Larval meal of the black soldier fly *(Hermetia illucens)* is suitable for feeding whiteleg shrimps *(Penaeus vannamei)* under practical conditions in a modern closed-loop system

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Abstract

Numerous studies have already demonstrated the suitability of protein meal from black soldier fly larvae *(Hermetia illucens)* as a substitute for fish or soybean meal in feed for multiple livestock species, including various fish species as well as poultry and pigs. However, this has not yet been extensively researched with regard to the Pacific white shrimp *(Penaeus vannamei)*. The aim of these studies was therefore to test under practical conditions the suitability of black soldier fly protein meal as a protein component for *P. vannamei.*

In four consecutive runs, an experimental feed containing 10% soldier fly meal and a control feed were tested under practical conditions in a recirculation system. In order to compare the production performance of both feeds, growth, feed conversion and mortality were compared. Performance was generally high, yet compared to the control group, insect meal tended to have positive effects on important parameters, or even had significant positive effects.

This work shows that black soldier fly larvae meal can be used at the tested proportion of 10% under practical conditions in recirculation systems without compromising production performance.

Citation
Stadtlander T, Deininger K, Sandrock C, Schindler F, Wecker B, Wohlfahrt J, Lemme A, Lambertz C: Larval meal of the black soldier fly *(Hermetia illucens)* is suitable for feeding whiteleg shrimp *(Penaeus vannamei)* under practical conditions in a modern closed-loop system. Ernahrungs Umschau 2025; 72(8): 146–54.

Open access
This article is available online: DOI: 10.4455/eu.2025.034

Peer reviewed
Manuscript (original) submitted: 4 September 2024; revision accepted: 17 February 2025

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Introduction

Livestock farming is increasingly criticized due to its high consumption of resources. This is primarily due to the necessary cultivation of feed, the large-scale use of fertilizers, and the resulting greenhouse gas emissions (carbon dioxide, methane, nitrous oxide). In the case of monogastric animals, especially pigs and poultry, the use of soybean meal as a protein component is at the center of criticism. In aquaculture, the main concern is the increasing demand for fishmeal and fish oil production and the associated environmental effects [1].
Insect meal, produced from the larval stages of various insect species, could contribute to improve the sustainability in animal production [2, 3]. In nature, insect larvae often feed on decaying organic material, and this provides the potential to use residual flows from food and feed production as substrate for the insect larvae. By using these substrates, the nutrients they contain can be transformed by the insect larvae into valuable feed (upcycling) [2, 4]. Globally, around one third of all food is thrown away (food waste) or lost during production (food loss) [5].
Recirculation of these nutrients can increase sustainability. Due to insufficient data and the precautionary principle, the EU currently only allows substrates for insects fulfilling feed quality standards and may therefore be used directly in feed for livestock or aquaculture (Regulation (EU) 2017/893 of May 24, 2017; Regulation (EU) 2021/1372 of August 17, 2021). Potentially problematic substrates, such as food waste from restaurants or commercial kitchens (post-consumer), which may also contain meat or fish, are not permitted. Similarly, excrements from animal husbandry (e.g. from poultry, pigs, or cattle) may not be used to feed insects.
The insect species approved in the EU and Switzerland differ depending on their intended use as feed or food. Accordingly, protein meals from the following insect species are currently approved in the EU as feed for aquaculture (fish and shrimp), poultry, and pigs: black soldier fly *(Hermetia illucens)*, housefly *(Musca domestica)*, yellow mealworm or mealworm beetle *(Tenebrio molitor)*, lesser mealworm *(Alphitobius diaperinus)*, house cricket *(Acheta domestica)*, Tropical house cricket *(Gryllodes sigillatus)*, Jamaican field cricket *(Gryllus assimilis)*, migratory locust *(Locusta migratoria)*, and silkworm *(Bombyx mori)*.
Internationally, the black soldier fly has established as the most widely and intensively produced insect species for livestock feed [6]. There are various reasons for this, including the fact that this species grows and develops faster than many other insect larvae and therefore has significantly shorter production cycles. In addition, the larvae can utilize a much wider range of organic materials very efficiently than, for example, mealworm larvae.
Black soldier fly larvae (BSFL) and BSFL protein meal are well established in aquaculture. The focus has been particularly on salmonids (salmon and trout) [7–9]. Other fish species on which BSFL meal has been tested as a protein component include turbot [10], tilapia [11], European perch [12, 13], and zander [14]. More recently, BSFL meal has also been tested for whiteleg shrimp under laboratory conditions [15–18], but not yet under practical conditions in a commercially operated shrimp farm.
Commercial insect meals produced for animal feed are generally rich in chitin [19]. Chitin is a complex polysaccharide that is difficult or impossible for many animals to digest. Aquatic freshwater and marine species in particular, whose natural diet includes insects, insect larvae, or zooplankton (especially small crustaceans such as daphnia or copepods), are better able to digest chitin than most terrestrial farm animals [20, 21]. Flagellate shrimp, which include the Pacific whiteleg shrimp *(Penaeus vannamei)*, feed on large amounts of zooplankton, but also on their own exuviae (shed animal skin) after molting. Whiteleg shrimp therefore exhibit increased chitinase activity, meaning they can utilize chitin better than animal species whose diet does not contain large amounts of insects or zooplankton [21, 22]. In addition to chitin, the amino acid composition of insect meals also determines their nutritional value. For example, a comparison of fish meals with comparable protein content shows lower lysine content than BSFL meals. The sulfur-containing amino acids are also lower, while the branched-chain amino acids isoleucine, valine, and leucine are more concentrated [23]. In addition, the protein content and amino acid profile of BSFL meals can vary considerably [24, 25].

