JOURNAL OF
EMERGING INVESTIGATORS

Article

Alloferon improves the growth performance and developmental time of mealworms (*Tenebrio molitor*)

Jimin Shon[1], David Shon[1], Woo Jin Kim[2]
[1]Portsmouth Abbey School, Portsmouth, Rhode Island
[2]Jeonbuk University, Jeollabuk-do, Republic of Korea

SUMMARY

Mealworms (*Tenebrio molitor*) are important food sources for reptiles, birds, and other organisms, as well as for humans. Due to their outstanding nutritional value, mealworms are in high demand as an alternative food source and are the subject of widespread curiosity. However, the slow growth and low survival rate of mealworms cause problems for mass production. Since alloferon, a synthetic peptide, showed long-term immunological effects on mealworms, we hypothesized that alloferon would function as a growth promoter to maximize mealworm production. We discovered that the overall weight of the alloferon-containing gelatin diet group was 39.5-90% heavier, and the development time of the experimental group was shortened up to 20.6-39.6% than the control group. In addition, 300 nM alloferon significantly boosted cell proliferation of Sf9 cells (insect cells) only after six days of the treatment. Also, we noticed that two proteins were induced in alloferon fed mealworm body tissue and found that one of the proteins was phenoloxidase 1, which may be responsible for increasing growth rate by regulating the production of melanin pigments. Overall, these findings have potential implications in mealworm insect farming because alloferon may foster a sustainable food supply industry.

INTRODUCTION

At least 690 million people around the world suffered from hunger and nutrient deficiencies in 2019 according to a recent report of the Food and Agriculture Organization of the United Nations (1). The constant increase in world population raises serious questions regarding our capacity to provide an adequate food source. Another study reported global demand for food is expected to grow by 70% as the population reaches approximately 9.6 billion by 2050 (2).

The ongoing human-driven encroachment on the environment, mainly due to the demands for urbanization and food production, also exacerbates the imminent food crisis. For example, livestock/meat production plays an important role in climate change, with huge CO2 emissions representing 14.5% of human-induced greenhouse gas emissions (3). The livestock sector also occupies 26% of the ice-free terrestrial surface of the planet as well as 70% of all agricultural land. This leaves negative effects behind on the grazed land, such as deforestation, terrain compaction, and erosion (3). In summary, the increasing demand for food, especially animal-based protein, is adversely impacting the environment in terms of greenhouse gas emissions, water, energy, and land usage (4).

As a solution for these problems, insects are considered to have the potential to be a new, environment-friendly food source (5). The practice of entomophagy has been practiced for hundreds of years. While European-derived populations in North America historically have placed taboos on entomophagous eating practices, native cultures in Asia, South America, Africa, and Europe include the consumption of various species of insects, approximately over 2,000 (6). The United Nations also recommended insects as a potential solution to the global food shortage (7). There are a few factors that account for previous historical practices: insects have a low environmental impact due to the limited need for arable land and water compared with livestock and low ecological cost (8, 9). Additionally, insects can provide nutritional benefits such as high-quality proteins, polyunsaturated fatty acids, dietary fibers, and various micronutrients (10). Therefore, insect farming, the practice of raising and breeding insects as a food/protein source, has been gradually developing over the past few years.

Mealworms (Tenebrio molitor) are the larval form of two darkling beetle species of the Tenebrionidae family, the yellow mealworm beetle and the dark or mini mealworm beetle. Tenebrio Molitor, like all holometabolous insects, goes through four growth stages: egg, larva, pupa, and adult. The whole life cycle spans from 280 to 630 days, and takes 10-12 days for the larvae to hatch and typically 3-4 months to mature after a variable number of stages (11). A mature larva is a light yellow-brown color, 20 to 32 mm long, and weighs 130-160 mg (11). After the larvae enter the pupal stage, which lasts about 7-9 days at room temperature, they eventually become adults that live for 2-3 months. Mealworms' diet is not strictly limited, as they are omnivorous and can devour various plant materials and animal products (12).

The advantages resulting from high protein content, well-balanced amino acid profile, and efficient feed conversion rate accompanied with available mass production technology ultimately allowed mealworms a promising candidate for insect rearing (8,10,11,13). However, despite these benefits, there is still a need for further improvements in mealworm feed efficiency. As a current caveat, further optimization is in need, especially when it comes to the nutritional composition, digestibility, and cost-effectiveness of mealworm feed. Also, the slow growth and low survival rate of mealworms cause problems for mass production.

