

Multifunctional use of insects in aquaculture

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Abstract: Insects are being explored as sustainable alternatives to fishmeal, a conventional component in aquaculture feed, due to their environmental advantages. However, the higher production cost of insect-based meals compared to fishmeal remains a significant challenge. The wider adoption of insect meals depends on effectively highlighting their functional benefits beyond sustainability, as well as reducing production costs.

Our research has shown that housefly (*Musca domestica*) pupae can enhance disease resistance in fish, suggesting the presence of immunostimulatory compounds in the pupae. These compounds, which activate phagocytic cells, are not unique to housefly but are found in various insect species. Studies have successfully isolated immunostimulatory substances from several insects, including melon fly (*Bactrocera cucurbitae*), black soldier fly (BSF; *Hermetia illucens*), Japanese oak silk moth (*Antheraea yamamai*), and silkworm (*Bombyx mori*). One such compound, “Silkrose”, derived from the silkworm, has been studied extensively and has been shown to improve not only disease resistance in fish but also muscle structure, stress tolerance, and heat resistance.

Gene expression analysis in fish treated with Silkrose has revealed changes in the expression of genes associated with immune function, redox balance, lipid metabolism, and protein processing. These genetic changes suggest that Silkrose reduces stress, enhances disease resistance, improves thermal tolerance, and may contribute to better flesh quality in fish. Notably, similar gene expression patterns have been observed in other animals, including mice, crustaceans, and bivalves, indicating that the effects of Silkrose extend beyond fish and apply to a wide range of vertebrates and invertebrates.

Other insect species, such as BSF, crickets, and mealworm, also contain similar bioactive compounds, suggesting they may offer comparable benefits. Insects, already recognized as promising protein sources for food and feed, are receiving increasing global attention. Their biofunctional properties are expected to further enhance their values as feed ingredients, potentially positioning them as ingredients superior to conventional fishmeal. Future research will aim to maximize the use of these bioactive compounds to fully realize the potential of insects as alternative protein sources in aquaculture feeds.

When insect-based meals become more cost-effective and their functional benefits are better understood, they could play a pivotal role in advancing sustainable aquaculture. By promoting fish health, improving stress resilience, and enhancing product quality, insect-derived ingredients may surpass fishmeal in both performance and environmental compatibility, supporting the growing demand for eco-friendly and nutritious aquaculture solutions.

Key words: insect for feed, functional property, fish culture, immunostimulant

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Introduction

Many fish species commonly farmed in Japan, such as red sea bream, yellowtail, greater amberjack, eels, and Pacific bluefin tuna, are carnivorous and rely on fishmeal derived from wild edible fish such as anchovies. These species have higher fishmeal requirements; approximately five kilograms of fishmeal are needed to produce one kilogram of red sea bream, seven to ten kilograms for yellowtail, and fourteen to seventeen kilograms for Pacific bluefin tuna. Therefore, producing high-value carnivorous fish through aquaculture requires a substantial amount of wild fish biomass. From a food sustainability standpoint, feeding these species with such feeds is both contradictory and unsustainable.

To address this issue, alternative protein sources to fishmeal are needed. In recent years, insect-based meals have gained attention as potential substitutes (Huis *et al.* 2013). Although approximately 950,000 insect species have been identified, only a few are currently utilized as protein sources. Given the growing concerns about the sustainability of fishmeal supply, it is essential to seriously consider the use of insects as sources of animal protein for aquafeeds.

Characteristics of insect meal as an aquafeed ingredient

Not all insect species are suitable for use in aquafeeds. Candidate species must be safe, available in stable quantities, and reared on substrates that do not compete with human food sources. Cost is also crucial; to compete with fishmeal, which is priced at around USD 1.70/kg, insect meal must be affordable below this level. Based on these criteria, three species of insect larvae currently stand out: the housefly (*Musca domestica*), the black soldier fly (BSF; *Hermetia illucens*), and the mealworm (*Tenebrio molitor*). Housefly larvae can be reared on livestock manure or food waste; BSF larvae thrive on food waste; and mealworm can be raised on cereal by-products or vegetable scraps. These characteristics make all three species strong candidates for inclusion as ingredients in aquafeeds.

However, insect meals may contain compounds that are toxic to fish. One such compound is catechol, a melanin precursor formed via catecholamines, which is present in relatively high concentrations (25 mg/kg in housefly, 16 mg/kg in mealworm, and 140 mg/kg in silkworm). Feeding catechol-supplemented diets to red sea bream resulted in suppressed growth and increased mortality. Because catechol is lipophilic, removing

lipids from housefly meal effectively reduces its levels. A diet formulated with defatted housefly meal instead of fishmeal supported the red sea bream growth and attained nearly equivalent to that achieved with a fishmeal-based control diet. Therefore, with appropriate detoxification, housefly meal represents a viable alternative to fishmeal (Hashizume *et al.* 2019).