Research question

The project "Sustainable and resilient cultivation of insects for innovative use in feed and food production" (reKultI4Food) was carried out as part of the NewFoodSystems innovation space [26]. In three work packages, important issues regarding the production and utilization of BSFL meal were addressed for the efficient production and use of the black soldier fly.
In the study reported here, insect meal was tested as a protein component for Pacific whiteleg shrimp *(P. vannamei)* under practical conditions in a commercially operated closed-loop system, in four replicated complete production cycles. While the nutritional suitability of BSFL meal for various edible fish, especially salmonids (salmon and trout), tilapia, carp, and perch, poultry, and pigs has been widely studied, there have been relatively few studies on the suitability and recommended maximum inclusion levels of BSFL meal in feed for *P. vannamei*, and none for shrimp production in recirculating aquaculture systems. The production of *P. vannamei* is a niche production in Europe, but it can provide high-quality and high-priced seafood regionally, thus avoiding long distance transports and energy-intensive cooling chains.

The following article examines how well a commercially extruded experimental feed containing 10% BSFL meal as a protein component for *P. vannamei* performs compared to a standard or control feed based on fish meal, produced under the same conditions.

Methods

Integrating feeding trials into ongoing production operations and conducting them with the necessary scientific accuracy always presents a certain challenge. The shrimp farm Oceanloop Munich (known as "CrustaNova" at the start of the project) produces Pacific whiteleg shrimp *(P. vannamei)*, the most widely produced shrimp in the world in terms of volume, in a modern closed-loop system [27]. The water in the system circulates continuously and is purified by a water treatment system consisting of a drum filter, skimming, biofilter, denitrification reactor, and degassing, enabling a water exchange rate of only 1–3% per day. In addition, there are other input systems for technical oxygen, buffer solutions, and heat to optimally adjust the water to the needs of the shrimp. The oxygen content in the fattening tanks is 5–7 mg/L, the pH value is 7.4–8.3, and the temperature is 29–31 °C. Feeding takes place hourly via automatic feeders and is based on a pre-defined feeding curve. The amount of feed is adjusted daily as needed to avoid feed residues in the tanks. Generally, commercial facilities are not designed to conduct many experimental replicates at the same time. However, the facility has sufficient capacity to feed control and experimental feed in parallel. Therefore, four temporal replicates were carried out. In the experimental feed mixtures, 10% of the fish meal was replaced by BSFL meal without making any further adjustments to the nutrient profile. The composition of the control feed mixture is shown in ◆ Table 1. In the experimental feed, the fish meal still had a reduced proportion of 11% (110 g/kg) after being partially replaced with BSFL meal. In addition, the krill meal content was increased to compensate for the difference in protein content between fish meal and BSFL meal.
Although the crude protein content remained approximately the same, the experimental feeds contained, for example, 0.10–0.13 percentage points less methionine + cysteine, 0.07–0.08 percentage points lower threonine, 0.11–0.14 percentage points lower arginine, 0.05–0.07 percentage points lower valine + isoleucine, and 0.19 percentage points lower leucine content, while lysine was only marginally affected. Among the essential amino acids, only the tyrosine content was 0.07% higher. These effects can mainly be explained by the fact that a higher proportion of the crude protein in the BSFL feed is due to chitin in the larval meal, while the amino acid concentration is lower. Both feeds were extruded in two different pellet sizes (1.6 and 2.2 mm diameter) to account for the increasing size of the shrimps.
