Replacement of soybean cake by *Hermetia illucens* meal in diets for layers

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RESEARCH ARTICLE

Abstract

Insects will likely play an important role as protein sources for livestock in the future. Many insect species are able to convert materials not suitable for human nutrition – or even waste – into valuable protein with a favourable amino acid composition for poultry and other livestock. A feeding trial with partly de-fatted meal of dried *Hermetia illucens* larvae (Hermetia meal) reared on vegetarian by-products of the pasta and convenience food industry was carried out in small groups of Lohmann Selected Leghorn laying hens (four rounds, 10 hens/round). Experimental diets H12 and H24 contained 12 and 24 g/100 g Hermetia meal replacing 50 or 100% of soybean cake used in the control feed, respectively. After three weeks of feeding experimental diets, there were no significant differences between feeding groups with regard to performance (egg production, feed intake). There was a tendency (P=0.06) for lower albumen weight in the H24 group; yolk and shell weights did not differ. No mortality and no sign of health disorders occurred. Plumage as well as wound scores remained stable during the feeding period and did not differ between treatments. Dry matter of faeces increased with increasing proportions of Hermetia meal in the diet, with a significant difference between H24 and the control (P=0.03). An increase of black faecal pads was observed in the H12 and H24 groups. Overall, these results suggest Hermetia meal can be a valuable component of layer diets. However, insect meal production still has to become economically more viable through upscaling production and, especially, legislative issues have to be solved.

Keywords: animal health, feed, insect meal, laying performance, poultry

1. Introduction

Soybean cake is a main protein source for pigs and poultry in Europe. Using this protein source as animal feed is controversial due to its potentially negative environmental and social impacts (Semino *et al.*, 2009; Von Witzke *et al.*, 2011). Calculations of the Food and Agricultural Organization of the United Nations predict the world's population will grow to around 9 billion people by 2050 (Alexandratos and Bruinsma, 2012), resulting in a higher consumption of food from animal origin and, therefore, an increased demand for protein to feed livestock. Alternative protein sources will play an important role to fill the resulting supply gap. A focus will be on sources which do not use arable land resources and are, therefore, not competing with food crops for human nutrition (Cassidy *et al.*, 2013).

Insect proteins are supposed to be valuable protein sources for monogastric animals (Makkar *et al.*, 2014). Many insect species can potentially be used for producing insect biomass, but most promising are the larvae of the black soldier fly (*Hermetia illucens*), the common housefly (*Musca domestica*), the yellow mealworm (*Tenebrio molitor*) as well as blue bottle (*Calliphora vomitoria*) and blow flies (*Chrysomya* spp.) (Charlton *et al.*, 2015). Insects can feed on a broad variety of materials including manure and they have a better feed conversion rate than most other animals, which reduces wastage and costs (Khusro *et al.*, 2012). *H. illucens* seems to be particularly promising, because it can reach high growth rates on materials that are unsuitable for human nutrition (e.g. by-products from food processing, organic waste; Diener *et al.*, 2011) and due to its amino acid composition, which is reported to be similar or even superior to soybean (Veldkamp *et al.*, 2012). However, knowledge about the actual suitability

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of *H. illucens*- and other insect-based feeds for poultry in meat and egg production is very scarce so far (Makkar *et al.*, 2014). Acceptance by the animals, digestibility, feed conversion rates, animal health issues and the quality of the resulting products are of particular importance and not yet investigated sufficiently.

So far, legal restrictions hinder the use of insect protein in feed for livestock intended for human consumption in many countries (Belluco *et al.*, 2013; Vantomme, 2015). This might be the main reason why, to the best of our knowledge, no feeding trials using insects as feed components for laying hens have yet been published. On the other hand, more data about application of insects as livestock feed (Makkar *et al.*, 2014) as well as safety issues (Belluco *et al.*, 2013; Charlton *et al.*, 2015) would probably be necessary to move political processes forward in a responsible way.

