

# Use of black soldier fly and house fly in feed to promote sustainable poultry production

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## Abstract

The growing human population, changing dietary habits and intensifying competition between food and feed production underline the urgent need to explore novel sustainable production chains. In the past, the poultry sector has gained popularity due to its superior environmental and economic benefits compared to other livestock production systems. Therefore, it is of special interest to focus on refinement and innovation along the value chain to further improve the sector's sustainability. One major issue is the transition towards sustainable protein sources in poultry feed. In this regard, insects are the secret rising stars. Insect species such as the black soldier fly (*Hermetia illucens*) and house fly (*Musca domestica*) have been proposed for farming as multifunctional mini-livestock for feed. One major property of these flies is that larvae can convert low-quality organic waste streams into valuable body mass containing high levels of high-quality protein and fat. Furthermore, the larvae are reported to have health- and welfare-promoting effects due to bioactive compounds and poultry having a natural interest in them. The aim of the current paper is to discuss the state-of-the-art of using black soldier fly and house fly larvae as components of poultry feed and to highlight knowledge gaps, future opportunities and challenges. Some first studies have focussed on the successful partial replacement of soybean meal or fishmeal by these insects on poultry performance. However, since the sector is still in its infancy several uncertainties remain to be addressed. More research is required on identifying optimal inclusion levels, clearly differentiating between insect products based on their nutritional value and health-stimulating effects, and comparing the potential of insect products across species.

**Keywords:** black soldier fly, *Hermetia illucens*, health and welfare, house fly, *Musca domestica*, nutritional value, poultry feed

## 1. Global challenges for the poultry feed industry

Producing insects as feed has started in recent years and this industry is expected to grow exponentially in the near future, given that insects are expected to soon be authorised as pig and poultry feed in the EU (IPIFF, 2018). The new insect sector provides animal protein through a sustainable production process with low-value inputs, high-value outputs and low environmental impact and can become an important element of the circular economy (Dicke, 2018). For a novel, circular and sustainable way of producing feed, insects provide an excellent opportunity because various species can be reared on organic left-over streams. Fly larvae are important insects used as feed because they have

excellent nutritional quality and can be grown sustainably on various organic waste streams (Bava *et al.*, 2019; Jucker *et al.*, 2019; Miranda *et al.*, 2020; Ocio *et al.*, 1979). Therefore, this review focuses on the application of two fly species that are currently produced as a natural ingredient in poultry feed: black soldier fly (BSF; *Hermetia illucens*) and house fly (HF; *Musca domestica*). Recent literature on BSF- and HF-based poultry feed is evaluated for effects on nutrient digestibility, growth performance, product quality, and health and welfare.

Within the last decade the global human population has grown to about 7.8 billion people (FAOSTAT, 2019) and is forecasted to increase to 9.7 billion in 2050 (FAO, 2018). This will increase the need for food and feed, while rising

incomes in especially East and Southeast Asia will shift consumer patterns towards enhanced meat consumption (OECD/FAO, 2019). Therefore, one of the major challenges the food and feed sectors face is the increasing demand for protein (OECD/FAO, 2019; Van Krimpen and Hendriks, 2019). Competition between food and feed production is expected to increase even more, because considerable protein quantities in animal feed originate from sources also edible for humans (Mottet *et al.*, 2017).

The protein component of feed may originate from different sources: crops (cereals, oilseeds and pulses) and non-plant sources (e.g. former foodstuff, fishmeal and processed animal protein) (EC, 2019). Currently, most commonly used protein sources in commercial livestock and fish feed formulations are soybean meal and fishmeal (FAO, 2008; Olsen and Hasan, 2012). The biggest downside of using plant-based protein sources in animal feed, such as soybean meal, is that their cultivation requires a lot of land and water (FAO, 2009). Soybean production leads to deforestation, especially in the Amazon (Hecht, 2005) and, additionally, soybean meal requires long-distance transportation. Because global arable land is limited, increasing food and feed demands will intensify the trade-off between crop land used for food versus feed (OECD/FAO, 2019).

Fishmeal represents a highly valuable protein source in animal feed that is mainly incorporated in aquafeed, but also in feed for other livestock such as poultry and pigs (FAO, 2008; OECD/FAO, 2019). In comparison to soybean meal, fishmeal reaches about four-fold higher market prices. It is expected that fishmeal will gradually be replaced by cheaper oilseed products (OECD/FAO, 2019). Moreover, due to overexploitation of the ocean, fishmeal bears the risk of becoming even rarer in the future (Van Huis *et al.*, 2013).

The production of protein sources for feed is unevenly distributed (Van Krimpen and Hendriks, 2019). Most countries are highly dependent on international trade. The EU, for instance, imports about one quarter of its feed protein. When focussing on the EU's self-sufficiency rate of soybean meal this value even drops to 3% (EC, 2019). The combined effect of land limitation and market dominance of export countries is predicted to amplify competition and to increase market prices (Clément *et al.*, 2018; OECD/FAO, 2019).

In conclusion, feed protein sources are important, but limited resources which will become even more valuable in the next decades. The current protein sources for feed, fishmeal and soybean meal, are not sustainable. Consequently, both products are not suitable to meet with the rapidly growing demand. The development of new, sustainable, protein-rich resources is therefore of major importance.

## 2. Poultry production: a rising sector

Animal products commonly incorporated in human diets are pork, beef, chicken, milk, and eggs. Of these, the poultry sector recently emerged as one of the most important agricultural branches experiencing rising global attention (Augère-Granier, 2019; OECD/FAO, 2019). Several facts favour the production and consumption of these products. One reason is the increasing public attention towards higher sustainability standards in livestock husbandry (OECD/FAO, 2019). The environmental impact of poultry products is comparatively low (De Vries and De Boer, 2010; Flachowsky *et al.*, 2018). In contrast to other livestock products, chicken meat and eggs have the lowest water and carbon footprint per kg edible protein. Also, land use efficiency per kg edible protein is very high and only exceeded by dairy milk production (Flachowsky *et al.*, 2018). An effective poultry production system should pay full attention to animal health and welfare.

The attractiveness of the two sub-sectors reflects their economic growth in 2008-2018. During this period, chicken meat and egg industries recorded by far the biggest global production increase of ~41 and ~24%, respectively (FAOSTAT, 2020a). This increase is expected to continue in the future (OECD/FAO, 2019). Eggs are highly nutritious and provide essential and valuable nutrients in addition to protein (Farrell, 2013). As global population growth is expected to be accompanied by economic growth, rising incomes are expected to promote dietary shifts towards higher levels of animal protein inclusion (OECD/FAO, 2019).

This is also true for the poultry meat industry. On a global scale, poultry meat greatly impacts the daily protein supply per capita, and exceeds the protein contribution of pig and cattle meat (FAOSTAT, 2020b). The attractiveness of the sector is equally shared amongst consumers and producers (OECD/FAO, 2019). Compared to other production systems such as cattle and pig husbandry, poultry offers several advantages. First of all, it supplies lean meat, which is increasingly meaningful to consumers due to their rising awareness of healthy foods. Secondly, chickens grown for meat have low feed conversion ratios and short life cycles, which promote flexible production, limited production costs and consequently relatively low product prices (FAO, 2018; OECD/FAO, 2019). Finally, unlike most other meat sources, poultry meat consumption has no religious barriers and therefore has a unique market position (EC, 2018). However, the one-sided selection for low feed conversion and fast growth in broilers has led to severe animal welfare and health problems, such as leg and metabolic disorders (EC, 2016; Wallenbeck *et al.*, 2016).

In conclusion, the poultry sector is important for the production of protein-rich nutritious food, largely

contributing to the global supply of animal protein. Its main branches are the production of chicken meat and eggs, both showing a steep upward trend in production. The poultry industry is predicted to fulfil a key position in satisfying future rising demands for animal protein. It is, therefore, an essential element to focus on refinement and innovation at every part of the value chain to improve the sector's sustainability.