After their quarantine period, 12,500 shrimp (post-larvae aged 10 days) of the same size and weight (average of 3.74 mg across all trials) were placed in the respective compartments. The normal standard feed was used as the control feed and the experimental feed with the same nutritional composition was used as the test feed. The feed amount was 5.9% of the biomass at the start of the trials and 3.8% of the biomass at the end. The feeding period (fattening days) was based on the size of the shrimps and the economic circumstances. At the end of the fattening period, both compartments were fished in parallel so that the number of fattening days was identical between control and test diet.

The nutritional values for the two pellet sizes (1.6 and 2.2 mm in diameter) are shown in ◆ Table 2. The nutritional values differ slightly between the two pellet sizes, but there were no differences between the control and test feed.

At the beginning of each replication, approximately 200 g of shrimp were caught and frozen as an initial sample for later amino acid determination. The average fattening period per cycle was 97 ± 8.5 days, ranging from 83 to 105 days. At regular intervals (every 1–2 weeks), random samples from each experimental group were weighed and the number of animals counted to document growth. At the end of each replication, 1 kg of shrimp were caught from the control and experimental tanks and frozen for later amino acid determination. At the end of the experiment, all shrimp from the initial, control, and experimental groups were freeze-dried and ground. The amino acid compositions of all control and experimental feeds in both pellet sizes were then determined in duplicate, and all sampled shrimp (initial, control,

Component[a]	Control feed [g/kg]
wheat middlings[b]	320
fishmeal	280
triticale	50
rapeseed meal	50
sunflower meal	50
krill meal	50
wheat germs	50
rapeseed	40
lime	35
fish oil	25
lecithin	5

Tab. 1: **Composition of the control diets**
[a] Vitamin premix and special components are not listed; BSFL feed contained 110 g/kg fishmeal and 100 g/kg BSFL meal.
[b] co-product from the production of meal from cleaned wheat

and experimental groups) were determined in four replicates at Evonik in Essen.

The data collected (growth, feed conversion, and mortality) was used to calculate productivity indicators and thus the performance of the two feeds. These included the following indicators:

Growth

In regular weighings, which were not carried out at the same times in the four replicates, between 3 and 126 animals (average 23 animals) were weighed per weighing. The number of weighed animals was higher at the beginning and smaller at the end of the fattening period. Animals were weighed between 3 and 6 times in each of the four replications. The animals were caught and weighed as a group. The individual shrimps per group were counted to calculate the average individual body weight. Since the shrimp from all four replications were not only weighed on different days, but also had different durations of replication, the specific growth rate (SGR, %/day) and average daily gain (g/day) are used to compare growth development, and the weighing data of both feeding treatments are compared in a mixed statistical model:

$$\text{SGR: } \frac{\ln \textit{final weight } (g) - \ln \textit{initial weight } (g)}{\textit{Days of fattening}} \times 100$$

Feed conversion

The feed conversion ratio was averaged for each treatment (control and BSFL) over the four replications. It was calculated based on total feed consumption (in g) and total biomass growth or final harvest of the control-fed shrimp and BSFL-fed shrimp.

