spranghers *et al.*, 2017) contain more than twice the amount of calcium than prepupae reared on chicken feed (Spranghers *et al.*, 2017), broiler chicken feed (Liu *et al.*, 2017), and vegetable waste (Spranghers *et al.*, 2017) but restaurant waste as substrate results in a low content of calcium (Spranghers *et al.*, 2017). Levels of phosphorus and sodium are comparable between diets for prepupae (Table 4). Literature is limited regarding trace minerals and vitamins.

Chitin

H. illucens contain one of nature's most abundant biopolymers; chitin, a naturally occurring

polysaccharide (Wasko *et al.*, 2016). Chitin may function as a gut microbiota substrate which influences composition and metabolites of microbial fermentation, i.e. functions as a prebiotic substance (Borrelli *et al.*, 2017). Therefore, it is possible that the chitin level present in defatted *H. illucens* larvae could have a prebiotic effect when inclusion level of the insect is 18.8% in the diet of laying hens, corresponding to a daily consumption of 1.02g chitin (Borrelli *et al.*, 2017). Larvae fed cereal by-products consist of 5% and 7% chitin in DM depending on degree of defatting (Schiavone *et al.*, 2017). Level of chitin in prepupae is about 6-9% in DM (Diener *et al.*, 2009; Cutrignelli *et al.*, 2017; Spranghers *et al.*, 2017). A level of around 6-7% chitin in DM has been observed without significant differences between prepupae reared on different substrates; digestate, chicken feed and vegetable waste from industry and restaurant (Spranghers *et al.*, 2017). Chitin content is likely related to life stage of *H. illucens* because of a possibly higher cuticular protein-sclerotization in pupae than larvae which entails lower digestibility of pupae (Bosch *et al.*, 2014).

Limiting factors of *H. illucens* in the diet of laying hens

There are ten essential amino acids and one essential fatty acid for laying hens which represent potentially limiting factors of feedstuffs (National Research Council, 1994). Requirement of CP of a laying hen consuming 90 g of feed in DM/day is 16.7%. Corresponding requirement of amino acids in DM is 0.78% arginine, 0.19% histidine, 0.72% isoleucine, 0.91% leucine, 0.77% lysine, 0.33% methionine, 0.52% phenylalanine as well as threonine, 0.18% tryptophan and 0.78% valine. Linoleic acid requirement is 1.1%, calcium 3.6%, chloride 0.14%, phosphorus 0.28%, and sodium 0.17%. Other macrominerals and trace minerals are also required but no studies with *H. illucens* have been found with those data, therefore those minerals are not included in this review. Larvae contain 2.0% linoleic acid in DM (Liu *et al.*, 2017) and prepupae varying amounts; 0.7-7.1% in DM (Jucker *et al.*, 2017; Spranghers *et al.*, 2017). In comparison to soybean meal *H. illucens* contain both lower and higher amounts of nutrients, except for tryptophan which is deficient in the larva stage and isoleucine, lysine, tryptophan and phosphorus which are deficient in the prepupa stage (Table 5). High levels of crude fat of *H. illucens* in comparison to soy (Table 5) may be a limiting factor, as well as the chitin content because the latter may impair digestibility of the insect (Bosch *et al.*, 2014).

Effects of including² *H. illucens* larvae in the diet of laying hens

Feed intake

The results are inconsistent regarding the effect of *H. illucens* on feed intake. Several studies showed a negative effect on feed intake with *H. illucens* included in the diet (Borrelli *et al.*, 2017; Cutrignelli *et al.*, 2017; Marono *et al.*, 2017; Secci *et al.*, 2018). However, there are

² Inclusion levels are always presented as “as is basis” and the percent of soybean meal replaced within parenthesis

also studies which showed no effect on the feed intake (Al-Qazzaz *et al.*, 2016; Maurer *et al.*, 2016). One of those studies focused on replacing fish meal in the diet instead of soybean meal (Al-Qazzaz *et al.*, 2016). Inclusion of 17% defatted *H. illucens* (as fed), corresponding to a total replacement of soybean meal, has resulted in reduced feed intake (Cutrignelli *et al.*, 2017; Marono *et al.*, 2017; Secci *et al.*, 2018). However, neither 1% (8) and 5% (-3) non-defatted *H. illucens* (Al-Qazzaz *et al.*, 2016) nor 12% (43) and 24% (100) partially defatted *H. illucens* (Maurer *et al.*, 2016) affected birds feed intake.