### 3. Poultry production systems

#### Broiler production

Within the poultry sector, chickens raised for meat production are known under the term 'broilers' (EC, 2016). Broiler husbandry can be categorised into rearing systems with an intensive and less intensive production focus. Within the latter category, production is realised with slow-growing broiler strains, while fast-growing broilers dominate in intensive systems (Van Horne, 2018; Vissers *et al.*, 2019). The main difference between the two breeding strategies is the longer growth period of slow-growing broilers required to reach a marketable weight of 2–2.5 kg. In practice, the growth period varies greatly and ranges between 35 to 81 d, while fast-growing strains are slaughtered at an age of 35–42 d (EC, 2016).

The quantity of alternative production systems keeping slow growing broilers has slightly increased within the past years (Van Horne, 2018). The increase of alternative systems is primarily reasoned by welfare issues emerging in conventional systems. Commonly reported issues are leg weaknesses, reduced active behaviour, disease susceptibility, prevalence of contact dermatitis, and ascites, which can result in mortality (EC, 2016; Wallenbeck *et al.*, 2016). However, fast-growing broiler systems represent the majority. Within the EU, for instance, fast-growing broiler systems cover 90–95% of broiler production (Augère-Granier, 2019; Van Horne, 2018).

Across EU countries, market share of both systems varies greatly. For instance, within The Netherlands 30% of marketed broiler meat is based on slow-growing strains (Saatkamp *et al.*, 2019; Stadig, 2019). Alternative husbandry systems using slow-growing broilers emerged rapidly in the Netherlands from 2013 onwards. As for now, this development is unique worldwide (Saatkamp *et al.*, 2019).

Slow-growing broilers experience better welfare (Vissers *et al.*, 2019), due to lower daily weight gain and altered morphological conformation, improved walking abilities (Corr *et al.*, 2003; Wallenbeck *et al.*, 2016), and higher active behaviour (Wallenbeck *et al.*, 2016; Wilhelmsson *et al.*, 2019). Moreover, slow-growing broilers exhibit lower mortality rates (Dixon, 2020) and similar (Sakkas *et al.*, 2018) or improved immune responses (Giles *et al.*, 2019).

Sustainability in animal husbandry has many elements, including animal welfare, economics, environmental impact and social justice (Fernyhough *et al.*, 2020). Within this context one may realise that farm systems that produce slow-growing strains also negatively impact some aspects of sustainability. The improved animal welfare compared to fast-growing strains is accompanied by a higher economic and ecological burden due to higher feed conversion ratios (Rezaei *et al.*, 2018), longer life cycles, higher feed and energy requirements (Tallentire *et al.*, 2018), lower breast meat yield, and meat quality (Fanatico *et al.*, 2008; Quentin *et al.*, 2003). The market price of meat of slow-growing strains is higher and by this the economic burden is reduced. Society in general has a more critical attitude to animal meat production because of ethics (Lund *et al.*, 2016). Thus, the poultry meat sector faces the dilemma of a trade-off between different elements of sustainability.

#### Laying hen production

The European egg sector is also of great importance because it is the second producer worldwide, behind China (Augère-Granier, 2019). Based on the annual production in 2018, about 7.1 million tonnes of hen eggs were produced, of which 9.9% were produced in The Netherlands (FAOSTAT, 2020a).

Laying hens are reared within four major husbandry systems: enriched cages, barn-, free range- and organic systems (Augère-Granier, 2019). Laying hens used within these production systems can be divided into two categories which reflect strains producing eggs with either white or brown eggshell colour (Leenstra *et al.*, 2012). In practice, the eggshell colour is commonly pre-indicated by the colour of the feather dress (white or brown, Leenstra *et al.*, 2012) or earlobe (white or red, Nie *et al.*, 2016). Several regions show a specific preference for one of the two eggshell colours. For example, within the EU brown eggs dominate the market reflecting consumer preference (International Egg Commission, 2019).

The genetic trait for brown eggshell colour in laying hens negatively impacts economic and environmental aspects. White strains for instance are known to have a lighter body weight (Bean and Leeson, 2003; De Haas *et al.*, 2013; Singh *et al.*, 2009), slightly lighter eggs, equal egg productivity (Bean and Leeson, 2003; Onbaşlılar *et al.*, 2015; Singh *et al.*, 2009), and more favourable feed conversion ratios (De Haas *et al.*, 2013; Fernyhough *et al.*, 2020; Onbaşlılar *et al.*, 2015) in comparison to their brown companions. Hence, from an economic and environmental point of view white egg-producing strains have a distinct advantage over brown egg-producing strains. The production of brown eggs seems, therefore, mainly driven by consumer perception and preference.

In some EU countries there is a noticeable change within the market share from brown towards white layers (International Egg Commission, 2015). While the ratio in The Netherlands in 2011 was 55:45, the ratio shifted in 2019 to 35:65, respectively. Also China, the global leader in egg production, produced in 2011, 82% of their eggs with brown eggshell colour, while this proportion reduced substantially to 65% in 2019 (International Egg Commission, 2011, 2019). One reason for this development is the reduced aggressive behaviour of white layers (International Egg Commission, 2015). Hence, these strains are of special interest in countries where beak trimming is prohibited (FERNYHOUGH *et al.*, 2020; International Egg Commission, 2015).

The literature comparing feather pecking and plumage scores between white and brown layers appears rather inconclusive. Some reports are in accordance with the previous statement and suggest less issues regarding feather loss in white layers (DAMME and URSELMANS, 2013; ODÉN *et al.*, 2002; STRUTHERS *et al.*, 2019). Other authors even indicate worse feather conditions in white layer flocks (DE HAAS *et al.*, 2013; DIXON and DUNCAN, 2010; LEENSTRA *et al.*, 2012; UITDEHAAG *et al.*, 2008). Variation in the literature can possibly be attributed to the multifactorial nature of feather pecking (NICOL *et al.*, 2013).

Equivalent to the debate about slow- vs fast-growing broiler strains, also eggshell colour in strains of laying hens differently affects components of sustainability. While economic and environmental characteristics favour strains laying white eggs, consumer beliefs drive production systems towards brown strains.

In summary, sustainability of the poultry sector has several dimensions (animal welfare, economics, environmental impact and social justice), that in many instances trade off. The main component appears to be a potential trade-off between animal welfare and resource friendly production. The main challenge will be to find a balanced solution or to explore new approaches combining those elements, such as environmental enrichment for resource-efficient poultry strains. Reducing pressure on the feed market by presenting an alternative and more environmentally sustainable protein

source such as insects as (part of) animal feed might be an option to positively impact poultry health and welfare while production efficiency is guaranteed.

#### 4. Insects as part of the solution

To improve sustainable production, the use of insects in animal feed has gained great interest over the past years, which is highlighted by the strong increase in academic publications on this topic (Van Huis, 2020). The increasing attention for the use of insects in animal feed is justified by their qualitatively high nutritional content, but also by their potential to act as health promoters for livestock.

##### Nutritional value

Currently, insect species including BSF, HF, yellow mealworm (TM), lesser mealworm, house cricket, banded cricket, and field cricket have been approved to be used in animal feeds for specific purposes (EC, 2017). Among these insects, the first three species have been proposed to be most promising for production as feed components (Sogari *et al.*, 2019; Van Huis, 2020; Veldkamp *et al.*, 2012). Although these insects have a high protein and fat content the proximate analysis demonstrates the prevalence of nutritional differences (Table 1). HF larvae for instance contain higher levels of crude protein and lower levels of crude fat than BSF (Makkar *et al.*, 2014). However, the composition can be highly variable dependent on the larval rearing substrate (Schmitt *et al.*, 2019).