Thus, we hypothesized that alloferon, a synthetic peptide, would act as a growth promoter to increase mealworm production because it had long-lasting immunological effects on mealworms. Alloferons, a group of naturally occurring immunomodulatory peptides, are chiefly isolated from the

hemolymph of maggots of the blowfly Calliphora vicina challenged with bacteria (14). Most hemocytes possess cytotoxic activity comparable to mammalian natural killer cell activity, another unique property uncovered in other insects (15). Alloferon, included in hemolymph, stimulated the natural cytotoxicity of mouse spleen lymphocytes in vitro and human blood mononuclear cells (16). However, synthetic alloferon administered in picomolar concentrations showed antiviral and anti-tumor activities in mice in vivo (17).

Other beneficial biological activities of alloferons have been reported, such as stimulation of interferon synthesis in mice and humans in vivo (18), suppression of herpes simplex virus in vitro proliferation (19), deblocking of the nuclear factor kappa-light-chain-enhancer mediated signaling pathway (20), and modification of proinflammatory cytokine production (21). In the present study, our goal was to evaluate the effect of alloferon on the growth performance and survival rate of mealworms and investigate the mechanism of alloferon. We found that feeding mealworms with alloferon led to increased growth, as the overall weight of the alloferon-containing gelatin diet group was 39.5-90% heavier. Also, the development time of the experimental group was shortened up to 20.6-39.6% than the control group. In addition, Alloferon also boosted cell proliferation in Sf9 cells and induced phenoloxidase 1 in mealworm body tissue. Overall, these findings suggest that alloferon improves the growth and development of mealworms.

RESULTS

To determine whether alloferon increases the weight of the mealworms, we analyzed the growth rate of the mealworms after feeding them alloferon. We prepared a wheat bran diet group, a gelatin diet group, and an alloferon-containing gelatin group (300 nM). For the three groups, 500 larvae each, the average weight of the wheat bran diet group increased by 140.2% over 10 weeks. The gelatin diet group experienced a growth rate of 189.4% ($p = 0.0003$, two-way ANOVA, **Figure 1C**), and the alloferon-containing gelatin supplementation group improved the growth rate to 267.3% ($p < 0.0001$, two-way ANOVA, **Figure 1C**). In week 10, mealworm larvae fed on gelatin and alloferon supplements were 39.5-90% heavier than those fed on wheat bran only ($p < 0.0001$, two-way ANOVA, **Figure 1C**).

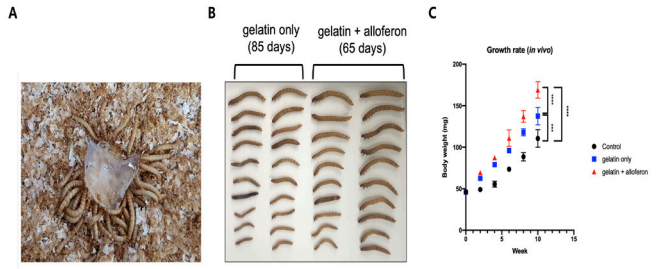

Figure 1: Bodyweight of mealworms (T. molitor) after gelatin and alloferon-containing gelatin diet. (A) Gelatin gel containing an alloferon supplement was provided to the mealworms (T. molitor). (B) The effect of gelatin and alloferon-containing gelatin on the body size change of mealworms was analyzed over 10 weeks of larvae development. (C) Bodyweight of mealworms was increased after the gelatin and alloferon-containing gelatin diet. The mean and standard deviation of body weight are represented in the graph. Two-way ANOVA with Tukey multiple comparisons test was used to analyze the statistical significance; $p < 0.001$(***) and $p < 0.0001$(****). Two biological replicate samples were analyzed.

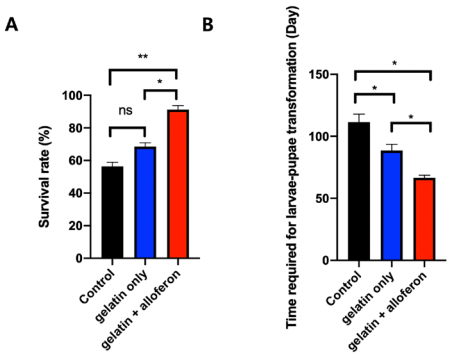

Figure 2: Effect of the gelatin and alloferon-containing gelatin diet on survival rate and development time (larvae-pupae transformation) of the mealworms (T. molitor). (A) The survival rate of mealworms fed on wheat bran, gelatin supplement, and alloferon-containing gelatin. The survival rate was calculated using the following equation: [(final number of lived mealworms) / (initial number of total mealworms)] x 100. The mean and standard deviation of percentage of survival are represented in the graph. (B) Development time (the number of days until 50% of mealworm larvae changed into the pupae stage) of mealworms fed on wheat bran, gelatin supplement, and alloferon-containing gelatin. Two biological replicate samples were analyzed in each condition. Unpaired t-test was used to analyze the statistical significance; $p > 0.05$ (ns) and $p < 0.05$ (*).