Feed conversion rate

Studies regarding laying hens with *H. illucens* included in the diet showed contradictory results regarding FCR; impaired (Al-Qazzaz *et al.*, 2016), unaffected (Al-Qazzaz *et al.*, 2016; Maurer *et al.*, 2016) and improved (Marono *et al.*, 2017). Layers fed a diet with low inclusion of non-defatted *H. illucens*, 1% (8) and 5% (-3) (increase in soy, i.e. fish meal was replaced, not soy), showed unaffected and impaired FCR respectively (Al-Qazzaz *et al.*, 2016). Higher inclusion levels of *H. illucens* (partially defatted), 12% (43) and 24% (100), showed no effect on FCR in laying hens (Maurer *et al.*, 2016) while an inclusion of 17% (100) defatted *H. illucens* has resulted in improved FCR (Marono *et al.*, 2017). Protein digestibility was negatively affected by 17% (100) inclusion of defatted *H. illucens* (Cutrignelli *et al.*, 2017).

Egg production

Results regarding egg production (laying percentage) are contradictive; both increased (Al-Qazzaz *et al.*, 2016), and decreased (Marono *et al.*, 2017) as well as unaffected (Maurer *et al.*, 2016) egg production have been observed. Inclusion levels of 1% (8) non-defatted (Al-Qazzaz *et al.*, 2016) and 17% (100) defatted *H. illucens* (Marono *et al.*, 2017) had a negative effect on egg production but 12% (43), as well as 24% (100) inclusion of partially defatted *H. illucens* was not associated with any adverse effects regarding egg production (Maurer *et al.*, 2016). Inclusion of 5% (-3) non-defatted *H. illucens* increased egg production (Al-Qazzaz *et al.*, 2016). The diet with 1% (8) inclusion of *H. illucens* contained 17.9% CP and metabolizable energy (ME) was 2836kcal/kg. For the diet with 5% (-3) inclusion the respective values were 19.1% and 2669kcal/kg and for the control diet in the study with the former diets the values were 17.9% and 2777kcal/kg. CP content was 17.9% and ME was 2745kcal/kg for the diet with 17% (100) inclusion with a control diet containing 18.1% CP and 2780kcal/kg. The diet with 12% (43) inclusion had values of 20.3% and 2701kcal/kg and the diet with 24% (100) inclusion had 21.4% and 2701kcal/kg. The control diet in the study with the two latter diets contained 20.0% CP and same ME as the experimental feeds.

Egg weight

The effect of *H. illucens* on egg weight is unclear. When *H. illucens* has been included in the diet it has resulted in decreased (Al-Qazzaz *et al.*, 2016; Marono *et al.*, 2017) or unaffected (Al-Qazzaz *et al.*, 2016; Maurer *et al.*, 2016; Secci *et al.*, 2018) egg weight. A variety of inclusion levels showed unaffected egg weight; 1% (8) (non-defatted) (Al-Qazzaz *et al.*, 2016), 12% (43) and 24% (100) (partially defatted) (Maurer *et al.*, 2016). Feed with 17% (100) inclusion of defatted *H. illucens* have resulted in reduced (Marono *et al.*, 2017) as well

as unaffected egg weight (Secci *et al.*, 2018). However, reduced egg weight has also been observed for hens with 5% (-3) non-defatted *H. illucens* included in the diet (Al-Qazzaz *et al.*, 2016).

Egg quality

H. illucens in the diet of laying hens seem to improve egg quality. An inclusion of 17% (100) defatted *H. illucens* improved content of carotenoids in the eggs and decreased the content of cholesterol in the yolks with almost 12% (Secci *et al.*, 2018). In a panel test evaluating sensory traits of eggs, the appearance, texture and acceptance of eggs produced by hens fed a diet with 1% (8) and 5% (-3) inclusion of non-defatted *H. illucens* were significantly improved (Al-Qazzaz *et al.*, 2016). Taste was also affected by inclusion of *H. illucens*; 5% inclusion significantly improved taste relative to 1% inclusion which in turn significantly improved taste compared to control diet (Al-Qazzaz *et al.*, 2016).