When focusing on amino acid (AA) profiles, comparable values have been reported for insect sources and soybean meal (Makkar *et al.*, 2014; Veldkamp and Van Niekerk, 2019). Insects are particularly rich in lysine and methionine (Makkar *et al.*, 2014), while cystine appears to be the most limiting AA (Makkar *et al.*, 2014; Veldkamp *et al.*, 2012). Regarding animal feed production, it is essential that insects fulfil the requirements of the target animal. In poultry diets, most limiting AAs are methionine, lysine, tryptophan, and threonine (Fernandez *et al.*, 1994; Harms and Ivey, 1993). According to the essential AA index for broilers, BSF, HF, and TM larvae exceed the required quantities of essential AA (Veldkamp and Bosch, 2015). Thus, insects have a great

**Table 1. Proximate composition (minimum and maximum) of yellow mealworm, black soldier fly, and house fly larvae.**

	Dry matter (DM) g/kg fresh	Crude protein g/kg DM	Crude fat g/kg DM	References
Yellow mealworm	364-424	494-661	245-360	Azagoh <i>et al.</i> , 2016; Elahi <i>et al.</i> , 2020; Ghaly and Alkoaik, 2009; Ochoa Sanabria <i>et al.</i> , 2019
Black soldier fly	300-388	371-492	72-387	Finke, 2013; Jucker <i>et al.</i> , 2020; Schmitt <i>et al.</i> , 2019; Star <i>et al.</i> , 2020; Veldkamp and van Niekerk, 2019; Woods <i>et al.</i> , 2020
House fly	205-276	579-646	156-245	Fitches <i>et al.</i> , 2019; Qi <i>et al.</i> , 2019; Wang <i>et al.</i> , 2013

potential to substitute soybean meal in poultry diets based on the AA profile.

### Potential health promoters

In addition to their excellent nutritional composition, insects recently gained further attention due to health promoting characteristics of specific components such as chitin, antimicrobial peptides and lauric acid (Figure 1).

#### Chitin

Chitin is, after cellulose, the most abundant polysaccharide in nature. Its molecular structure is nearly identical to cellulose; chitin possesses acetamide groups attached at the C-2 position, thus chitin is cellulose's 2-acetamido derivative (Rinaudo, 2006). Chitin is a main component of the exoskeleton of insects (Rinaudo, 2006), which fulfils structural functions and protects the organism against environmental hazards (Lee *et al.*, 2008). It is naturally embedded within a matrix with proteins, lipids and minerals (Józefiak *et al.*, 2016). Enzymes such as chitinase break down the molecule into chitin derivatives like chitosan (Shahidi and Abuzaytoun, 2005). Due to the polyanionic nature of chitosan, the molecule can turn into a strong chelator and bind metal ions (Rinaudo, 2006). Since most animals are unable to produce chitinase in the gastrointestinal tract (Shahidi and Abuzaytoun, 2005), chitin is considered as an indigestible fibre (Tabata *et al.*, 2017). However, mRNA expression of the gene encoding for chitinase within the

glandular stomach of chickens, indicates that chickens are able to digest chitin to a certain extent (Suzuki *et al.*, 2002; Tabata *et al.*, 2017).

Chitin and its derivatives have been found to positively impact the health status of animals. For instance, chitosan oligosaccharides (COS) supplemented to broiler diets significantly increased the relative weight of important immunological organs such as the spleen, bursa of Fabricius and thymus (Chi *et al.*, 2017). Similar experiments with COS in broiler diets highlighted its potential to enhance IgM levels and proinflammatory cytokine expression in blood serum (Deng *et al.*, 2008). Moreover, chitin derivatives can also serve as a substrate for caecal bacteria in chickens. The alteration of the caecal microbial community influences the health status of chickens, because microorganisms play a key role in shaping the immune system (Li *et al.*, 2007). Based on these findings, provision of chickens with insects containing chitin is expected to impact health parameters. Moreover, because the structure of chitin varies between insect species (Kaya *et al.*, 2015) and the effect of chitin on the immune system is complex and size-dependent (Lee *et al.*, 2008), their health promoting properties might differ.

To evaluate the impact of insect chitin as feed component on the immune system of animals, the analysis of ingested chitin levels is indispensable. Currently, several methods are available for quantification of chitin in whole insects (Finke, 2007, 2013; Hahn *et al.*, 2018; Woods *et al.*, 2020). However, due to the application of different methodological

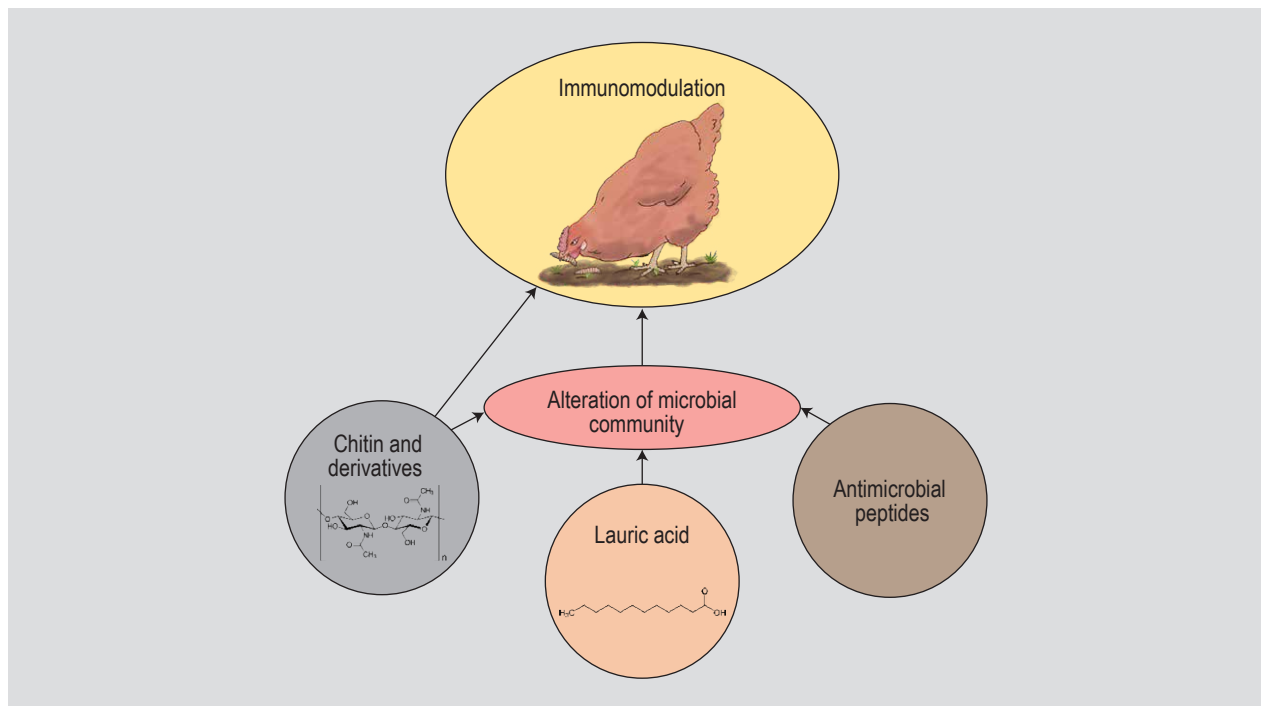


Figure 1. Potential direct and indirect modulation of the poultry immune system by insect chitin, lauric acid, and antimicrobial peptides.

approaches, results are rather variable (Hahn *et al.*, 2018). For example, reported chitin contents in BSF larvae range from 54 g/kg dry matter (DM) (Finke, 2013) to 106 g/kg DM (Hahn *et al.*, 2018). Consequently, there is a need for an accurate, practically applicable and uniformly accepted method to determine chitin levels in insects. Moreover, within the cited studies neither the instar nor the age in days of the harvested larvae was mentioned. Chitin levels increase with development of the larvae (Wong *et al.*, 2019). It is of utmost importance to facilitate the comparability of chitin levels among literature.

#### Lauric acid

Lauric acid (C12:0) belongs to the group of medium-chain fatty acids (MCFAs), which are known to have antibacterial properties (Skrivanova *et al.*, 2006). Insects such as BSF and HF larvae contain considerable amounts of crude fat (Veldkamp *et al.*, 2012). Especially the fat fraction of BSF larvae has been highlighted to contain great quantities of MCFAs and particularly lauric acid (Cullere *et al.*, 2019a; Gasco *et al.*, 2018). The mechanism underlying MCFA disturbance of bacterial development is not fully known to date. Yet, *in vitro* experiments indicate that MCFAs are able to enter bacterial cells through their membrane (Kim and Rhee, 2013; Skrivanova *et al.*, 2006). The entry process is assumed to lead to membrane damage, which consequently changes the cell's permeability. This damage may further promote the entry of other antibacterial compounds into the cell (Kim and Rhee, 2013).