Mortality

To calculate mortality, the number of shrimp stocked (determined using average weights from sample counts and the total weight of stocked animals or post larvae) and harvested (determined using total harvested biomass and sample weighings to determine the average individual weight) or the reduction in animal numbers from start to finish (number of animals at end/number of animals at start * 100) were used.

	Diameter: 1.6 mm	Diameter: 2.2 mm
crude protein (g/kg)	400	360
crude fat (g/kg)	100	95
crude ash (g/kg)	103	100
crude fibre (g/kg)	25	30
gross energy (MJ/kg)	19.3	18.0

Tab. 2: **Nutritional composition for both pellet diameters (1.6 and 2.2 mm) of the control and BSFL feed**
BSFL: black soldier fly larvae

Statistics

The growth curve of the two feeding treatments over time was examined using successive model simplifications based on a linear mixed model that included the interaction between the feeding group and a nonlinear function for modeling weight over time (natural splines) as fixed effects, as well as the successive trial repetitions as a random factor. A comparative analysis was performed to determine the extent to which the complex or simplified models better explained the data. Statistical comparisons based on mixed models were applied for the target variables harvest weight, feed conversion, specific growth rate, mortality, and protein content of the shrimp, with the feeding group considered as a fixed effect and the trial repetitions as a random factor. All model diagnostics were visually checked. The proportions of individual amino acids in the protein content were examined both between feeding groups and in relation to the composition of the shrimp initially used, using one-way analysis of variance and subsequent post-hoc comparison.

Results

The comparative model analysis of growth showed that the effect of the feeding group (control vs. BSFL) was negligible (both interactive and additive) and that the temporal function alone explained the data sufficiently and significantly compared to a zero model (◆ Figure 1). No effect of feeding on mortality was found across the four runs ($p = 0.229$). While there was only a trend in favor of the BSFL-fed group for feed conversion ($p = 0.109$), a significantly positive effect ($p = 0.036$) was found for the specific growth rate per day. The harvest weights of the BSFL-fed shrimp were significantly higher than those of the control group ($p = 0.003$), but the protein contents of the harvested shrimp from both groups did not differ ($p = 0.153$). The corresponding data are shown in ◆ Table 3. The total fattening period ranged from 83 to 105 days, with an average of 97.8 days. The initial weight is identical for both groups, as they were placed in the tanks on the same day and were therefore of equal weight. The fattening period is also identical for both groups, as they were harvested on the same day.

The amino acid content in the BSFL feed mixtures was lower than in the control feed (◆ Table 4). The amino acid profiles of the harvested shrimp showed that many BSFL-fed animals had higher amino acid content in the dry matter. At the end of the fattening period, the lysine, arginine, leucine, and glycine contents of BSFL-fed shrimp were significantly ($p < 0.05$) higher than those of the initial animals and the control-fed animals. For tyrosine, both the control and BSFL-fed animals had significantly higher values than the initial animals (◆ Table 5).