Discussion

The aim of this literature review was to evaluate limiting factors of replacing soybean meal with *H. illucens* in the diet of laying hens, its effect on feed intake, feed conversion rate, egg production and egg quality as well as the optimal level of inclusion. Searches were conducted in the databases Web of Science, Scopus and Google Scholar. The main findings of this review are: 1) nutrient composition of *H. illucens* can be similar or superior to that of soybean, in particular content of CP and composition of essential amino acids 2) life stage, processing and type of diet fed to the larvae all affect nutrient composition of *H. illucens* 3) effects of replacing soybean meal with *H. illucens* on production parameters are inconsistent and probably dependent on life stage, processing and diet of the larvae and 4) the optimal level of inclusion in the laying hen diet is not clear from the existing literature. An inclusion level of 17% seems to improve egg quality, in terms of content of carotenoids and cholesterol, but effects on other parameters are unclear as well as contradictory in some cases. It appears to be better with an inclusion level of 12% than 17% or 24% since no adverse effects have been reported on production parameters at the lower inclusion level.

H. illucens contain varying amounts of nutrients depending on life stage (Liu *et al.*, 2017) and diet (Jucker *et al.*, 2017), therefore it is of importance to consider age and rearing substrate in order to obtain the desired nutrient composition. There is a possible bias in Table 1-4 because age is stated in less than half of the studies which entails difficulties to know whether age or diet had the major impact on the nutrient composition of the insect. Furthermore, this results in difficulties to compare nutrient levels in *H. illucens* between studies. Also, age is in some studies not specified to an exact day but to an interval of days. Generally, *H. illucens* contain adequate amounts of most essential amino acids in comparison to soy, the only amino acid which occurs in an insufficient amount in both larvae and prepupae compared to soy is tryptophan (Table 5). Prepupae reared on vegetables result in a low amount of the essential fatty acid linoleic acid (Jucker *et al.*, 2017), which might be due to the lower level of crude fat. Though, linoleic acid is most likely present in adequate amounts in other feed ingredients

or supplements. Imbalances in macromineral content and absent data on trace minerals and vitamins in *H. illucens* can be compensated by supplements with suitable composition.

An inclusion level of 17% *H. illucens* decreased feed intake in three studies whereas no adverse effects were observed regarding feed intake in studies which had inclusion levels of 1%, 5%, 12% and 24%. In all studies reporting a reduced feed intake a defatted *H. illucens* was used (Borrelli *et al.*, 2017; Cutrignelli *et al.*, 2017; Marono *et al.*, 2017; Secci *et al.*, 2018) whereas feed intake was maintained in studies using partially defatted *H. illucens* (Maurer *et al.*, 2016) or not defatted (Al-Qazzaz *et al.*, 2016). This indicates a benefit of non-processed *H. illucens*, and it would therefore be interesting to feed live larvae to laying hens. A possible explanation to the reduced feed intake when using defatted meals of *H. illucens* is the increase in concentration of chitin.

Impaired FCR has only been presented in a study in which soybean meal was increased with 3% and 5% of *H. illucens* was included (Al-Qazzaz *et al.*, 2016). The focus in that study was to replace fish meal, instead of soy, with *H. illucens*. Therefore, it entails difficulties to compare the result of an impaired FCR with results from studies with a focus on replacing soy. It is more likely that the removal of fish meal is the reason behind the impaired FCR and not the low inclusion of *H. illucens*, because 12% and 24% (Maurer *et al.*, 2016) inclusion of *H. illucens* have not resulted in any adverse effects and 17% inclusion has been observed to improve FCR (Marono *et al.*, 2017). However, it is important to keep in mind that protein digestibility was impaired in a study in which the inclusion level of *H. illucens* was 17%, with 5.4% chitin as fed (Cutrignelli *et al.*, 2017), and it might be related to chitin content of insects.