*In vitro* analysis of the effects of lauric acid on *Clostridium perfringens* indeed shows that the MCFAs enter and disrupt the cytoplasmatic structure of the cell, although the bacterial membrane integrity remained intact (Skrivanova *et al.*, 2006). Additional testing with different concentrations of MCFA on *C. perfringens* development identified 0.5 mmol/l lauric acid as the minimum inhibitor concentration required to induce antibacterial activity. However, when *Escherichia coli* bacteria were treated with 0.125 to 1.5 mmol/l lauric acid, only negligible bactericidal effects were recorded (Kim and Rhee, 2013). Possibly membrane differences between Gram-negative and -positive bacteria may play a role in this. Gram-positive bacteria are more susceptible to damage caused by MCFAs, whereas it seems rather challenging to successfully inactivate Gram-negative bacteria with MCFAs (Kim and Rhee, 2013; Skrivanova *et al.*, 2006). Also, the type of MCFAs appears to play an important role. The application of the MCFA caprylic acid, for instance, had dose-dependent lethal effects on Gram-negative *E. coli* bacteria (Kim and Rhee, 2013; Skrivanova *et al.*, 2006). Furthermore, lower pH values resulting from MCFAs promoted bacterial cell death (Kim and Rhee, 2013).

Because especially Gram-positive bacteria seem to be sensitive to MCFAs, this property could be used to

reduce pathogen loads in livestock. For example, in broiler chickens challenged with *C. perfringens*, supplementation of experimental diets with MCFA (C6-C12), significantly reduced the prevalence of necrotic enteritis lesions in the small intestine. The authors suggest that this was due to growth inhibition and lethal effects of MCFAs on the bacterium (Timbermont *et al.*, 2010). Thus, the provision of insects in chicken feed may modify the microbial community and reduce the prevalence of pathogens in the gastrointestinal tract.

#### Antimicrobial peptides

An important category of molecules with immunomodulatory functions contained in insects are antimicrobial peptides (AMPs; Bulet *et al.*, 1999), also known as host defence peptides (Cole and Nizet, 2016). AMPs are part of the innate immune system of various living organisms besides insects, including plants, fungi, bacteria, and animals (Józefiak and Engberg, 2017). Their specific mode of action varies on the basis of their amino acid sequence and structural organisation (Bulet *et al.*, 1999).

AMPs currently gain immense global attention, due to their antibacterial function (Gasco *et al.*, 2018) and successful action against multi-drug resistant pathogens, which are a rising threat for society (Bahar and Ren, 2013). For instance, HF larvae and pupae express AMP's such as defensin in several tissues, which act upon invading Gram-positive bacteria (Andoh *et al.*, 2018; Dang *et al.*, 2010). Experiments with HF AMPs even showed that the administration to *Salmonella pullorum* infected chickens can serve as effective treatment of the intestinal disease, without having negative effects on mucosal epithelial cells (Wang *et al.*, 2017). The novel defensin-like peptide 4 (DLP4) detected in BSF larvae exhibits activity against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (Park *et al.*, 2015). While insects contain a wide spectrum of AMPs which have been identified to function against Gram-positive bacteria, most of them show no or little activity against Gram-negative bacteria (Dang *et al.*, 2010; Meylaers *et al.*, 2004; Park *et al.*, 2015; Vogel *et al.*, 2018).

To benefit from insect AMPs in animal feed it is important to understand their biosynthesis. For example, although defensin in HF larvae and pupae is continuously expressed at a low level, injury of HF larvae and pupae by a needle or immunised with a needle dipped in a bacterial suspension sharply elevates defensin levels (Andoh *et al.*, 2018; Dang *et al.*, 2010). Due to larval and pupal immunisation, defensin can reach values up to 300 and 600 times higher than before immunisation (Andoh *et al.*, 2018). A similar infection-dependent synthesis of DLP4 is observed in BSF larvae, though DLP4 expression is absent in unchallenged larvae (Park *et al.*, 2015). Besides, AMP expression has been found to be diet-dependent (Vogel *et al.*, 2018). AMP levels in

insect products might be further influenced by larval processing. Although some AMPs have been found to be thermal stable (Hao *et al.*, 2008; Li *et al.*, 2017), heat treatment might largely impact heat labile AMPs and reduce their presence in the final product. Currently, various insect products in addition to live larvae are applied in animal feed. This includes the use of non-defatted or defatted insect meal. To the best of our knowledge, there is no information available on AMP activity in those manufactured insect products. Such information would be highly valuable.

In conclusion, current knowledge indicates that AMP production in insects is variable and is affected by injury-, infection- or diet-dependent induction. Rearing conditions and processing of insect larvae therefore likely influence their impact on immunomodulatory functions when used in livestock feed. It is, therefore, important to determine AMP levels in insect products to properly estimate their effect on chickens.

## 5. BSF and HF in poultry feed

Although there are yet only few studies available examining the use of BSF and HF larvae in animal feed, some studies already reported their successful inclusion in poultry diets. To prevent that conclusions are affected by nutritional imbalances between experimental diets, the following paragraphs focus only on literature explicitly indicating the comparison of isonitrogenous and isocaloric diet formulations. Table 2, 3 and 4 report the effect of dietary BSF and HF products inclusion in broiler, laying hen and other poultry feed and their effect on digestibility, performance, product quality, and animal health.

## Broilers

### Production performance

Few publications are available regarding the application of BSF or HF larvae in broiler nutrition. Incorporation of up to 10% of defatted BSF larval meal in broiler diets revealed an increased feed intake (FI) and average daily gain (ADG) during the first phase of rearing. On the other hand, the development of broilers receiving higher levels of partially defatted BSF larvae were rather hampered (Dabbou *et al.*, 2018) and further led to the reduction of bacterial diversity in the caeca and intestinal mucin production (Biasato *et al.*, 2020). Also inclusion of 6 and 8% BSF larval meal improved the animal performance by reducing the FI and FCR while the final BW was unaffected (Attivi *et al.*, 2020). The findings give an indication that in order to maintain favourable performance results, insect inclusion in broiler diets should be restricted to an optimal inclusion level which needs to be defined by further research (Dabbou *et al.*, 2018). Inclusion of HF larval meal lower than 2% increased the final BW, and BWG at 1.6, and 2% of inclusion. Also FI was influenced which led to higher uptake of feed at 0.8 and 1.2% HF larval meal inclusion. The FCR also indicated a positive development by showing lower values at all inclusion levels (Okah and Onwujiariri, 2012). In contrast, inclusion of up to 22% HF larval meal did not alter nutrient retention (Adeniji, 2007) and performance parameters of broilers (Adeniji, 2007; Ocio *et al.*, 1979). Furthermore, results for the apparent ileal digestibility and true ileal digestibility of AA of HF larval meal and fishmeal investigated in broilers identify AA digestibility in HF larval meal comparable with AA digestibility in fishmeal (Hall *et al.*, 2018). Because

**Table 2. Inclusion of black soldier fly (BSF) and house fly (HF) products in broiler diets and their effect on digestibility, performance, product quality, and animal health, as reported in studies that compare diets that are isonitrogenous and isocaloric.**

Age	Insect product	Target replacement	Insect inclusion %	Results (digestibility, performance, product quality and animal health) <sup>1</sup>	Reference <sup>2</sup>
1-35 d	partially defatted BSF larval meal	soybean meal, soybean oil, corn gluten meal	0, 5, 10, 15	Improved LW and DFI during starter period, improved final LW, no effect on FCR at <10% BSF inclusion. Lower LW during growing and finisher period and increased FCR at 15% BSF meal inclusion.	Dabbou <i>et al.</i> , 2018
1-35 d	partially defatted BSF larval meal	soybean meal, soybean oil, corn gluten meal	0, 5, 10, 15	Mucin staining intensity in the intestinal villi reduced at 10 and 15% meal inclusion. Shift in caecal microbiota.	Biasato <i>et al.</i> , 2020
14- 56 d	BSF larval meal	fishmeal	0, 2, 4, 6, 8	Lower feed intake and FCR at 6 and 8% inclusion. Lower BWG at 2, 4 and 6% inclusion. Higher BWG at 8% inclusion. No difference in final BW. Lower Gizzard weight and intestinal length at 4, 6, and 8% inclusion. No difference in total protein, lower albumin at 2, 4, and 6% inclusion and higher values at 8% inclusion. Lower level of uric acid at any inclusion level and higher triglyceride values for 4, 6, and 8% inclusion. Lower apparent crude protein digestibility at 2, 4, and 6% inclusion level.	Attivi <i>et al.</i> , 2020