This paper describes two experimental *H. illucens*-based diets for layers and a trial carried out with small experimental layer flocks. With the experiment, we aimed at gaining knowledge about the suitability of Hermetia meal as a replacer for soybean cake in poultry diets. The focus of the experiment was on feed intake and conversion, laying performance and welfare scores.

2. Animals, materials and methods

Experimental setup

A feeding experiment was carried out with experimental flocks of 10 laying hens at the end of their laying period. For each round, 30 white hens (Lohmann Selected Leghorn classic; 64-74 weeks old) were purchased from commercial organic flocks and randomly distributed to one of three feeding groups. The experiment was repeated four subsequent times with hens from different commercial flocks for each round. By choosing hens from different origins we expected a larger variability and thus a better external validity of results. Each feeding group was housed in an experimental unit equipped with perches, litter, nests, feeders and drinkers. Space allowance in the experimental houses corresponded to the Swiss Animal Welfare Ordinance and to organic standards (BioSuisse, 2014). Hens had permanent access to a covered outdoor area ('wintergarden'), but not to pasture. The experiment started after one week of adaptation to housing conditions and to experimental diets (adaptation period). Hens were then fed experimental diets for another 3 weeks (feeding period).

Hens started the adaptation period with no significant difference in live weight between treatments (P=0.68). Hens of round 2 were heavier at the start of the adaption period than those of round 1 and 3 (P<0.01), with no differences in start weight between rounds 1, 3, and 4. Overall, weights (1,735±180 g) were within the standard for non-cage production systems as given by the breeding organisation (Lohmann, 2013). Wound scores (see below) and laying performance (%) did not differ between the treatment groups, initially.

All animal-related procedures were in compliance with the Swiss Animal Welfare Act, the Animal Welfare Ordinance, and the Animal Experimentation Ordinance (experiment no. 75645).

Hermetia meal production

Larvae of *H. illucens* were produced by modified methods based on Sheppard *et al.* (2002). Freshly deposited eggs were transferred to incubation boxes where larvae hatched within three days. Young larvae were first fed a powdered compound feed for layers (composition and nutrient concentration corresponding to control feed used in this experiment; see below). After a maximum developmental time (egg to larval stage L2/L3) of 10 days, larvae were transferred to grow-out units. Larvae were fed on vegetarian by-products of the pasta and convenience food industry until they reached the end of larval stage L5 after another 2-3 weeks. Larvae were harvested before they reached the pre-pupal stage due to the lower degree of sclerotisation of the cuticle and the resulting higher digestibility (Bosch *et al.*, 2014). They were then harvested, killed (by freezing) and stored at -20 °C. Finally the larvae were washed, dried at 60 °C for 24-34 h depending on the water content, ground and defatted by pressing. A commercial oil press (KK 20 F Universal; Screw Press, Reut, Germany) was used. The resulting product was a brown Hermetia meal (colour comparable to fishmeal) with a crude fat concentration of 110 g/kg and a crude protein concentration of 590 g/kg (Table 1).

Table 1. Nutritional values of *Hermetia illucens* meal after drying at 60 °C only and drying followed by partly defatting (g/100 g fresh matter).

	Dried, full fat	Dried, partly defatted
Dry matter	96.4	95.9
Crude protein	41.5	59.0
Methionine	not analysed	0.98
Lysine	not analysed	3.09
Crude fat	26.5	11.0
Crude ash	4.3	5.0
Calcium	0.80	0.98
Phosphorus	0.50	0.63
Sodium	0.08	0.08
Chloride	0.33	0.28

Feed composition

Three isonitrogenous and isoenergetic experimental feeds were produced by a commercial organic feed manufacturer: a standard control feed containing 36 g/100 g soybean cake ('control'), a feed containing 12 g/100 g Hermetia meal and 15.6 g/100 g soybean cake ('H12'), and a feed with 24 g/100 g Hermetia meal replacing 100% of the soybean ('H24'). Apart from Hermetia meal, all components used were certified organic and no synthetic amino acids were added. Table 2 summarises composition and main analytical results for the three compound feeds.