Parameter	Control	BSFL
individual final weight (g)	22.4 ± 4.4	24.0 ± 4.2
mortality (%)	53 ± 20	51 ± 19
feed conversion ratio (g feed/g weight gain)	1.56 ± 0.15	1.52 ± 0.18
SGR (%/Tag)	9.16 ± 0.25	9.24 ± 0.25
weight gain (g/Tag)	0.23 ± 0.029	0.25 ± 0.026

Tab. 3: **Overview of important performance parameters**
The individual final weight is presented as range and due to the different durations of replications and ages at harvest averages are not shown. Mortality, feed conversion ratio and weight gain are presented as mean ± standard deviation (N = 4).
BSFL: black soldier fly larvae; SGR: specific growth rate

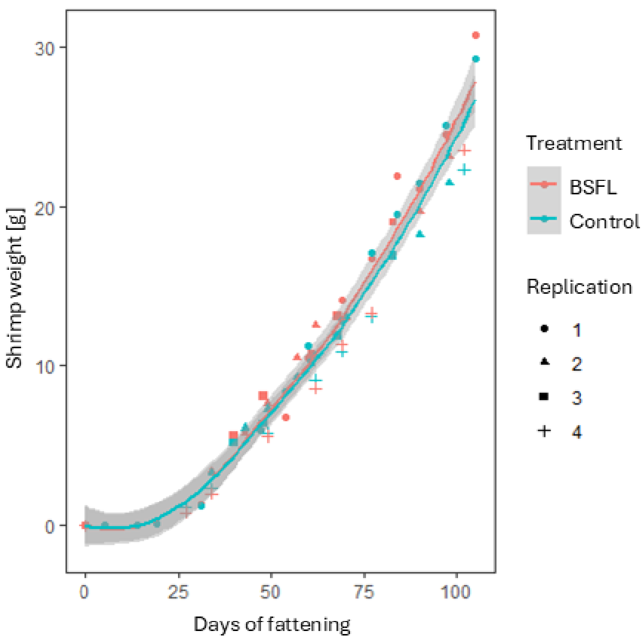

Fig. 1: **Growth development of the BSFL-fed (red) and control-fed (green) shrimps over time in the four replications (symbols)** (own diagram)
BSFL: black soldier fly larvae

Discussion

The overall production performance did not differ between the two groups (mortality, feed conversion ratio) or was improved in the BSFL group (growth). Compared to previously published results on feeding *P. vannamei* from standardized feeding trials [15–18], the results of this field trial are similar.

Mortality was very similar in both groups, but on average tended to be at the upper end of the mortality rates reported in published studies. Survival rates (100% – survival rate = mortality) of 86.7–95.6% were reported by Cummins et al. [15], while Chen et al. [16] observed even higher survival rates of over 95%. Only He and colleagues [18] reported mortality rates (26.7–62.7%) similar to those observed in this study. In the aforementioned study, the survival rates in the control and in a group with a low proportion of BSFL in the feed (25%) were significantly better compared to 100% fish meal replacement with BSFL, where only 26.7% of the shrimp survived [18]. However, it is important to consider the fattening period in the present study. None of the above studies covered the entire fattening cycle from stocking shrimp to harvesting marketable shrimp, but lasted only between 4 [17] and 9 weeks [15]. Considering the mortalities in the present study under practical conditions, they can be assessed as good, even though an increased mortality rate was observed in one of the replications. No difference was found between the control and the BSFL feed. The specific growth rate (SGR, %/day) is very high across the entire experiment, regardless of the group or individual replications (control: 9.16 ± 0.25%/day; BSFL: 9.24 ± 0.25%/day). Compared to other studies, it is significantly above the respective published ranges (3.06–4.07%/day [14]; 4.15–4.34%/day [16]; 6.01–7.53%/day [17]) and can therefore be rated as very good, especially when considered over the entire production period. This can be explained primarily by the genetics used in the present study, which are characterized by very high growth potential. In Richardson et al. [18], SGR even increased significantly with increasing proportions of BSFL meal, and no plateau with decreasing growth values was observed, as was the case in the other studies, which showed clear negative effects when the proportion of BSFL meal in the feed was too high or when 100% BSF