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Table 2. Continued

Age	Insect product	Target replacement	Insect inclusion %	Results (digestibility, performance, product quality and animal health) <sup>1</sup>	Reference <sup>2</sup>
1-35 d	defatted BSF larval meal	soybean meal, soybean oil, corn gluten meal	0, 5, 10, 15	Increased carcass weight and breast yield at <10% BSF meal inclusion. Linear increase of meat redness to a maximum at 15% BSF inclusion. Linear decrease of meat yellowness, CP, MUFA, and reduced moisture and PUFA as inclusion level was increased.	Schiavone <i>et al.</i> , 2019
1-42 d	live BSF	–	0, 5, 5, 10, 10	Live BSF were given at inclusion levels of 5 and 10% of the dietary FI. Larvae were provided 2 (BSF52, BSF102) or 4 (BSF54, BSF104) times a day. Final BW lower in BSF102. No influence on ADMI and ADMEI. No effect on shuffling, perching, stretching and comfort behaviour. All remaining behaviours (standing idle, walking, eating, drinking, foraging, ground pecking, resting, and activity) were affected by treatment, week, and/or interaction. Higher ground pecking and total foraging at any inclusion level and provision level at any timepoint (week). Despite week 1 group BSF104 had consistently lower values for resting and higher values general activity than the control group. Food pad dermatitis was not affected. Hock burn score was higher in control group than BSF102 and BSF104. Higher gait scores in control group compared to BSF54, BSF102 and BSF104.	Ipema <i>et al.</i> , 2020
1-35 d	HF larval meal	fishmeal	0, 0.8, 1.2, 1.6, 2	Increased BW at 0.8, 1.2, and 1.6% HF meal inclusion. Higher BWG at 1.6 and 2% inclusion. Higher feed intake at 0.8 and 1.2% inclusion. FCR lower at any inclusion level. Lower dressing % at 0.8% inclusion. Lower % of the heart at 1.2, 1.6, and 2% inclusion. Larger % of Gizzard at 0.8, 1.6, and 2% inclusion. Higher % inguinal fat at 1.2 and 1.6% of inclusion.	Okah and Onwujiariri, 2012
1-42 d	HF larval meal	groundnut cake	0, 5.5, 11.0, 16.5, 22.0	No effect on nutrient retention and performance parameters.	Adeniji, 2007
1-28 d	HF larval meal	fishmeal	0, 4	No effect on any performance parameter.	Ocio <i>et al.</i> , 1979
21-28 d	HF larval meal	fishmeal	0, 0, 0, 20, 40, 60	Regression analysis was used to investigate the digestibility of AA. Therefore, diets contained either 20, 40, and 60% fishmeal, or 20, 40, and 60% HF larval meal. AIDC and TIDC of fishmeal and HF larval meal was comparable.	Hall <i>et al.</i> , 2018
1-31 d	HF larval meal	soybean meal	0, 4, 5, 6	Higher DM, EE, and ash digestibility and lower crude fibre digestibility at 6% inclusion, higher CP digestibility at 4 and 6% inclusion. Increased BW, reduced FI, and FCR, higher dressing %, and higher apparent metabolisable energy at any level of meal provision. Unchanged organoleptic meat properties.	Khan <i>et al.</i> , 2018a
1-31 d	HF larval meal	soybean meal	0, 1, 2, 3	Apparent metabolisable energy increased at 2 and 3% meal inclusion. Digestibility of nutrients remained unchanged. Reduced FI and FCR at any inclusion level. Increased weight gain at 2 and 3% inclusion. Dressing % of meat increased at 3% meal inclusion. Improved organoleptic properties due to meal provision. Improved meat tenderness at 2% meal inclusion, higher juiciness at 1% meal inclusion. Reduced colour at 3% meal inclusion, better meat flavour at 1 and 2% meal inclusion.	Khan <i>et al.</i> , 2016
1-35 d	HF larval meal	soybean meal	0, 8	No effect on FI, mortality, dressing %, and sensory meat characteristics. Increase in BW and reduction of FCR with administration of HF meal.	Khan <i>et al.</i> , 2018b
1-42 d	HF larval meal	soybean meal	0, 4, 8	Linear reduction of BW, ADG, and increase in FCR during starter phase. No effect on carcass quality. Alteration of blood parameters were in physiological range.	Elahi <i>et al.</i> , 2019
1-42 d	HF larval meal	soybean meal	0, 5 (1-7 d), 4 (8-42 d)	Increased BW, ADG, ADFI, and reduced FCR. Increased breast and leg flavour, aroma and desirability.	Radulović <i>et al.</i> , 2018

<sup>1</sup> AA = amino acid; ADG = average daily gain; ADMI = average daily dry matter intake; ADMEI = average daily metabolizable energy intake; BW = body weight; BWG = body weight gain; CP = crude protein; DFI = daily feed intake; DM = dry matter; EE = ether extract; FCR = feed conversion ratio; FI = feed intake; LW = live weight; MUFA = monounsaturated fatty acids; PUFA = poly unsaturated fatty acids; TIDC = true ileal digestibility coefficient

<sup>2</sup> Diet formulation by Hall *et al.* (2018) is not in accordance with the criterion of iso-nitrogenous and iso-caloric diet formulation. Therefore, only results regarding the AA digestibility were include in the table.



**Table 3. Inclusion of black soldier fly (BSF) and housefly (HF) products in laying hen diets and their effect on digestibility, performance, product quality, and animal health, as reported in studies that compare diets that are isonitrogenous and isocaloric.**