All feeds as well as the dried and dried, partly defatted Hermetia meal were analysed for nutrient composition following the methods defined by the EC Commission Regulation no. 152/2009 (EC, 2009).

Feed intake, production and animal health

Feed intake was measured during the 3-week feeding periods at group level. Egg production at group level and mortality were recorded daily. Body weight as well as feather and wound scores according to Tauson *et al.* (2005) were recorded weekly, starting at the beginning of the adaptation period. Individual scores of neck feathers, breast feathers, belly feathers, back feathers, tail feathers and wing feathers were summed up to a new overall plumage score ranging from 6 to 24, with 24 being the highest achievable overall plumage score. Hens of the four rounds had different levels of plumage scores, both at the beginning and at the end of the trial ($P<0.001$). Wound scores remained at the same level during the feeding period in all rounds and treatments.

Faeces were assessed weekly during the 3-week feeding period. Assessment took place 24 h after cleaning the dung board below the perches in order to ensure that only fresh faeces were present. Faeces were scored in a semi-quantitative way, counting numbers of black faecal pads and of liquid faeces present on the dung board. Then, 20 faecal pads per group were collected from the dung board for determination of dry matter (drying at 105 °C for 12 h).

Table 2. Composition and nutrient concentration of the experimental feeds.[1]

	Control	H12	H24
Feed components (g/100 g fresh matter)			
Hermetia meal, partly defatted	0.0	12.0	24.0
Soybean cake	36.0	15.6	0.0
Corn	35.0	40.9	34.3
Wheat	0.0	4.6	14.6
Wheat bran	3.1	2.2	2.1
Mixed bran	2.1	0.0	0.0
Sunflower cake	2.6	8.1	8.2
Alfalfa meal	3.1	3.0	3.0
Grass meal	2.1	2.2	2.1
Granulated cereals	4.1	0.0	0.0
Minerals, limestone, vitamins	11.8	11.3	11.7
Nutrient concentrations (g/100 g dry matter)			
Crude protein	20.0	20.3	21.4
Methionine	0.32	0.36	0.39
Lysine	1.20	1.03	1.01
Crude fat	4.50	5.72	6.44
Metabolisable energy (MJ/kg)	11.3	11.3	11.3
Crude ash	14.0	13.4	13.7
Calcium	4.00	4.08	4.26
Phosphorus	0.53	0.52	0.53
Sodium	0.20	0.19	0.22
Chloride	0.22	0.23	0.28

[1] Control = a standard control feed containing 36 g/100 g soybean cake; H12 = a feed containing 12 g/100 g Hermetia meal and 15.6 g/100 g soybean cake; H24 = a feed with 24 g/100 g Hermetia meal replacing 100% of the soybean.

Egg composition

Eggs were weighed as a whole and then broken. Adhering albumen (including chalazae) was removed from the yolk and the shells before weighing yolk and shells. Albumen weight was then calculated as the difference between the total egg weight and the shell and yolk weights. The proportion of each component was determined for all the eggs laid on the last day of the feeding period of each flock (resulting in 5 to 10 eggs per flock, depending on actual egg production).

Statistical analyses

Data on egg production and feed intake were analysed by univariate ANOVA (n=4 rounds). Effects on live weight change were calculated using a linear mixed effect model, where animal in group in round was considered as nested random effect to correct for repeated measurements, dependencies within group and round, and different initial values among rounds. Fixed effects were start weight, treatment and week, including the interaction between treatment and week. Likelihood ratio tests were performed to compare full and reduced models. Plumage score was analysed accordingly with fixed effects treatment and week and their interaction. No significant interaction between treatment and week was found in any of the models; therefore, further calculations were done only with main effects. Estimates of start weight, egg weight, egg composition and faecal dry matter were calculated using linear mixed effects models and likelihood ratio tests with group nested in round as random effect and treatment as fixed effect. If treatment was significant, Tukey post-hoc tests were performed. Residuals of all models as well as random effects were graphically inspected for normality and homoscedasticity. No model was calculated for injury scores due to very large skewness in the data. Since faecal scoring was done semi-quantitatively, no statistics could be applied to those parameters. They remain descriptive. All statistical analyses were computed with the statistical software R 2.15 (R Core Team, 2014).