Egg production was negatively affected with both 1% (Al-Qazzaz *et al.*, 2016) and 17% (Marono *et al.*, 2017) inclusion of *H. illucens*, but no adverse effects were observed in another study where the inclusion level was 12% and 24% (Maurer *et al.*, 2016). Marono *et al.* (2017) discuss that the lower productive performance of hens fed a diet including *H. illucens* could be due to lower feed intake which they believe may be correlated to the darker colour of the feed containing *H. illucens* compared to the control diet. There might be a connection between feed intake and colour of the feed which requires further investigation.

No adverse effects have been observed regarding egg quality. An inclusion of 17% *H. illucens* has resulted in improved content of carotenoids in the eggs and lower cholesterol content in the yolks (Secci *et al.*, 2018). The improved appearance, texture, acceptance and taste of eggs produced by hens with 1% and 5% inclusion level (Al-Qazzaz *et al.*, 2016) could possibly be due to the removal of fish meal in the diet instead of the low levels of *H. illucens* in the feed.

In conclusion, the optimal level of inclusion of *H. illucens* is most likely higher than 12% but lower than 17% because the former does not entail any deleterious effects regarding the studied parameters and the latter entails unclear results regarding production parameters. Further research is necessary to evaluate what level of inclusion is optimal.

Table 1. Age (days), crude protein (% in DM) and composition of essential amino acids (% in DM) of *H. illucens* larvae fed different diets

Diet	Age	Crude protein	Arginine	Histidine	Iso-leucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine	Reference
Broiler starter diet	NA	56.1	2.1	2.5	2.2	3.4	3.0	0.79	1.7	2.0		3.1	Bosch <i>et al.</i> (2014)
NA	NA	54.8 ^a	1.7 ^a	0.50 ^a	2.4 ^a	3.5 ^a	2.1 ^a	0.66 ^a	1.8 ^a	2.0 ^a	0.021 ^a	3.8 ^a	Cullere <i>et al.</i> (2016)
NA	NA	62.7 ^a			3.2 ^a		4.1 ^a	1.3 ^a		2.4 ^a	0.31 ^a	5.1 ^a	Cutrignelli <i>et al.</i> (2017)
Broiler chicken feed	1		3.0	5.3	2.1	3.7	2.9	2.4	1.8	2.3		2.4	Liu <i>et al.</i> (2017)
Broiler chicken feed	4		2.9	4.9	2.0	3.4	3.0	1.9	2.1	2.3		2.5	Liu <i>et al.</i> (2017)
Broiler chicken feed	6		2.0	5.5	2.0	3.5	2.8	2.2	1.9	2.2		2.4	Liu <i>et al.</i> (2017)
Broiler chicken feed	7		2.0	4.7	1.8	3.0	2.4	2.3	1.7	2.0		2.1	Liu <i>et al.</i> (2017)
Broiler chicken feed	9		1.7	4.1	1.6	2.8	2.1	1.9	1.7	1.8		1.9	Liu <i>et al.</i> (2017)
Broiler chicken feed	12		1.8	3.5	1.5	2.5	2.1	2.2	1.7	1.7		1.8	Liu <i>et al.</i> (2017)
Broiler chicken feed	14	39.2	2.1	3.2	1.6	2.7	2.3	2.2	1.9	1.8		1.9	Liu <i>et al.</i> (2017)
NA	NA	62.7 ^a			3.2 ^a		4.1 ^a	1.3 ^a		2.4 ^a	0.31 ^a	5.1 ^a	Marono <i>et al.</i> (2017)
Chicken feed & vegetarian by-products	14-31	43.0											Maurer <i>et al.</i> (2016)
Chicken feed & vegetarian by-products	14-31	61.5 ^b					3.2 ^b	1.0 ^b					Maurer <i>et al.</i> (2016)
Cereal by-products	NA	55.3 ^b	2.2 ^b	1.2 ^b	1.9 ^b	2.9 ^b	2.1 ^b	6.5 ^b	1.7 ^b	1.7 ^b		2.7 ^b	Schiavone <i>et al.</i> (2017)
Cereal by-products	NA	65.5 ^c	2.7 ^c	1.6 ^c	2.4 ^c	3.7 ^c	2.5 ^c	8.6 ^c	2.2 ^c	2.2 ^c		3.5 ^c	Schiavone <i>et al.</i> (2017)
NA	NA	55.0											Smetana <i>et al.</i> (2016)