Since previous studies indicated that alloferon enhanced insect immune response, we hypothesized that the survival rate and time to development of the alloferon fed group would increase. Compared to the wheat bran diet, the average survival rates of pupated mealworms were not significantly increased when mealworms were fed with gelatin ($p < 0.08$, unpaired t-test, **Figure 2A**). However, when the mealworms were fed with an alloferon-containing supplement diet, the survival rate significantly increased ($p < 0.005$, unpaired t-test, **Figure 2A**). In this study, mealworm larvae transformed into pupae in 9 to 17 weeks. Development time was defined as the number of days until 50% of mealworm larvae changed into the pupae stage. The development time of mealworms in the experimental group was shortened to 20.6-39.6% on gelatin and alloferon-containing gelatin diet compared to the control group with wheat bran diet (**Figure 2B**).

Next, we next determined whether increased cell proliferation was responsible forthe increase in the body weight of mealworms fed alloferon. We performed an in vitro Presto blue proliferation assay, which is colorimetric assay ased on the reduction of a dye called PrestoBlue by metabolically active cells using Sf9 insect cells. Sf9 cells, originally established from ovarian tissue, are commonly used in insect cell culture for in vitro assay. The assay revealed that 300 nM alloferon significantly boosted cell proliferation of Sf9 cells by about 35% after six days of the treatment ($p = 0.0006$, two-way ANOVA, **Figure 3**).

To analyze the effect of the alloferon diet on the protein expression level in mealworm body tissue, the total protein extracts from mealworm body tissue were analyzed in each group by SDS-PAGE. The result showed that two different proteins (~135 kDa and 80 kDa) were induced in mealworm

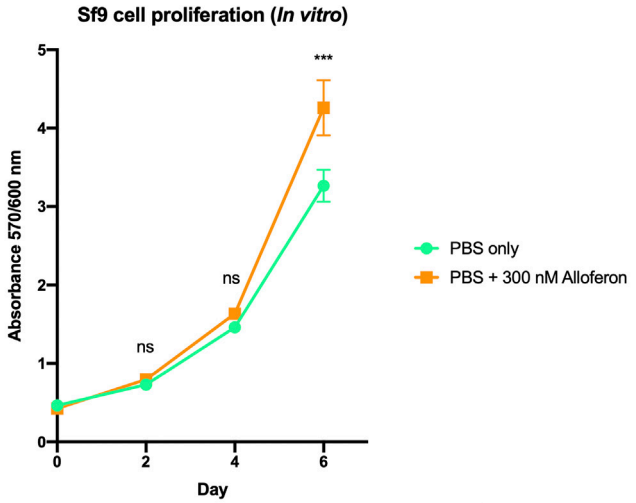

Figure 3: Cell proliferation of Sf9 cells after 300 nM of the allofero treatment. The mean and standard deviation of absorbance 570/600nm indicating the normalized cell proliferation, are represented in the graph. Two-way ANOVA with Tukey multiple comparisons test was used to analyze the statistical significance; degrees of freedom = 8, $p > 0.01$(ns) and $p < 0.01$(***). Three biological replicate samples were analyzed.

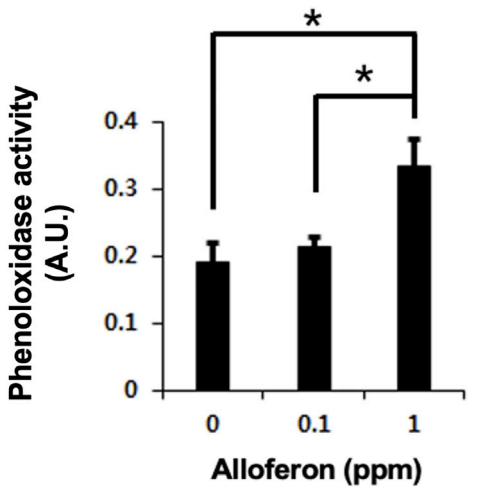

Figure 5. Phenoloxidase activity after alloferon treatment. The mean and standard deviation of phenoloxidase activity are represented in the graph. Oxidation of phenols to form quinines was used to measure phenoloxidase activity. Duplicate samples were analyzed in this assay. Unpaired t-test was used to analyze the statistical significance; $p < 0.05$ (*).