3. Results

Parameters of production and egg composition

The parameters of egg production, feed intake, and feed conversion are presented in Table 3. There were no significant differences between treatments for any of these parameters.

Table 4 presents the egg composition determined at the end of each round. Treatments with Hermetia meal did not significantly differ from the control group concerning the weight of yolk, albumen, egg shell and the total egg weight. A tendency was found for a lower weight of the albumen with the treatment H24.

Parameters of animal health and welfare

Except for sporadic diarrhoea in the H groups (see below), no signs of clinical diseases (e.g. digestive or respiratory symptoms) were recorded and mortality was zero in all rounds and treatments. From week 0 to 3, an average increase in live weight of 8.7 g was observed (P=0.01) with no significant difference between treatments (Figure 1). Live weight during the feeding period was clearly dependent on the weight in week 0 (start weight; P<0.001).

There was no significant difference among treatments in overall plumage score ($\chi^2(2)$=0.84; P=0.66; Table 5). Means and standard errors of injury scores were similar across treatments at the end of the trial.

Round 1 was excluded from analysis of faeces properties, because the assessment protocol was changed slightly afterwards. During the subsequent rounds, faeces were always considered 'normal' (consistency, colour) in the control groups, whereas black faeces with or without diarrhoea (liquid consistency) were seen in the Hermetia groups. Descriptive data are shown in Table 6. Creamy, foamy or extremely watery faeces were not observed.

Table 3. Performance of layers fed diets containing 0% (control), 12% (H12), and 24% (H24) of *Hermetia illucens* meal during 3 weeks of experimental feeding (n=4; mean ± standard error).[1]

	Treatment			Statistical values	
Item	Control	H12	H24	*F*-value (2,9)	*P*-value
Laying performance (%)	79.0±4.4	84.4±8.4	83.4±3.2	0.25	0.785
Feed intake (g/d)	116±5.5	131±5.3	107±9.4	2.95	0.103
Feed intake (g/egg)	148±8.0	159±14.9	134±11.6	1.16	0.356
Feed intake (g/g egg weight)	2.15±0.08	2.38±0.25	2.03±0.13	1.12	0.369
Feed intake (g/g egg without shell)	2.51±0.09	2.78±0.30	2.38±0.12	1.03	0.397

[1] Control = a standard control feed containing 36 g/100 g soybean cake; H12 = a feed containing 12 g/100 g Hermetia meal and 15.6 g/100 g soybean cake; H24 = a feed with 24 g/100 g Hermetia meal replacing 100% of the soybean.

Table 4. Weight of egg components and total egg weight (g) (mean ± standard error) of layers fed diets containing 0% (control), 12% (H12), and 24% (H24) of *Hermetia illucens* meal; (n=4 rounds per treatment; 5-10 eggs per round).[1]

	Treatment			Statistical values	
	Control	H12	H24	χ^2(2)-value	*P*-value
Weight of egg yolk	19.2±0.30	18.6±0.23	18.6±0.23	3.8	0.15
Weight of albumen	39.6±0.89	39.2±0.53	36.6±0.81	5.6	0.06
Weight of egg shell	9.4±0.19	9.3±0.21	9.3±0.24	0.2	0.89
Total egg weight	68.5±1.1	67.3±0.66	64.8±1.07	3.7	0.15

[1] Control = a standard control feed containing 36 g/100 g soybean cake; H12 = a feed containing 12 g/100 g Hermetia meal and 15.6 g/100 g soybean cake; H24 = a feed with 24 g/100 g Hermetia meal replacing 100% of the soybean.