	Control (1,6 mm)	Control (2,2 mm)	BSFL (1,6 mm)	BSFL (2,2 mm)
crude protein	39.7	39.2	38.9	38.3
methionine	1.05	1.04	1.01	0.97
cysteine	0.54	0.54	0.49	0.48
methionine + cysteine	1.59	1.58	1.49	1.45
lysine	2.26	2.26	2.29	2.23
threonine	1.51	1.50	1.45	1.42
tryptophan	0.45	0.45	0.46	0.46
arginine	2.22	2.26	2.13	2.11
isoleucine	1.51	1.51	1.47	1.44
leucine	2.70	2.67	2.52	2.47
valine	1.92	1.90	1.88	1.84
histidine	1.05	1.05	1.02	0.99
phenylalanine	1.56	1.56	1.47	1.46
tyrosine	1.14	1.16	1.21	1.22
glycine	2.28	2.23	2.14	2.07
serine	1.65	1.64	1.52	1.51
proline	2.01	1.98	1.93	1.93
alanine	2.10	2.06	2.07	2.02
asparagine	3.13	3.19	3.04	3.02
glutamine	5.57	5.67	5.37	5.36

Tab. 4: **Crude protein content and amino acid profile of the control and BSFL feed mixtures** for the two pellet diameters 1.6 mm and 2.2 mm (values in %)
BSFL: black soldier fly larvae

larvae were fed [18]. It should be noted here that the larvae were fed fresh and not in extruded form as in the present experiment.

Similarly, both groups showed very similar feed conversion ratios, with 1.56 g of feed per g of growth in the control group and 1.52 g of feed per g of growth in the BSFL test group. This is slightly below the global feed conversion ratio (FCR) of 1.6 predicted for shrimp in 2020 [28]. However, compared to studies using BSFL meal in *P. vannamei* feed, the FCR is significantly lower than the FCR reported by Cummins et al. [15], which ranged from 2.01 (7% BSFL meal) to 4.51 (36% BSFL meal). Similar, but slightly higher FCR compared to our study were achieved by Chen et al. [16] and ranged from 1.55 with 20% fish meal protein replacement by BSFL protein to 1.7 with 30% fish meal protein replacement by BSFL protein. Slightly lower FCRs are reported by Richardson et al. [17]. These decreased with increasing BSFL content in the feed from 1.42 to 1.23 and were, thus, significantly lower than the control (1.70) [17].

Low feed conversion has a direct impact on sustainability, both financial and environmental. Reducing FCR from 1.6 to 1.2 directly translates into a 25% reduction in feed costs at the same feed price and also a direct reduction in nutrient emissions (e.g., nitrogen and phosphorus). This, however, is also influenced by the digestibility of the feed and nutrient retention in the animal. In general, animals at lower trophic levels (e.g., herbivorous animals) are considered to be more financially and ecologically sustainable and stable to produce, but not necessarily more profitable than animals at higher trophic levels (e.g., carnivorous animals) [29]. Pacific whiteleg shrimp, with a relatively low trophic level [29], are more sustainable in production. On the other hand, FCR are higher for shrimp than for fish due to the way shrimp feed. Fish usually swallow pellets as whole, and any mechanical breakdown takes place in the throat or often pellets are not at all broken. Shrimps, on the other hand, "nibble" at the feed pellets, which leads to so-called "sloppy feeding". Depending on the pellet stability and the degree of grinding of the feed components (the finer the pellets, the more stable they are), this can lead to particle formation and corresponding abrasion (◆ Figure 2). Poor mechanical pellet properties therefore not only increase feed costs due to lower feed intake and higher abrasion but also increase the need to treat the water in the recirculation system with drum filters and biofilters. The pellet properties were not examined in the experiment, but observations at the tanks suggest that there were no fundamental differences between the two feed variants.