^a Defatted ^b Partially defatted ^c Highly defatted, NA=not available

Table 2. Age (days or stage), crude protein (% in DM) and composition of essential amino acids (% in DM) of *H. illucens* prepupae fed different diets

Diet	Age	Crude protein	Arginine	Histidine	Iso-leucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine	Reference
Chicken feed 12,5 ^a	NA	42.5											Diener <i>et al.</i> (2009)
Chicken feed 25 ^a	NA	32.6											Diener <i>et al.</i> (2009)
Chicken feed 50 ^a	NA	32.8											Diener <i>et al.</i> (2009)
Chicken feed 100 ^a	NA	34.4											Diener <i>et al.</i> (2009)
Chicken feed 200 ^a	NA	28.2											Diener <i>et al.</i> (2009)
Fruit & vegetables	37	48.9											Jucker <i>et al.</i> (2017)
Fruit	52	30.8											Jucker <i>et al.</i> (2017)
Vegetables	48	60.0											Jucker <i>et al.</i> (2017)
Broiler chicken feed	Early ^b	40.2	2.1	3.5	1.7	2.8	2.3	2.7	1.9	1.9		2.0	Liu <i>et al.</i> (2017)
Broiler chicken feed	Late	40.4	2.0	3.7	1.6	2.8	2.1	3.1	1.9	1.8		1.9	Liu <i>et al.</i> (2017)
Chicken feed	NA	43.9											Nguyen <i>et al.</i> (2015)
Pig liver	NA	47.0											Nguyen <i>et al.</i> (2015)
Fruit & vegetables	NA	45.7											Nguyen <i>et al.</i> (2015)
Fish rendering	NA	41.6											Nguyen <i>et al.</i> (2015)
Manure from laying hens	NA	42.0											Sheppard <i>et al.</i> (1994)
Chicken feed	20-29	38.8	2.0	1.4	1.7	2.9	2.3	0.76	1.7	1.6	0.67	2.4	Spranghers <i>et al.</i> (2017)
Digestate	20-29	40.1	2.0	1.4	1.8	3.0	2.6	0.87	1.9	1.7	0.62	2.5	Spranghers <i>et al.</i> (2017)
Vegetable waste	20-29	37.7	2.0	1.2	1.7	2.8	2.3	0.76	1.6	1.5	0.58	2.5	Spranghers <i>et al.</i> (2017)
Restaurant waste	20-29	40.7	2.0	1.4	1.9	3.1	2.3	0.71	1.6	1.6	0.54	2.8	Spranghers <i>et al.</i> (2017)

^a mg/larva/day ^b16 days, NA=not available

Table 3. Age (days and/or stage), crude fat and composition of the most common fatty acid and the essential fatty acid (% in DM) of *H. illucens* fed different diets