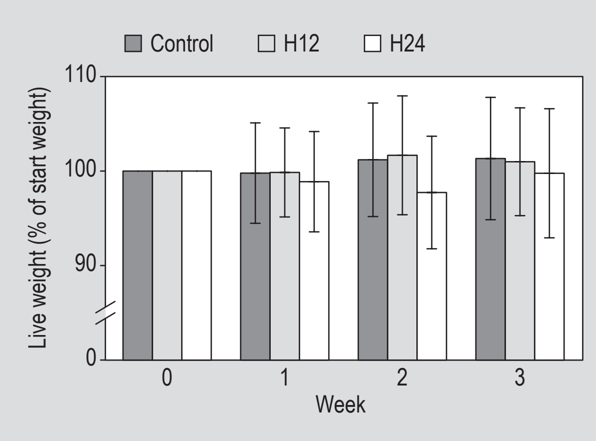

Figure 1. Live weight in percentage of start weight (averages ± standard deviations) of hens fed diets containing 0% (control), 12% (H12) and 24% (H24) of *Hermetia illucens* meal at the beginning of the adaptation period (week 0) and during the feeding period (weeks 1-3).

Table 5. Plumage and injury scores and number of missing toes for all treatments.[1] Mean ± standard error of all rounds in experimental week 3.

	Treatment		
Scores	Control	H12	H24
Plumage	16.5±0.52	17±0.49	16.6±0.40
Comb wound	3.25±0.07	3.17±0.06	3.15±0.06
Belly wound	4.00±0.00	3.98±0.02	3.90±0.05
Foot pad lesion	3.20±0.14	3.20±0.14	3.31±0.13
Keel bone fracture	2.88±0.20	3.33±0.12	2.95±0.18
Missing toes (N)	0.08±0.04	0.10±0.05	0.08±0.04

[1] Control = a standard control feed containing 36 g/100 g soybean cake; H12 = a feed containing 12 g/100 g Hermetia meal and 15.6 g/100 g soybean cake; H24 = a feed with 24 g/100 g Hermetia meal replacing 100% of the soybean.

Table 6. Numbers of black faecal pads and of liquid faeces present on the dung board 24 h after the last removal of faeces (sum of rounds 2-4).[1]

Description of faeces	Treatment		
	Control	H12	H24
Black, liquid consistency	0	16	20
Black, medium consistency	1	4	9
Black, firm consistency	12	21	21

[1] Control = a standard control feed containing 36 g/100 g soybean cake; H12 = a feed containing 12 g/100 g Hermetia meal and 15.6 g/100 g soybean cake; H24 = a feed with 24 g/100 g Hermetia meal replacing 100% of the soybean.

Dry matter content of faeces increased with increasing proportion of Hermetia meal in the diets (Figure 2), with a significant difference between control and H24 ($\chi2(2)=6.8$; $P=0.03$); one outlier had to be excluded due to implausibility (group H24).

4. Discussion and conclusions

Production parameters of layers

The partial or full replacement of soybean cake by partly defatted meal from *H. illucens* in a diet for layers did not affect their feed intake, nor laying performance, egg weights and feed efficiency, if compared to the treatment with an organic standard diet for layers. The fact that intake did not differ and there was no significant weight loss during the experimental period gives reason to assume that no severe endogenous imbalances occurred, and that hens did not develop metabolic disorders. Feed intake of the control and H24 treatments were in accordance with the standard given by the breeder (105-115 g/day); intake of H12 treatments was slightly above the standard (Lohmann, 2013). Laying performance of both treatments fed *Hermetia*-based feed corresponded to the standard given by the breeder for this age (84.4%; Lohmann, 2013), while performance of the control treatment was numerically 5% lower. This is an important result, because it indicates the equivalence of *Hermetia*-based feed with an organic standard feed on the nutritional level. Generally, feed intake and feed conversion were in the range of data reported for high performance conventional layers (Li *et al.*, 2013; Wu *et al.*, 2007) as well as to the standard indicated by the breeder (Lohmann, 2013). However, it has to be stated that the crude protein concentrations in the organic diets of the present experiment were higher (20%) than in the diets used in the quoted studies (14.5-18.7%). This might mean that protein efficiency was lower than in conventional systems.