body tissue only when 0.1 and 1.0 ppm of alloferon were fed. We further analyzed these two induced bands by LC-MS. The 135 kDa protein did not match with any reference proteins and therefore was not identified. The 80 kDa protein was identified as phenoloxidase 1. Overall, this result indicated that the alloferon-containing gelatin diet increased phenoloxidase 1 expression level in mealworm body tissue (**Figure 4**).

Since the alloferon diet increased the phenoloxidase expression level, phenoloxidase activity was analyzed in mealworm body tissue. Even though 0.1 ppm of alloferon supplemented diet did not affect phenoloxidase activity compared to 0 ppm of alloferon supplemented diet, 1 ppm of alloferon diet significantly increased phenoloxidase activity in mealworm body tissue (**Figure 5**). This result indicated that the alloferon diet increased phenoloxidase activity in mealworm body tissue.

DISCUSSION

Considering world population increases, food shortages, and environmental pollution, insect proteins are expected to be a favorable alternative (5). Edible insects have nutritional advantages because they are composed of high levels

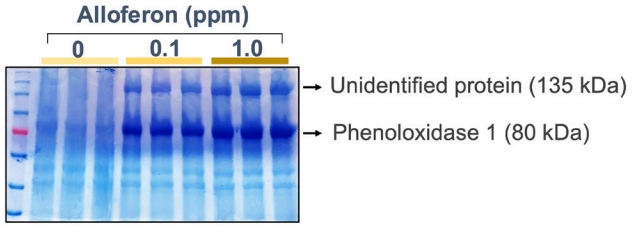

Figure 4: Phenoloxidase 1 was presented in mealworms (T. molitor) body tissue after alloferon-containing gelatin diet. SDS-PAGE gel image is represented with the total protein extracts of mealworm groups fed with 0, 0.1, and 1.0 ppm alloferon.

of protein (50–60%) and considerable levels of fat, fiber, vitamins, and minerals (22). Mealworms (Tenebrio molitor) are well-known to contain a substantial amount of protein and unsaturated fatty acids (23). Therefore, the attempt to develop and apply mealworms for protein supply to patient diets has been continued. Notably, a recent study reported that a mealworm diet both increased muscle mass and body fat and activated the immune cells of cancer patients (24). Overall, the nutrient contents of mealworms are considered a valuable source of nutrition for patients who need high nourishment, even in small amounts.

In this study, we observed that the alloferon-containing gelatin diet group was 39.5-90% heavier overall, and the experimental group's development period was decreased by up to 20.6-39.6% compared to the control group. Furthermore, 300 nM alloferon greatly increased cell proliferation of Sf9 cells (insect cells) six days after treatment. In addition, we discovered that two proteins were elevated in alloferon-fed mealworm body tissue, one of which was phenoloxidase 1, which may be involved in enhancing growth rate via modulating melanin pigment formation.

Sf9 insect cells are a type of cell line commonly used in insect cell culture. They were derived from the ovaries of the fall armyworm Spodoptera frugiperda and are frequently used to express recombinant proteins for research purposes. In this study, we used Sf9 cells to test whether alloferon, a peptide derived from the blowfly Calliphora vicina, could increase cell proliferation (**Figure 3**). Our results indicate that 300nM alloferon did indeed increase the proliferation of Sf9 cells in vitro, suggesting that it may have a similar effect on other insect cells as well. However, since we only tested 300 nM of alloferon, we should investigate the effect of alloferon on cell proliferation with different concentrations. This finding supports our hypothesis that alloferon may promote the growth and development of insects such as mealworms (**Figure 1**).

The antibacterial effects of alloferon on mealworms

have been reported in previous studies. In particular, the correlation between the increased activity of phenoloxidase and the innate immunity of insects has been evaluated (14). The present study is the first to speculate that alloferon may potentially increase the survival rate of mealworms by increasing the expression of phenoloxidase. In other words, alloferon may increase the survival rate of the mealworm by strengthening the innate immunity of insects and preventing bacterial infection. Moreover, further research is necessary to reveal a more definite mode of action of alloferon for mealworm's growth to clarify the connection between changes in body mass and immune function.