Furthermore, in some cases, insect meals even outperform fishmeal. For example, replacing 100% of fishmeal in a red sea bream diet with mealworm meal resulted in weight gains of 1.4- and 1.8-fold without and with mealworm lipid removal, respectively. Thus, when processed properly to eliminate toxic compounds such as catechol, insect meals can match or exceed the performance of fishmeal (Ido *et al.* 2019).

Field trial of aquaculture using insect meal

Promising results in controlled laboratory experiments do not always guarantee the success in the field. Therefore, a field trial was conducted at a red sea bream farm in Uwajima, Ehime, in collaboration with a farmer. Mealworm meal, which had shown the most favorable results in laboratory tests, was used. From July to the following March, approximately 8,000 fish per cage (with an initial average weight of about 700 g) were fed a diet containing 10% mealworm meal. On March 16, the fish grew to an average weight of 1.63 kg, matching the growth of fish fed a control diet without mealworm.

Functional properties of insect meal

Replacing merely 5% of fishmeal with housefly meal can enhance feed intake, growth, and disease resistance in red sea bream (Ido *et al.* 2015). This immunostimulatory effect has been observed in several insect species, suggesting that insect meals offer an additional value beyond simply providing protein.

To identify the compounds responsible for this effect, we screened 13 mass-reared insect species for activity on RAW264 mouse macrophages by measuring nitric oxide (NO) production (Fig. 1), a common biomarker of immune activation and function in fish. While housefly extracts showed moderate activity, pumpkin fly (*Bactrocera cucurbitae*) pupae demonstrated stronger NO-inducing effects. Using the pupal extracts, two-stage chromatography enabled the isolation of a novel acidic polysaccharide (molecular weight ~1 MDa) named “Dipterose BC” (Ohta *et al.* 2014). Similar polysaccharides were later purified from the silk moth (*Antheraea yamamai*, “Silkrose AY”) (Ohta *et al.* 2016), the silkworm (*Bombyx mori*, “Silkrose

BM”) (Ali *et al.* 2018), and the BSF (“Dipterose BSF”) (Ali *et al.* 2019).

Aquaculture practice often adopts high-density stocking, which increases the risk of disease outbreak. Approximately 10% of production costs are related to fish disease management. Although vaccines can be effective, species diversity and small market sizes hinder their development. Antibiotics pose risks such as resistance and environmental impact. Therefore, natural immunostimulants such as Dipterose and Silkrose could offer an eco-friendly and promising approach to disease control.

Silkworm pupae, a by-product of silk production that is estimated to be produced at around 520,000 tonnes annually in the world, are a readily available and cost-effective source of

functional polysaccharides, making them well-suited for large-scale immunostimulant production.

The effects of insect-derived functional compounds on fish

We investigated the molecular mechanisms underlying Silkrose’s immunostimulatory effects using the medaka fish (*Oryzias latipes*). After feeding medaka Silkrose-enriched diets for one week and then performing an intraperitoneal challenge with *Edwardsiella tarda*, we observed reduced infection rates and significantly lower mortality (Fig.2). Gene expression profiling revealed modulation of innate immunity

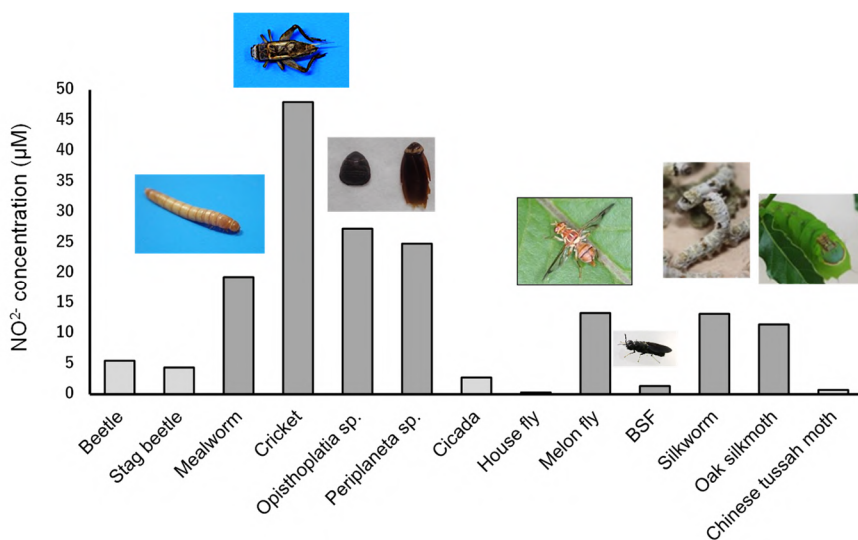


Fig.1 Search for insect species with high levels of immune-activating substances

The immune-activating capacity of insect species is indicated by the amount of nitric oxide (NO) produced by macrophage-derived RAW264.7 cells.