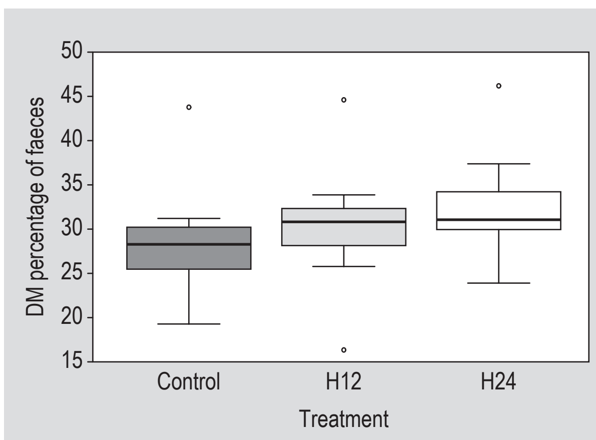

Figure 2. Faecal dry matter content of hens fed diets containing 0% (control), 12% (H12) and 24% (H24) of *Hermetia illucens* meal. Control and H24 groups differ significantly (*P*=0.03).

Under experimental conditions, higher protein contents in layer feed have been linked to higher ammonia emissions per animal as well as per unit of egg output (Rahman *et al.*, 2012). However, if Hermetia meal production could be based on the use of waste materials as main protein sources (Belluco *et al.*, 2013; Makkar *et al.*, 2014) – thus reintroducing wasted N into the food chain – feeding poultry with such materials appears as a reasonable option, also in terms of efficiency. This would be a significant contribution towards de-escalation of global ecological and social issues (Cassidy *et al.*, 2013; Semino *et al.*, 2009).

Weights of egg compounds (albumen, yolk, shell) did not differ between the treatments, although there was a tendency for less albumen. The Ca content of the Hermetia meal was very low (0.98 compared to 7.56 mentioned by Makkar *et al.* (2014). This might be due to harvest before the pre-pupal stage. However, it did not affect the total Ca content of the diets and did therefore not result in lower shell weights.

Higher dry matter of faeces and a larger proportion of dark, firm faecal pads with H24 give reason to assume that in this diet the proportion of Hermetia was too high. The causes of these differences are not fully understood; there might have been a lower water intake in the H24 groups. Another reason could be the inclusion of wheat in the Hermetia treatments that had been done to reach nutrient equivalence between all diets. The non-starch polysaccharides, which are comparably highly concentrated in wheat, may cause changes in intestinal viscosity, enzyme activity and digestibility (Maisonnier *et al.*, 2001; Mirzaie *et al.*, 2012). Theoretically, this could have confounded with the Hermetia meal and influenced faeces properties as well as feed conversion efficiency. However, Mirzaie *et al.* (2012) reported that such effects were still absent in diets containing 23% wheat, and the maximum of wheat inclusion in the current study has been 14.5%. Therefore, a considerable effect of wheat inclusion on faecal properties and feed conversion is not assumed for the diets H12 and H24.

Health and welfare parameters of layers

There was no mortality in the animals fed either diet. With average wound scores ranging between 3 and 4, the hens can be considered to be in a good or even excellent state (Tauson *et al.*, 2005). There were no new injuries and older injuries (mainly missing toes and broken keel bones) remained as they were at installation of the flocks.