Diet	Age	Crude fat	C12:0	C18:2	Reference
<i>Larvae</i>					
Broiler starter diet	NA	12.8			Bosch <i>et al.</i> (2014)
NA	NA	15.6 ^a			Cullere <i>et al.</i> (2016)
NA	NA	4.7 ^a			Cutrignelli <i>et al.</i> (2017)
Broiler chicken feed	1	4.8	3.4	0.24	Liu <i>et al.</i> (2017)
Broiler chicken feed	4	5.8	0.44	1.3	Liu <i>et al.</i> (2017)
Broiler chicken feed	6	9.6	1.6	3.0	Liu <i>et al.</i> (2017)
Broiler chicken feed	7	13.4	4.6	2.0	Liu <i>et al.</i> (2017)
Broiler chicken feed	9	22.2	8.5	3.7	Liu <i>et al.</i> (2017)
Broiler chicken feed	12	22.6	12.2	2.3	Liu <i>et al.</i> (2017)
Broiler chicken feed	14	28.4	17.4	2.0	Liu <i>et al.</i> (2017)
NA	NA	4.7 ^a			Marono <i>et al.</i> (2017)
Chicken feed & vegetarian by-products	14-31	11.5 ^b			Maurer <i>et al.</i> (2016)
Chicken feed & vegetarian by-products	14-31	27.5			Maurer <i>et al.</i> (2016)
Cereal by-products	NA	18.0 ^b			Schiavone <i>et al.</i> (2017)
Cereal by-products	NA	4.6 ^c			Schiavone <i>et al.</i> (2017)
NA	NA	45.0			Smetana <i>et al.</i> (2016)
<i>Prepupae</i>					
Fruit & vegetables	37	33.3	13.8	7.1	Jucker <i>et al.</i> (2017)
Fruit	52	55.3	37.6	1.3	Jucker <i>et al.</i> (2017)
Vegetables	48	9.1	2.3	0.68	Jucker <i>et al.</i> (2017)
Broiler chicken feed	16/Early	28.0	17.5	2.7	Liu <i>et al.</i> (2017)
Broiler chicken feed	Late	24.2	17.8	1.2	Liu <i>et al.</i> (2017)
Chicken feed	NA	12.0			Nguyen <i>et al.</i> (2015)
Pig liver	NA	18.8			Nguyen <i>et al.</i> (2015)
Fruit & vegetables	NA	7.9			Nguyen <i>et al.</i> (2015)
Fish rendering	NA	24.9			Nguyen <i>et al.</i> (2015)
Manure from laying hens	NA	35.0			Sheppard <i>et al.</i> (1994)
Chicken feed	20-29	33.6	19.3	3.9	Spranghers <i>et al.</i> (2017)
Digestate	20-29	21.8	9.5	1.7	Spranghers <i>et al.</i> (2017)
Vegetable waste	20-29	37.1	22.6	1.7	Spranghers <i>et al.</i> (2017)
Restaurant waste	20-29	38.6	22.2	3.0	Spranghers <i>et al.</i> (2017)

^a Defatted ^b Partially defatted ^c Highly defatted, NA=not available

Table 4. Age (days or stage) and macromineral content (% in DM) of *H. illucens* fed different diets

Diet	Age	Calcium	Chloride	Phosphorus	Sodium	Reference
<i>Larvae</i>						
NA	NA	7.1		0.93	0.12	Cutrignelli <i>et al.</i> (2017)
Broiler chicken feed	14	2.9		0.35	0.10	Liu <i>et al.</i> (2017)
Chicken feed & vegetarian by-products	14-31	0.83	0.34	0.52	0.08	Maurer <i>et al.</i> (2016)
Chicken feed & vegetarian by-products	14-31	1.0 ^a	0.29 ^a	0.66 ^a	0.08 ^a	Maurer <i>et al.</i> (2016)
<i>Prepupae</i>						
Broiler chicken feed	Early ^b	3.0		0.62	0.05	Liu <i>et al.</i> (2017)
Chicken feed	20-29	2.9		0.50	0.07	Spranghers <i>et al.</i> (2017)
Digestate	20-29	6.6		0.44	0.09	Spranghers <i>et al.</i> (2017)
Vegetable waste	20-29	2.9		0.40	0.06	Spranghers <i>et al.</i> (2017)
Restaurant waste	20-29	0.12		0.41	0.07	Spranghers <i>et al.</i> (2017)

^a Partially defatted ^b16 days, NA=not available

Table 5. Nutrient composition (% in DM) of soybean meal, *H. illucens* larvae and prepupae

Nutrient	Soybean meal ^a	<i>H. illucens</i> larvae ^b		<i>H. illucens</i> prepupae ^b	
		Minimum	Maximum	Minimum	Maximum
Crude protein	48.2	39.2	65.5	28.2	60.0
Arginine	NA	1.7	2.7	2.0	2.1
Histidine	NA	0.50	3.2	1.2	3.5
Isoleucine	2.6	1.6	3.2	1.7	1.9
Leucine	NA	2.7	3.7	2.8	3.1
Lysine	3.2	2.1	4.1	2.3	2.6
Methionine	0.68	0.66	8.6	0.71	2.7
Phenylalanine	NA	1.7	2.2	1.6	1.9
Threonine	1.9	1.7	2.4	1.5	1.9
Tryptophan	0.81	0.021	0.31	0.54	0.67
Valine	2.3	1.9	5.1	2.0	2.8
Crude fat	1.2	4.6	45.0	7.9	55.3
Linoleic acid	NA	0.24	3.7	0.68	7.1
Calcium	3.1	0.83	7.1	0.12	6.6
Chloride	NA	0.29	0.34	NA	NA
Phosphorus	0.63	0.35	0.93	0.40	0.62
Sodium	NA	0.08	0.12	0.05	0.09