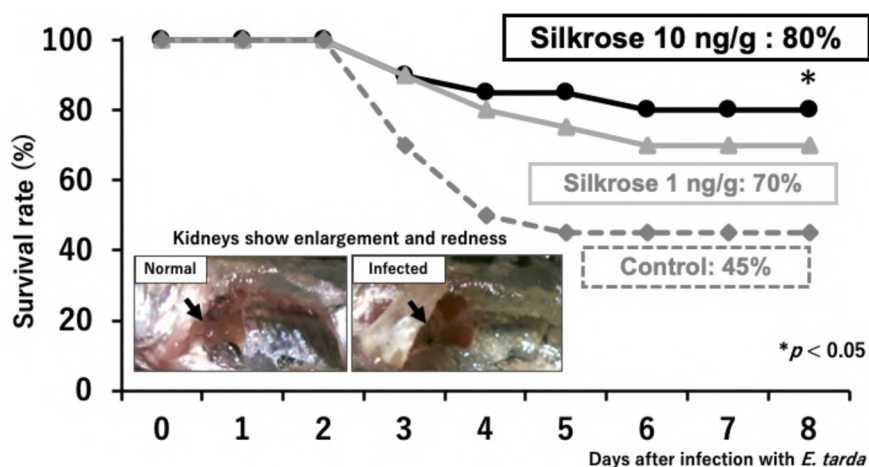


Fig.2 Survival rate of medaka following oral administration of Silkrose and then immersion exposure to *Edwardsiella tarda* (Ali *et al.* 2021)

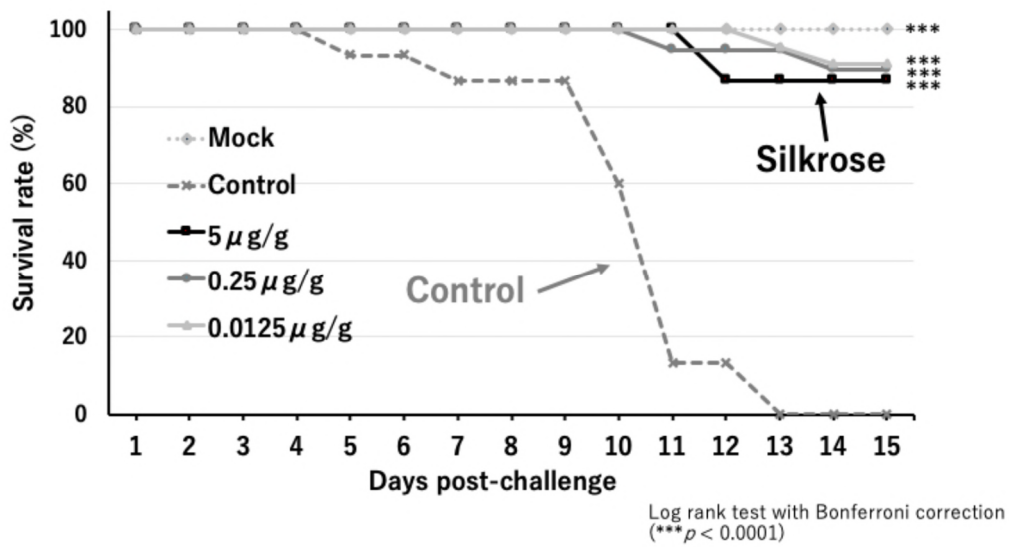


Fig.3 Survival rate of *Litopenaeus vannamei* following oral administration of Silkrose and then immersion exposure to the *Vibrio penaeicida* IAYKG13-1 strain (Ali *et al.* 2018)

(including complement activation, NO synthesis via Toll-like receptors, and antimicrobial peptide production), acquired immunity (antigen presentation and killer T-cell pathways), and mucosal barrier enhancement (Ali *et al.* 2021).