Age	Insect product	Target replacement	Insect inclusion %	Results (digestibility, performance, product quality and animal health) <sup>1</sup>	Reference
20-40 wk	partially defatted BSF larval meal	soybean meal	0, 7.3, 14.6	Lower apparent digestibility of DM and OM at 14.6% inclusion and reduced apparent CP digestibility at any inclusion level. No effect on weight gain, FI, FCR, and egg weight. Higher lay% with 7.3% inclusion of meal and higher egg mass at all inclusion levels. Higher entire intestinal length and of the jejunum as % of LW. Higher serum magnesium, potassium, chlorine, globulin and lower albumin to globulin ratios with 14.6% meal inclusion. Lower bilirubin, cholesterol, triglyceride and blood urea nitrogen levels at any inclusion level.	Bovera <i>et al.</i> , 2018
67-78 wk	live BSF larvae	soybean meal	0, 10	No effect on FI, FCR, BW, laying rate, egg weight, egg mass, mortality, and egg quality parameters. Reduction of feather damage. Higher counts of hens on the floor during the morning and lower counts in the afternoon when larvae were provided.	Star <i>et al.</i> , 2020
24-45 wk	defatted BSF larval meal	soybean meal	0, 17	Reduced albumen weight, higher proportion of yolk, and lower albumen proportion in eggs. Redder egg yolk, higher total carotenoid and $\gamma$ -tocopherol levels. Lower MUFA and higher PUFA n-6 in egg yolk.	Secci <i>et al.</i> , 2018
20-36 wk	partially defatted BSF larval meal	soybean meal	0, 7.3, 14.6	Higher egg weight, yolk weight. Higher eggshell weight with 7.3% meal inclusion. Lower eggshell % and eggshell thickness with 14.6% inclusion. Alteration of the yolk colour. Lower foaming capacity of egg albumen. Modified physical and chemical characteristics of angel cake.	Secci <i>et al.</i> , 2020
24-45 wk	defatted BSF larval meal	soybean meal	0, 17	Reduced final LW, and weight gain, FI, FRC, lay %, egg weight, and egg mass. High variation in egg size. Higher serum globulin and lower albumin to globulin ratio. Lower serum cholesterol and triglycerides. Higher serum calcium and lower chlorine levels.	Marono <i>et al.</i> , 2017
24-45 wk	defatted BSF larval meal	soybean meal	0, 17	Increased diversity of the microbial population and altered caecal microbiota in type and relative abundance of microbes. Increased concentrations of SCFA in ceca.	Borrelli <i>et al.</i> , 2017
25-45 wk	defatted BSF larval meal	soybean meal	0, 17	Lower apparent ileal digestibility of DM, OM, and CP. Lower LW. Heavier full digestive tract as % of LW, higher specific activity of duodenum maltase, lower activity of jejunum IAP, higher activity of ileal maltase and saccharase, and lower activity of IAP and $\gamma$ GT. Higher villi height in duodenum, lower villi height in jejunum and ileum. Higher crypt depth and lower villi to crypt ratio in ileum Higher volatile fatty acid production in caecum. Higher acetate, butyrate and total volatile fatty acid levels in caecum.	Cutrignelli <i>et al.</i> , 2018
18-26 wk	fresh BSF, dried BSF, BSF extract	fishmeal	0, 8, 8, 8	Inclusion of any of the BSF products increased the phagocytotic activity of peritoneal macrophages towards non-protein A <i>Staphylococcus aureus</i> .	Irawan <i>et al.</i> , 2019
20-40 wk	partially defatted BSF larval meal	soybean meal	0, 7.3, 14.6	Lower villi height, villi to crypt ratio in duodenum and jejunum at any inclusion level. Higher crypt dept in jejunum at 14.6% inclusion. No effect compared to the control group on ileal level. Lower maltase activity in duodenum at 14.6% inclusion and lower IAP in jejunum at any inclusion level. Lower $\gamma$ GT activity in ileum at 7.3% inclusion. Higher concentration of acetate, butyrate and total VTA in caecum at 14.6% inclusion. Lower concentration of valerianic in caecum at any inclusion level. Higher level of Isobutyrate and lower level of valerianic as % of total VFA in caecum at any inclusion level. Higher level of butyrate in caecum at 14.6% inclusion.	Moniello <i>et al.</i> , 2019
50-58 wk	undefined larval meal	fishmeal	0, 2.4, 4.7, 7.1, 9.4	No effect on FI, weight gain, FCR and mortality. Lower hen-day production and eggshell weight at highest level of meal inclusion. Lower shell thickness at 4.7% meal inclusion and shell weight at 9.4% meal inclusion. Other egg quality parameters unaffected.	Agunbiade <i>et al.</i> 2007
36-44 wk	HF larval meal	fishmeal	0, 0.9, 1.8, 2.7, 3.6	BW, FI, egg production, egg weight, feed efficiency, and any tested egg quality characteristics were unaffected. Egg yolk cholesterol, triglyceride, and calcium lower with inclusion of HF meal, while the concentrations of phosphorus was increased.	Akpodiete <i>et al.</i> , 1998

<sup>1</sup> BW = body weight; BWG= body weight gain; CP = crude protein; DM = dry matter; FA = fatty acid; FCR = feed conversion ratio; FI = feed intake;  $\gamma$ GT =  $\gamma$ -glutamyl transpeptidase; IAP = intestine alkaline phosphatase; LW = live weight; MUFA = monounsaturated fatty acids; OM = organic matter; PUFA = poly unsaturated fatty acids; SCFA = short chain fatty acids; VFA= volatile fatty acids.

**Table 4. Inclusion of black soldier fly (BSF) and housefly (HF) products in other poultry diets and their effect on digestibility, performance, product quality, and animal health, as reported in studies that compare diets that are isonitrogenous and isocaloric.**

Poultry species/type	Age	Insect product	Target replacement	Insect inclusion %	Results (digestibility, performance, product quality and animal health) <sup>1</sup>	Reference
Muscovy duck	3-50 d	partially defatted BSF larval meal	corn gluten meal	0, 3, 6, 9	No effect on LW, ADG, FI, and FCR. Haematological traits were unaffected. Linear reduction of serum triglycerides, cholesterol, magnesium, alkaline phosphatase, creatinine, malondialdehyde, and nitrotyrosine. Linear increase of serum iron. No effects on histopathological scores.	Gariglio <i>et al.</i> , 2019a
Japanese broiler quail	10-28 d	BSF larval meal	conventional protein/fat sources	0, 10, 10	Increased apparent digestibility of DM, OM and reduced apparent digestibility of ether extract of quails fed BSF2. Higher apparent digestibility of starch of quails fed meal of larvae reared on 100% layer mash (BSF1). Higher apparent digestibility of gross energy, higher metabolisable energy. Reduced slaughter weight and BWG of quails fed meal of larvae raised on 50:50 fish offal and layer mash (BSF2). Improved carcass and breast meat traits of quails fed BSF1. Lower carcass weight of quails fed BSF2. Lower FI of larval meals when given a choice.	Woods <i>et al.</i> , 2019
Turkey	1-35 d	live BSF larvae	soybean meal	0, 12	Lower FCR, higher FI and BWG and reduced aggressive pecking towards the back and tail-base in the final week. More feed pecking during first week, less feed pecking and drinking during the third week and less object pecking during the fifth week.	Veldkamp and van Niekerk, 2019
Japanese broiler quail	10-28 d	BSF larval meal	conventional protein/fat sources	0, 10, 10	Higher meat cooking losses when fed. Higher protein, lipid, and cholesterol levels in quail meat of birds fed BSF1. Alteration of AA content and FA profile of quail meat fed BSF1. Modified meat juiciness and fibrousness and colour of quails fed BSF2.	Cullere <i>et al.</i> , 2019b
Muscovy duck	3-50 d	partially defatted BSF larval meal	corn gluten meal	0, 3, 6, 9	Apparent digestibility of CP linearly reduced during the starter phase. Positive linear response of apparent digestibility of EE during the whole trial. Final LW, ADG, FI, and FCR was not affected. Intestinal morphology remained unaffected.	Gariglio <i>et al.</i> , 2019b
Japanese laying quail	6-13 wk	BSF larval meal	fishmeal	0, 3.18, 6.37, 9.56	Egg production was reduced at 6.37 and 9.56% BSF meal inclusion. Egg shell thickness was reduced at 9.56% of inclusion. Egg Haugh unit, egg weight, egg yolk index, and egg yolk colour was unaffected.	Suparman <i>et al.</i> , 2020
Japanese broiler quail	10-28 d	BSF larval meal	conventional protein/fat sources	0, 10, 10	Secondary response increased in BSF1. Lysozyme activity unaffected at day 27 but at day 37 activity was higher in BSF2. Bacterial activity unaffected at both timepoints. Wing web swelling index significantly higher in both BSF groups. At day 27 higher $\alpha$ 2-globulin in both BSF groups and lower $\gamma$ -globulin in BSF2 group. At day 37, $\alpha$ 2 globulin higher in both BSF groups. Other blood parameters and microbial count in caecal content remained unaffected.	Pasotto <i>et al.</i> , 2020

<sup>1</sup> AA = amino acid; ADG = average daily gain; BW = body weight; BWG = body weight gain; CP = crude protein; DM = dry matter; EE = ether extract; FA = fatty acid; FCR = feed conversion ratio; FI = feed intake; OM = organic matter.

diet formulation by Hall *et al.* (2018) is not in accordance with the above mentioned criterion (iso-nitrogenous and iso-caloric diet formulation), it was chosen to only report results regarding the AA digestibility. Significant positive effects on broiler development, also during later growth stages, have been reported when broilers were provided with up to 8% of HF larval meal in their diet (Khan *et al.*, 2016, 2018a,b).

Nonetheless, also between studies investigating the same insect species and using comparable inclusion levels, outcome parameters can be highly variable. For instance, broilers fed diets containing in total 4 and 8% of HF larval meal showed, in contrast to prior findings, reduced ADG during the starter phase, which resulted in higher feed conversion ratios (FCR). Darker colour of HF larval meal is assumed to be responsible for this adverse development

(Elahi *et al.*, 2019). However, it is also worth noticing that the crude fat (CF) content of HF meal used within these studies varied tremendously, with values of 5.6 (Elahi *et al.*, 2019) and 27.9% (Khan *et al.*, 2016). Varying levels of CF in insect products may be responsible for differences in performance of broilers (Elwert *et al.*, 2010). This condition furthermore hampers the comparability of results between studies, because the insect products have different nutritional and likely also bioactive values.