Feather pecking is still one of the main welfare issues encountered in commercial egg production (Huber-Eicher and Sebö, 2001; Lambton *et al.*, 2015). Feather pecking can have several reasons, among them feed composition, in particular inappropriate amino acid supply (Kjaer and Bessei, 2013; Van Krimpen *et al.*, 2005). Repeated scoring revealed no indication of a decrease of plumage scores linked to an increase of feather pecking in either feeding group during the experimental feeding, even though the risk for feather pecking is increased after re-housing (Cloutier and Newberry, 2002) and diet changes (Dixon and Nicol, 2008).

In summary, except for sporadic diarrhoea, there was no indication of impaired animal health and welfare during the three-week experimental feeding period.

General issues / future perspectives

The present experiment demonstrated that it is possible to produce a Hermetia meal on the basis of food industry by-products for the main growing period (L3-L5), after rearing the young larvae on compound feed for chicken. This insect meal is very suitable to serve at least as a partial replacer of soybean in layers diets. However, there are several issues to be considered, if this new pathway in the food-chain should be taken.

Several studies indicate that insects are able to transmit pathogens to poultry if untreated (Banjo *et al.*, 2005; Belluco *et al.*, 2013; Despins *et al.*, 1994; Erickson *et al.*, 2003; Hazeleger *et al.*, 2008). Therefore, suitable protocols for reducing microbial and parasitic risks (e.g. by heat treatment) in order to ensure feed and food safety need to be developed and strictly followed. Bioaccumulation of cadmium presents a main risk which cannot be addressed by processing (Charlton *et al.*, 2015). The European Food Safety Authority is concerned with the problem of food and feed safety (Van der Spiegel *et al.*, 2013) but at present no official statement is available.

The production of larvae of the black soldier fly on food- and farm-waste was re-discovered recently after the issue had been extensively examined and published already in the early seventies and eighties of the last century (e.g. Hale, 1973; Newton *et al.*, 1977; Sheppard, 1983). While the focus of early research was more on waste reduction than on feed production, nowadays the issue of using available waste resources in a re-cycling and sustainability context is predominant (Popa and Green, 2012; Ramos-Elorduy *et al.*, 2002; Van Zanten *et al.*, 2015). Investigation of the environmental impact when using insect larvae fed on organic waste (e.g. house fly larvae; Van Zanten *et al.*, 2015) as livestock feed, showed slightly higher global warming potential compared to soybean meal (mainly due to the energy input during production and processing) but lower land use than soybean meal. Also, the considerable indirect environmental impact (mainly through reduced availability of food waste to be used for anaerobic digestion) has to be included in an overall impact assessment of insect proteins (Van Zanten *et al.*, 2015). Regarding sustainable protein production in general, many insect species can be produced with much lower direct greenhouse gas emissions compared to cattle production (Oonincx *et al.*, 2010).

H. illucens larvae meals have proven to be nutritionally valuable in certain fish species including tilapia and catfish (Bondari and Sheppard, 1981, 1987), trout (St-Hilaire *et al.*, 2007; Stamer *et al.*, 2014), and turbot (Kroeckel *et al.*, 2012) as well as in poultry (Hale, 1973). Thus, while the nutritional value of insect meal is unquestioned, its sustainability-related value and economic feasibility strongly depend on the future opportunities to develop the production systems (Van Zanten *et al.*, 2015) and related opportunities of economy of scale. The application in the target species (e.g. proportion in the diet, need to process the harvested larvae into a suitable raw material) will be of predominant importance for the economic success of any insect meal.

5. Conclusions

This study shows that *H. illucens* larvae can be processed to a feed which can serve as a valuable replacer for soybean products in diets for layers. Feed efficiency was maintained on a level equivalent to soy-based feeds and there were no indications for an affected metabolic and health status of the hens.

However, further research on long-term feeding effects including hens before and during peak of lay and on resulting egg quality is necessary to approve insect larvae as a practicable source of feed protein.

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