^a Values from Cutrignelli *et al.* (2017) and Marono *et al.* (2017) ^b Values extracted from Table 1, Table 2 and Table 3, NA=not available

References

- Al-Qazzaz, M.F.A., Ismail, D., Akit, H. & Idris, L.H. (2016). Effect of using insect larvae meal as a complete protein source on quality and productivity characteristics of laying hens. *Revista Brasileira De Zootecnia-Brazilian Journal of Animal Science*, 45(9), pp. 518-523.
- Borrelli, L., Coretti, L., Dipineto, L., Bovera, F., Menna, F., Chiariotti, L., Nizza, A., Lembo, F. & Fioretti, A. (2017). Insect-based diet, a promising nutritional source, modulates gut microbiota composition and SCFAs production in laying hens. *Scientific Reports*, 7.
- Bosch, G., Zhang, S., Oonincx, D.G.A.B. & Hendriks, W.H. (2014). Protein quality of insects as potential ingredients for dog and cat foods. *Journal of nutritional science*, 3, pp. e29-e29.
- Brammer, C.A. & von Dohlen, C.D. (2007). Evolutionary history of Stratiomyidae (Insecta : Diptera): The molecular phylogeny of a diverse family of flies. *Molecular Phylogenetics and Evolution*, 43(2), pp. 660-673.
- Castanheira, E.G. & Freire, F. (2013). Greenhouse gas assessment of soybean production: implications of land use change and different cultivation systems. *Journal of Cleaner Production*, 54, pp. 49-60.
- Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., De Smet, S. & Zotte, A.D. (2016). Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. *Animal*, 10(12), pp. 1923-1930.
- Cutrignelli, M.I., Messina, M., Tulli, F., Randazzo, B., Olivotto, I., Gasco, L., Loponte, R. & Bovera, F. (2017). Evaluation of an insect meal of the Black Soldier Fly (*Hermetia illucens*) as soybean substitute: Intestinal morphometry, enzymatic and microbial activity in laying hens. *Research in veterinary science*, 117, pp. 209-215.
- da Silva, V.P., van der Werf, H.M.G., Spies, A. & Soares, S.R. (2010). Variability in environmental impacts of Brazilian soybean according to crop production and transport scenarios. *Journal of Environmental Management*, 91(9), pp. 1831-1839.
- Diener, S., Zurbrugg, C. & Tockner, K. (2009). Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Management & Research*, 27(6), pp. 603-610.
- Friedman, M. & Brandon, D.L. (2001). Nutritional and health benefits of soy proteins. *Journal of Agricultural and Food Chemistry*, 49(3), pp. 1069-1086.
- Jucker, C., Erba, D., Leonardi, M.G., Lupi, D. & Savoldelli, S. (2017). Assessment of Vegetable and Fruit Substrates as Potential Rearing Media for *Hermetia illucens* (Diptera: Stratiomyidae) Larvae. *Environmental Entomology*, 46(6), pp. 1415-1423.
- Li, Q., Zheng, L., Qiu, N., Cai, H., Tomberlin, J.K. & Yu, Z. (2011). Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Management*, 31(6), pp. 1316-1320.
- Liu, X., Chen, X., Wang, H., Yang, Q., Rehman, K.U., Li, W., Cai, M., Li, Q., Mazza, L., Zhang, J., Yu, Z. & Zheng, L. (2017). Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLoS ONE*, 12(8).
- Mariotti, F., Mahe, S., Benamouzig, R., Luengo, C., Dare, S., Gaudichon, C. & Tome, D. (1999). Nutritional value of N-15 -soy protein isolate assessed from ileal digestibility and postprandial protein utilization in humans. *Journal of Nutrition*, 129(11), pp. 1992-1997.
- Marono, S., Loponte, R., Lombardi, P., Vassalotti, G., Pero, M.E., Russo, F., Gasco, L., Parisi, G., Piccolo, G., Nizza, S., Di Meo, C., Attia, Y.A. & Bovera, F. (2017). Productive performance and blood profiles of laying hens fed *Hermetia illucens* larvae meal as total replacement of soybean meal from 24 to 45 weeks of age. *Poultry Science*, 96(6), pp. 1783-1790.
- Maurer, V., Holinger, M., Amsler, Z., Fruh, B., Wohlfahrt, J., Stamer, A. & Leiber, F. (2016). Replacement of soybean cake by *Hermetia illucens* meal in diets for layers. *Journal of Insects as Food and Feed*, 2(2), pp. 83-90.