### Product quality

Besides affecting broiler performance, inclusion of partly defatted BSF larvae improved carcass traits, such as breast yield (Schivone *et al.*, 2019). Also the use of HF larval meal beneficially affected broiler dressing percentage (Khan *et al.*, 2018a), while olfactory properties of the meat remained unaffected (Khan *et al.*, 2018a,b) or turned out even more favourable for consumers (Khan *et al.*, 2016; Radulović *et al.*, 2018). In some cases the analysed carcass characteristics were not significantly affected (Elahi *et al.*, 2019). It can be concluded that low levels of inclusion of these insect meals in broiler diets do not negatively influence carcass traits compared to standard feed. A closer analysis of the fatty acid (FA) composition of broiler meat revealed that partly defatted BSF larval meal increased the content of medium unsaturated FA (MUFA) and reduced the occurrence of poly unsaturated FA (PUFA). Since the FA profile of broiler meat is dependent on the feed source (Schivone *et al.*, 2019) and larval FA composition is dependent on the substrate on which the larvae were reared (Cullere *et al.*, 2019b), the unfavourable development might be adjusted by the choice of larval substrate (Schivone *et al.*, 2019). Also, the killing method might have an influence on nutritional characteristics of the insect end product (Larouche, 2019).

### Health and welfare

Inclusion of 2, 4, and 6% of BSF larval meal in broiler diets reduced their blood albumin levels, while 8% inclusion resulted in higher concentrations. Also triglyceride levels were affected resulting in higher values for 6 and 8% insect meal inclusion, while uric acid was affected at any inclusion level, indicating efficient utilisation of protein from the diet. However, protein digestibility was adversely influenced at any BSF meal inclusion level lower than 8% (Attivi *et al.*, 2020). Also the development of intestinal organs was affected by the treatment. Higher inclusion levels reduced the gizzard weight and intestinal length (Attivi *et al.*, 2020). On the other hand, increased gizzard weight was reported when broilers were fed HF larval meal. The reason for this development remains unknown (Okah and Onwujariri, 2012). Other studies report, in contrast to the expected positive effects of bioactive compounds in insect products on broiler health parameters, that immune organs in broilers were not altered by the inclusion of larval meal of either of

the two fly species. Moreover, haematological parameters were within the common physiological range (Dabbou *et al.*, 2018; Elahi *et al.*, 2019). Nonetheless, albumin to globulin ratios were not measured in the studies cited. Because in laying hens these parameters showed significant improvements (Bovera *et al.*, 2018; Marono *et al.*, 2017), it would be an interesting addition to test the effects of BSF and HF products in broiler feed on those parameters as well. Strong positive effects on foraging and general activity behaviour have been found when providing broilers with live BSF larvae. Most pronounced were the effects when the larvae were included to cover 10% of the DM intake and the provision was spread over 4 feeding moments a day. These behavioural changes likely also reduced the incidence of hock burns and improved gait score (Ipema *et al.*, 2020).

### Laying hens

#### Production performance

Including 10% live BSF larvae, up to 9.4% undefined larval meal and up to 14.6% of partially defatted BSF larval meal in layer nutrition showed no change in FI, weight gain and FCR (Agunbiade *et al.*, 2007; Bovera *et al.*, 2018; Star *et al.*, 2020). Moreover, egg weight remained either unaffected (Secci *et al.*, 2018) or increased by administration of BSF meal in layer diets (Secci *et al.*, 2020). However, because higher egg weight was accompanied by lower eggshell thickness, this might impact the fragility of the eggs (Secci *et al.*, 2020). Likewise administration of 9.4% undefined larval meal reduced the eggshell weight, but also hen egg production (Agunbiade *et al.*, 2007). Furthermore, performance parameters were reduced when higher inclusion levels of defatted BSF larval meal were administered (Marono *et al.*, 2017), which can be explained by either feed colour or chitin levels. Chickens are sensitive to feed colour (Heshmatollah, 2007). Hence, feed colouration may play a role at higher levels of insect meal inclusion, because BSF meal is darker than SBM (Bovera *et al.*, 2018). On the other hand, inclusion of up to 3.6% HF larval meal did not affect laying hen performance (Akpodiete *et al.*, 1998).

#### Product quality

In terms of consumer health, the binding capacity of chitin to nutrients can have favourable effects on end products for humans by reducing serum triglyceride and cholesterol (Bovera *et al.*, 2018; Marono *et al.*, 2017) and therefore egg cholesterol levels (Secci *et al.*, 2018). The same results were found when HF larval meal was administered (Akpodiete *et al.*, 1998). Further egg quality parameters influenced by the inclusion of BSF meal were the alteration of the yolk colour (Secci *et al.*, 2018, 2020), higher proportions of the yolk, lower albumen proportions in the egg, and less egg shell thickness (Secci *et al.*, 2020). The latter might be caused due to the higher egg weight, since calcium resources for

shell production are limited. In contrast laying hens fed undefined larval meal showed lower egg shell weight at the highest inclusion level (Agunbiade *et al.*, 2007).

### Health and welfare

The production of short-chain fatty acids (SCFA) in the cecum proved to have a positive effect when defatted BSF larval meal was included in the laying hen diet (Borrelli *et al.*, 2017; Cutrignelli *et al.*, 2018). The assumed mechanism is that chitin serves as a fermentation source for caecal microbiota, which leads to the production of SCFA (Borrelli *et al.*, 2017; Cutrignelli *et al.*, 2018). SCFA such as butyric acid serve as an energy source for epithelial cells in the colon and promote mucosal growth. Additionally, SCFA inhibit the growth of acid-intolerant pathogenic bacteria such as *C. perfringens*, *E. coli* and *Salmonella* (Borrelli *et al.*, 2017; Elnesr *et al.*, 2020). However, whether insect products indeed reduce the prevalence of these bacteria requires further investigation.

The better health status of laying hens has been confirmed by serum analysis. Elevated globulin levels and lower albumin to globulin ratios indicate higher resistance and improved immune responses in layers fed partially defatted or defatted BSF meal (Bovera *et al.*, 2018; Marono *et al.*, 2017). However, the inclusion of 7.6% of partially defatted BSF meal did not alter these immune parameters. Therefore, low levels of insect meal may be insufficient to induce immunological changes at the serum level (Bovera *et al.*, 2018). Besides administration of various BSF products showed that those larval products have the potential to increase the phagocytic activity of macrophages, potentially improving the innate immunity (Irawan *et al.*, 2019). Further promotion of laying hen welfare and health may be achieved by the provision of live larvae instead of larval meal. Insects are part their natural diet and may enable laying hens to express natural behaviour. Feather pecking is a well-known issue in layer flocks. Due to the provision of live larvae as a natural pecking substrate, feather conditions of laying hens were successfully improved. This achievement was likely caused by laying hens spending more time pecking on larvae rather than their pen companions. Besides pecking and ingestion of larvae might be rewarding for laying hens, thus satisfying their need to peck (Star *et al.*, 2020).

In addition to the beneficial prebiotic properties, it was also found that chitin can hinder the digestion of various nutrients (such as protein) by chitin-protein matrixes (Bovera *et al.*, 2018). This is supported by profound reduction of the apparent ileal digestibility of protein, but also organic matter, due to administration of defatted BSF larval meal (Cutrignelli *et al.*, 2018). Also morphological changes such as lower duodenal villi height and lower enzymatic activity are a result of the lower digestibility of the feed (Moniello *et al.*, 2019). Thus, to successfully

include insect products in layer diets it seems crucial to determine optimal inclusion levels.

In conclusion, inclusion of BSF and HF larvae in feed improves the performance, product quality, health, and welfare of layer hens dependent on the inclusion level. Furthermore, provision levels for optimal development of hen performance and health do not necessarily coincide. Also storability of insect products may play a role in the future regarding the choice of larval product. It should be considered that processing of live insects can improve its storage properties (Veldkamp *et al.*, 2012). Therefore, insect meals are storable for longer periods than live insects and further defatting can reduce the risk of lipid oxidation (Schiavone *et al.*, 2017).