Mealworms can be fed on wheat bran to obtain all the required nutrients for growth, development, and reproduction (25). Previous studies have shown that larval survival is improved when additional ingredients are provided (26). However, most studies have focused on the balance and replenishment of macronutrients, including protein, fat, and starch. The present study provides a novel link between alloferon and phenol oxidase, suggesting the biological mechanism of how alloferon may enhance the growth rate of mealworms.

To prevent cannibalism and increase productivity in mealworm breeding, sufficient moisture supply is an important factor. Previously, supplements such as vegetables and fruits were mainly used for hydration (11). However, this study did not detect growth disturbance when moisture supply was provided with gelatin containing alloferon peptide without any conventional supplements such as vegetables. This might be attributable to the composition of gelatin containing hydrolyzed collagen peptide and water. Thus, it is expected that this type of gelatin might be applied to the insect farming industry as a vehicle to supply hydrophilic peptide, alloferon, and moisture simultaneously.

This study showed 1 ppm of alloferon peptide decreased mealworm development time by 40.3% and increased survival rate by over 61.8% (**Figures 1 and 2**). This result can be applied to increase productivity required from a large-scale smart insect farming system and used as an essential basis for providing economic value to the insect production industry.

Phenoloxidase is an enzyme involved in the insect's melanization immune response, and it is responsible for the production of melanin pigments and the formation of melanin-based protective structures. (14) The effects of phenoloxidase on the faster growth of the mealworm are not well understood. The study found that feeding mealworms with alloferon led to an increase in growth and development time, and it also induced phenoloxidase 1 protein. However, it is not clear how the induction of phenoloxidase specifically contributes to the increased growth and development of mealworms. More research is needed to understand the specific mechanisms by which phenoloxidase may affect the growth of mealworms.

Earlier development and increased survival in insect farming can lead to faster turnover of crop cycles and higher yields, resulting in increased profitability for farmers. Larger worms can also be more desirable for certain end uses, such as animal feed or fertilizer, as they may contain more protein or other nutrients. Additionally, larger worms may be easier to harvest and process, leading to increased efficiency and profitability for the farmer.

In the present study, analysis of nutrient components, including protein contained in mealworms, was not performed for each experimental group. Therefore, further study about the nutrient composition, growth rate, and survival rate of mealworms when alloferon peptide is applied is required. The limitation of this study was the lack of a positive control group with scrambled peptides, a small sample size, and short-term follow-up periods. It will be necessary to verify the efficacy of alloferon through preliminary experimental studies for mass production.

MATERIALS AND METHODS

Fabrication of feeding gelatin

The feeding gelatin was made by combining 200 grams (g) of distilled water and 4 g of paper gelatin (Gelita HG, Eberbach, Germany), and was solidified in the refrigerator using an ice cubic stick tray with the lid closed to minimize loss of moisture. The same process was performed for the experimental group of gelatin containing 1 pars per million (ppm) alloferon peptide (H-His-Gly-Val-Ser-Gly-His-Gly-Gln-His-Gly-Val-His-Gly-OH) (Biomatik, Delaware, USA). The tray had 10 slots to make each approximately 20 g gelatin cubes and was kept in the refrigerator with the lids closed.

Mealworm larvae feeding

Yellow mealworms (Tenebrio molitor) were provided by a private local insect breeder (Mealworm village, Seoul, Korea). Mealworm larvae were divided into 3 different dietary groups: (1) about 500 larvae were placed in a plastic container (18.5 x 12 x 5.5 cm) with 30 g wheat bran only (control group), (2) about 500 larvae with wheat bran supplemented with 20 g gelatin three times per week (positive control group), (3) about 500 larvae with wheat bran supplemented with 20 g gelatin containing 1ppm alloferon peptide three times per week (experimental group). Two containers per each dietary group were set up, after which the containers were placed in a climate chamber at 25°C with a relative humidity of 60%.

Analysis of mealworm larvae body mass change, survival rate, and development time (larvae-pupae transformation)

Mealworm pupae and dead larvae were counted and removed daily from each experimental group, and the numbers of live larvae were recorded to evaluate survival rates. The average weight of the mealworm larvae was recorded every week with 20 randomly selected mealworms. The development time of mealworms was the number of days until 50% of mealworm larvae changed into pupae stage. The harvested mealworm larvae were killed by freezing, and all mealworms were stored at -20°C.