- National Research Council (1994). *Nutrient Requirements of Poultry*. 9. ed. Washington, D.C.: National Academy Press.
- Nguyen, T.T.X., Tomberlin, J.K. & Vanlaerhoven, S. (2015). Ability of Black Soldier Fly (Diptera: Stratiomyidae) Larvae to Recycle Food Waste. *Environmental Entomology*, 44(2), pp. 406-410.
- Paulk, A. & Gilbert, C. (2006). Proprioceptive encoding of head position in the black soldier fly, *Hermetia illucens* (L.) (Stratiomyidae). *Journal of Experimental Biology*, 209(19), pp. 3913-3924.
- Rehman, K.U., Cai, M., Xiao, X., Zheng, L., Wang, H., Soomro, A.A., Zhou, Y., Li, W., Yu, Z. & Zhang, J. (2017). Cellulose decomposition and larval biomass production from the co-digestion of dairy manure and chicken manure by mini-livestock (*Hermetia illucens* L.). *Journal of Environmental Management*, 196, pp. 458-465.
- Schiavone, A., De Marco, M., Martinez, S., Dabbou, S., Renna, M., Madrid, J., Hernandez, F., Rotolo, L., Costa, P., Gai, F. & Gasco, L. (2017). Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology*, 8.
- Secci, G., Bovera, F., Nizza, S., Baronti, N., Gasco, L., Conte, G., Serra, A., Bonelli, A. & Parisi, G. (2018). Quality of eggs from Lohmann Brown Classic laying hens fed black soldier fly meal as substitute for soya bean. *Animal : an international journal of animal bioscience*, pp. 1-7.
- Sheppard, D.C., Newton, G.L., Thompson, S.A. & Savage, S. (1994). A value-added manure management-system using the black soldier fly. *Bioresource Technology*, 50(3), pp. 275-279.
- Smetana, S., Palanisamy, M., Mathys, A. & Heinz, V. (2016). Sustainability of insect use for feed and food: Life Cycle Assessment perspective. *Journal of Cleaner Production*, 137, pp. 741-751.
- Sprangers, T., Ottoboni, M., Klootwijk, C., Owyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. & De Smet, S. (2017). Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture*, 97(8), pp. 2594-2600.
- Tomberlin, J.K., Sheppard, D.C. & Joyce, J.A. (2002). Selected life-history traits of black soldier flies (Diptera : Stratiomyidae) reared on three artificial diets. *Annals of the Entomological Society of America*, 95(3), pp. 379-386.
- van Gelder, J.W., Kammeraat, K. & Kroes, H. (2008). *Soy consumption for feed and fuel in the European Union*. Castricum, Netherlands.
- Wasko, A., Bulak, P., Polak-Berecka, M., Nowak, K., Polakowski, C. & Bieganski, A. (2016). The first report of the physicochemical structure of chitin isolated from *Hermetia illucens*. *International Journal of Biological Macromolecules*, 92, pp. 316-320.
- Veldkamp, T., van Duinkerken, G., van Huis, A., Lakemond, C.M.M., Ottevanger, E., Bosch, G. & van Boekel, M.A.J.S. (2012). *Insects as a sustainable feed ingredient in pig and poultry diets - a feasibility study*. (Report 638). Lelystad.