### Other poultry

#### Production performance

BSF larvae have also been successfully incorporated in diets of other poultry species. The performance of Muscovy ducks remained unaffected by 9% inclusion of partially defatted BSF meal (Gariglio *et al.*, 2019a). Likewise, performance parameters of Japanese quails remained unchanged when fed similar levels of BSF larval meal, of larvae that had been reared on layer mash. In contrast, meal of larvae raised on 50:50 fish offal and layer mash adversely affected quail performance, likely due to the odour and palatability of the meal (Woods *et al.*, 2019). Turkeys showed higher FI and ADG, and lower FCR when they were provided with live BSF larvae (Veldkamp and van Niekerk, 2019).

#### Product quality

As reported in broilers (Schiavone *et al.*, 2019), the administration of larval meal increased MUFA and decreased PUFA in quail meat (Cullere *et al.*, 2019b). Furthermore, provision of BSF meal improved carcass and breast meat traits of quails fed meal, of larvae reared on layer mash, while meal of larvae raised on 50:50 fish offal and layer mash lowered the carcass weight of quails. In layer quails BSF meal provision affected egg production at levels higher than 3.18%, this might have occurred due to lower palatability or darker colour of the feed. Moreover, egg shell thickness was negatively influenced at the highest level of BSF meal inclusion, likely caused by the shifted calcium and phosphorus levels in BSF meal compared to fishmeal (Suparman *et al.*, 2020).

#### Health and welfare

In Japanese broiler quails, administration of BSF larval meal had a significant effect on health-related blood parameters. The wing web swelling index, an indicator for the immune system's lymphoproliferative capacity, was improved in BSF-

fed groups. Also  $\alpha$ 2-globulin levels measured at day 27 and 37 were increased in the blood. Albumin-to-globulin ratio was unaffected. However, the results indicate a tendency of BSF-meal-fed groups to have lower values. Besides, the blood profile of quails was affected dependent on the prior rearing substrate of the BSF larvae. Broilers fed BSF larval meal of larvae reared on layer mash showed a higher secondary response, while quails fed larval meal of larvae reared on 50:50 layer mash and fish offal had lower  $\gamma$ -globulin levels at day 27 and higher lysozyme activity at day 37 compared to the control group. While blood parameters were altered, the treatments recorded no alteration of the microbial composition within the caeca. The results emphasise the immunomodulatory properties of BSF larval meal in quails (Pasotto *et al.*, 2020). Furthermore, in Muscovy ducks BSF meal had only minor effects on the digestibility of nutrients. For instance, crude protein (CP) digestibility was only reduced within the starter phase, likely due to the chitin content of the insect meal. However, with increasing age, birds seemed to be able to adapt to the feed source (Gariglio *et al.*, 2019a). The health status of the ducks was unaffected, showing no effects on haematological traits (Gariglio *et al.*, 2019b). Also in Japanese quails digestibility parameters were affected by the diet (Woods *et al.*, 2019). When quails were given a choice between a diet containing insect meal and a control diet, birds preferred to consume the control feed. Feed colour might have played a role in this because BSF larval meal had a darker colour than SBM. Hence, these results emphasise once more the importance of appropriate feed properties when presented to poultry and highlight the power to modulate those characteristics through the choice of larval substrate (Woods *et al.*, 2019). In this regard it is important to distinguish between the attractiveness of different insect products such as live insects and insect meal. Insects are a natural feed source for poultry. Visual cues and intrinsic mechanisms trigger poultry to eagerly ingest larvae. It was reported that turkeys consumed live larvae completely within two minutes after they were provided. In future studies this eagerness of consuming live larvae might be used to improve poultry welfare by stimulating natural behaviour (Veldkamp and van Niekerk, 2019). It is, therefore, important to investigate and distinguish the effect of different insect products on poultry production and behaviour.

## 6. Future prospects of insects in poultry feed

Insects have a good potential to be used in animal feed. Nonetheless, some hurdles need to be overcome. Especially in terms of legislation, new regulations need to be adopted to enable insect protein to be used in commercial poultry farming. Although currently only the provision of live insects is allowed in the EU, new legislation is expected within 2020 (IPIFF, 2018).

Feed and food safety are of fundamental importance when considering insects as feed. Major concerns are addressed regarding the provision of waste materials as larval substrate as a potential entrance route of chemical and biological contaminants into the food chain (Van Huis, 2020). At present, feeding of farm animals is merely permitted using vegetable materials, apart from a few exceptions (IPIFF, 2019). To maintain sustainable production and to obviate competition with human food and animal feed production new innovative ideas are required. Latest approaches identify the valorising of plant waste streams from industrial sectors for insect rearing, for instance by recycling brewery by-products (Bava *et al.*, 2019; Jucker *et al.*, 2019; Miranda *et al.*, 2020; Ocio *et al.*, 1979). Moreover, as reported for BSF, insects may add additional value to crop products, by metabolising undesired chemical contaminants such as mycotoxins. Mycotoxins are a major feed hazard because contaminated products are no longer suitable for feeding animals (Camenzuli *et al.*, 2018). Investigations focussing on the fate of mycotoxins in insects and effects on larvae would be especially important to evaluate the suitability of the larvae for animal feed.

Recently, questions arose regarding the welfare of insects in mass production systems. In the past, insects have been considered non-sentient beings, unable to experience emotions (Van Huis, 2019). Responsible insect farming and feeding, however, includes ethical considerations. Studying insect welfare is a rather new field. While literature focussing on insect performance is available (Miranda *et al.*, 2020; Schmitt *et al.*, 2019), information considering insect welfare is scarce (but see Boppré and Vane-Wright, 2019). Also the effects of rearing condition on insect health are rarely investigated. To provide insect breeding and rearing companies with the tools to assess well-being and insect health and to develop appropriate killing methods in the future it is important to initiate studies on these aspects.

The use of plant waste products for insect rearing further promotes the establishment of a circular economy (Dicke, 2018). Various designs for business models have been discussed, including implementations on-farm to benefit primarily smallholder farmers in low and middle income countries (Chia *et al.*, 2019) and large-scale farming in Europe (Veldkamp *et al.*, 2012). Multiple options arise, benefitting different stages of the insect chain. Nonetheless, probably the most powerful factor determining the success of insects in feed is societal acceptance. Insect products are rather new to the western society. However, initial consumer studies turned out positive. While consumer attitudes for insect products as direct part of their diet are still rather low, consumers are especially willing to accept insects in animal feed (Onwezen *et al.*, 2019; Verbeke *et al.*, 2015). Consequently, the inclusion of insects in feed might pave the way for faster acceptance in human food.

To support the inclusion of insects as feed, it is important to perform research into consumer acceptance and evaluate the economic robustness of using insects in feed considering the entire value chain from production to consumption.

## 7. Conclusions

The poultry sector is one of the most important providers of animal protein to humans. To satisfy future demands the sector is in transition towards more sustainable production. The main problems facing the industry are the need for sustainable feed protein sources and higher animal welfare, while maintaining economic viability. Both goals can potentially be met by using insects in poultry diets.

The use of insects in poultry feed is still subject to several uncertainties regarding legislation, substrate choice, ethical considerations and consumer attitude. On the other hand, insects have the potential to greatly contribute to the sustainable development goals (Barragán-Fonseca *et al.*, 2020; Dicke, 2018). For instance, the use of insects in feed contributes to food security, circular agriculture, health, economic efficiency, and animal well-being, health and nutritional satisfaction. Because the insects-as-feed sector is still in its infancy more research is required. The current state of knowledge indicates that partial replacement of fishmeal or soybean meal by fly larvae in feed is beneficial for poultry and therefore these insects can be used as a valuable replacement for conventional protein sources. In this regard, one of the rather undiscovered fields is the use of live fly larvae for welfare enhancement. To contribute to a stable sector there is a need for standardised procedures for insect production and processing, clear distinction between insect products based on their nutritional composition, determination of optimal inclusion levels for insect products per poultry species, and production aim and the direct comparison of products from different insect species.

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## Conflict of interest

The authors declare no conflict of interest.

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