In red sea bream, feeding a 0.1% Silkrose diet for one week followed by an *E. tarda* challenge (5×10^6 cells via injection) resulted in a survival rate of 63.6%, compared to 22.7% in the control group. This demonstrates a significant improvement in disease resistance linked to systemic immunomodulation. Silkrose also reduced mortality in shrimp (both kuruma and Pacific white) through oral administration (Fig.3) (Ali *et al.* 2018). Because shrimp rely solely on innate immunity and antibiotic overuse is common in world shrimp aquaculture (6,560,000 tonnes produced in 2019), Silkrose offers a safer alternative for disease management.

Additionally, Silkrose reduced ectoparasite loads (*Benedenia* and *Caligus*) on yellowtail and white trevally, while promoting epithelial cell proliferation and likely strengthening skin barrier defenses (Fig.4) (Miura *et al.* 2022).

Gene expression analysis in medaka revealed that Silkrose modulates pathways beyond immunity, including lipid metabolism, redox processes, and oxygen transport. These physiological effects are likely significant in aquaculture species and warrant further molecular investigation.

Recent data indicate that Silkrose enhances thermal tolerance in zebrafish exposed to heat stress; while control fish died, treated fish survived. The upregulation of heat-shock proteins in the liver suggests a protective mechanism that may help mitigate the risks associated with ocean warming.

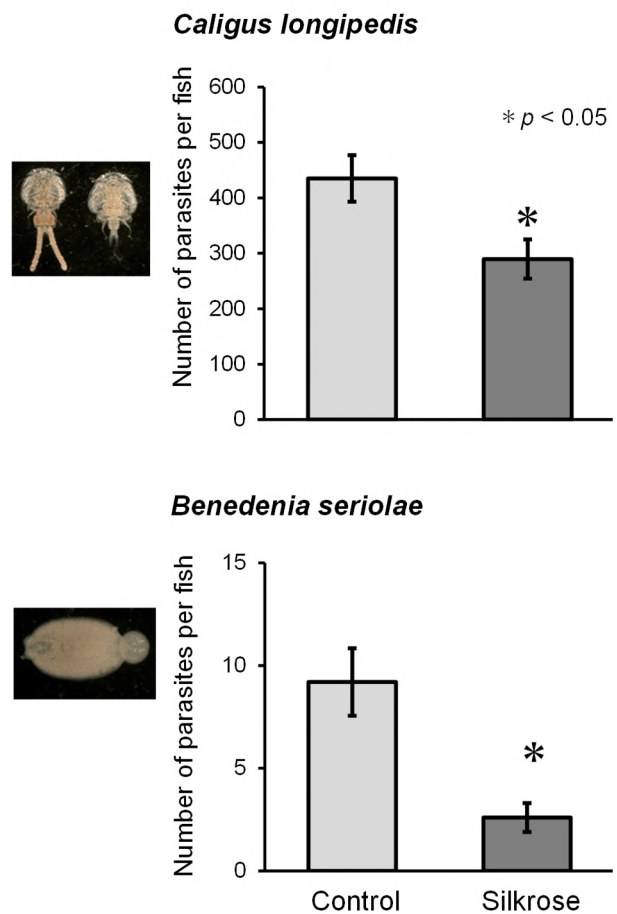


Fig.4 Effect of Silkrose treatment on the load of *Caligus longipedis* and *Neobenedenia girollae* parasites on white trevally at an aquaculture field site (Miura *et al.* 2022)

Effects on non-aquatic animals

Silkrose also benefits other animal species. In mice, oral administration lowered serum low-density lipoprotein cholesterol and reduced weight gain on a high-fat diet. In broiler chickens, it promoted the growth during heat stress. These findings suggest that Silkrose may have applications beyond aquaculture, including in livestock, pets, and possibly even humans.

Insect meal: “beyond the protein”

In summary, both silkworm- and BSF-derived products contain bioactive polysaccharides that enhance immunity, disease resistance, thermal tolerance, and more. Mealworm and housefly likely harbor similar molecules. Although insect meals currently struggle to compete with fishmeal on price due to the lower cost of marine-derived fishmeal, their functional properties elevate them to a “beyond the protein” category. Scientifically characterizing these bioactive compounds and quantifying their modes of action could enable insect meals to rival—or even surpass—fishmeal in aquaculture.

Although the use of insects in animal feed has been discussed for over a decade, and BSF products are now commercially available worldwide, Japan continues to lag behind in industrial adoption, largely due to persistent negative perceptions. As more companies enter the market, the production costs will fall. A rich, nutritious animal feed should not come at the cost of environmental destruction. Insects offer a sustainable solution by converting human-generated waste into valuable fertilizer and high-quality, functional protein for aquaculture. Growing awareness of the environmental and social benefits of insect-based feed is also essential to drive domestic uptake of insect products. Often overlooked, they could become the unsung heroes of tomorrow’s food systems.

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