	Initial	Control	BSFL
crude protein	71.9 ±0.91	75.7 ± 2.63	77.55 ±2.32
methionine	1.58 ± 0.04	1.53 ± 0.04	1.68 ± 0.10
cysteine	0.79 ± 0.02	0.76 ± 0.02	0.83 ± 0.05
methionine + cysteine	2.36 ± 0.06	2.30 ± 0.06	2.51 ± 0.15
lysine	4.72 ± 0.09[a]	4.78 ± 0.09[a]	5.10 ± 0.19[b]
threonine	2.56 ± 0.05	2.50 ± 0.05	2.67 ± 0.16
tryptophan	0.75 ± 0.02	0.76 ± 0.01	0.78 ± 0.01
arginine	5.38 ± 0.17[a]	5.62 ± 0.08[a]	6.13 ± 0.32[b]
isoleucine	2.69 ± 0.06	2.64 ± 0.05	2.85 ± 0.16
leucine	4.65 ± 0.09[a]	4.68 ± 0.07[a]	4.97 ± 0.21[b]
valine	3.10 ± 0.06	3.03 ± 0.06	3.22 ± 0.19
histidine	1.60 ± 0.05	1.52 ± 0.03	1.59 ± 0.09
phenylalanine	2.89 ± 0.07	2.82 ± 0.06	2.99 ± 0.17
tyrosine	2.32 ± 0.06[a]	2.64 ± 0.06[b]	2.63 ± 0.08[b]
glycine	4.37 ± 0.17[a]	4.83 ± 0.27[a]	5.53 ± 0.25[b]
serine	2.65 ± 0.05	2.62 ± 0.04	2.77 ± 0.16
proline	4.38 ± 0.21	4.66 ± 0.24	4.66 ± 0.36
alanine	4.24 ± 0.16[a, b]	4.13 ± 0.13[a]	4.52 ± 0.14[b]
asparagine	6.59 ± 0.13	6.49 ± 0.13	6.98 ± 0.39
glutamine	9.83 ± 0.16	9.49 ± 0.21	10.3 ± 0.58

Tab. 5: **Crude protein content and amino acid profile of the shrimps** at the start (initial) and end of the fattening period of the control-fed and BSFL-fed animals (N = 4; values in %)
BSFL: black soldier fly larvae
[a, b] significant differences between the two groups (significance level 5%)

Fig. 2: **Feeding shrimp *(P. vannamei)* in recirculation system at the university of Hohenheim**

Conclusions

Overall, the results of this field trial are very promising, as they show that the BSFL meal used here can replace some of the fish meal without any negative effects on performance. However, the cost of insect meal is currently still higher than that of other protein components such as fish, krill, and soybean meal. Nevertheless, the external costs (environmental damage caused by production) are not included in the price of "traditional" protein meals. Although global fishmeal prices are also lower than those of insect meal, at around US$ 1,700 per metric ton (varying according to quality and origin, as of July 2, 2024), there is much criticism of the use of fishmeal as livestock feed. A large proportion (90%) of the fish caught globally directly for fishmeal production is of food or even prime food quality [30], thereby also contributing to competition between feed and food. Locally or regionally produced insect meal could therefore contribute to the sustainable production of animal-based foods. However, data on sustainability is still unclear at present, although a recent review on insect meal as a fish feed component points to disadvantages in terms of sustainability [31]. Sustainability could potentially be improved by allowing other residual streams or food waste to be used as feed substrate for insects, provided that their safety has been proven.

Funding
Contribution as part of the publication series of the Innovation Space NewFoodSystems – funding measure "Bioeconomy Innovation Spaces" as part of the "National Bioeconomy Research Strategy 2030" of the Federal Ministry of Education and Research (BMFTR).

NewFoodSystems Neue Lebensmittelsysteme

Gefördert durch: Bundesministerium für Forschung, Technologie und Raumfahrt

Acknowledgement
The authors acknowledge the BMFTR Innovation Space NewFoodSystems for funding the project reKultI4Food (Grant number: 031B1206A).
Further information: → www.newfoodsystems.de

Disclosures on Conflicts of Interest and the use of AI
Franziska Schindler is employed at Hermetia Baruth GmbH, which is commercially producing insect meal. The company is partner within the project consortium, but does not finance this publication.
The authors declare that there is no conflict of interest and AI was not used to draft this manuscript.

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