Mealworm larvae tissue protein analysis

For harvested mealworm larvae tissue fixation, 10% picric acid was used as a fixative solution and incubated for 12 hours. The mealworm larvae were homogenized with tris-buffered saline (TBS). The supernatant was isolated from the sample slurry after centrifugation at 13,000 revolutions per minute (rpm) for 5 minutes (min) at 4oC. Then, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 12% acrylamide gels. The protein samples were stained with Coomassie blue R250 (Bio-Rad, CA, USA).

Liquid chromatography combined with mass spectrometry (LC-MS) was used to analyze the homogenized protein sample from mealworm larvae. The full-scan mass spectrometer data were obtained to analyze the fragmentation spectra of each compound. The resulting fragmentation spectra provide information about peptide sequence and post-translational modifications. Then, acquired mass spectra were analyzed to theoretical spectra generated from the protein database. By matching the observed spectra to the theoretical spectra, the peptides were identified present in the sample.

Phenoloxidase activity analysis

Phenoloxidase activity in homogenized larvae tissue was analyzed with a 96 well microplate reader using catechol as the substrate. After the sample was incubated for 37 oC for 10 min, the 420 nm absorbance was measured with a microplate spectrophotometer (Bio-Rad, CA, USA). The assay was performed with three replicates and each experiment was repeated three times. The phenoloxidase activity was expressed as units of enzyme activity per mg protein.

In vitro cell proliferation analysis by PrestoBlue assay

The proliferation of Sf9 insect cells was analyzed by the PrestoBlue assay (Invitrogen, CA, USA). 8,000 cells/well were prepared in 96 well plates (Sigma-Aldrich, MI, USA). After cells were seeded, the cells were incubated with PBS with alloferon (300 nM). The control group received PBS without alloferon. At 0, 2, 4, and 6 days after applying 10% PrestoBlue, the color switchover of resazurin to resorufin (absorbance 570 nm/600 nm) was measured with the microplate reader (BioTeck, VT, USA). Three biological replicates were analyzed.

Statistical test

Unpaired t-test and two-way ANOVA with Tukey multiple comparisons test were used for all statistical analyses using GraphPad Prism 8 (GraphPad, CA, USA). Statistical significance was set at $p < 0.05$.

Received: December 22, 2021
Accepted: April 12, 2023
Published: October 20, 2023

REFERENCES

1. "The State of Food Insecurity in the World." United Nations Food and Agriculture Organization (FAO). https://www.unicef.org/reports/state-of-food-security-and-nutrition-2020. Accessed 26 Dec. 2020.
2. Wu, Guoyao, et al. "Production and supply of high-quality food protein for human consumption: sustainability, challenges, and innovations." *Annals of the New York Academy of Sciences*, vol. 1321, Aug 2014, pp. 1-19. doi:10.1111/nyas.12500
3. Sejian, Veerasamy, et al. "Global Warming: Role of Livestock." *Springer*, Jan 2015, pp. 141-169, doi:10.1007/978-81-322-2265-1_10
4. Henchion Maeve, et al. "Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium." *Foods*, vol. 6,7 no. 53, Jul 2017, doi:10.3390/foods607005320
5. Van Huis A, et al. "Edible Insects: Future Prospects for Food and Feed Security." *Food and Agriculture Organization of the United Nations*, no. 171, Jan 2017, pp. 187
6. Woolf E, et al. "Willingness to consume insect-containing foods: A survey in the United States." *LWT Food Sci Technol*, vol. 102, Dec 2018, pp. 100-105, doi:10.1016/j.lwt.2018.12.010.
7. Liu C, Zhao J. "Insects as a Novel Food." *Encyclopedia of Food Chemistry*, 30 Nov 2018, pp. 428–436. doi:10.1016/B978-0-08-100596-5.21782-4.
8. Cortes Ortiz JA, et al. "Insect mass production technologies." *Insects as Sustainable Food Ingredient*s, 1 July 2016, pp. 153–201, doi:10.1016/B978-0-12-802856-8.00006-5.
9. Van Huis A. "Potential of insects as food and feed in assuring food security." *Annu Rev Entomol*, vol. 58, Jan 2013, pp. 563-583
10. Rumpold BA, Schlüter OK. "Nutritional composition and safety aspects of edible insects." *Mol Nutr Food Res*, vol. 57, no.5, 1 May 2013, pp. 802-23, doi:10.1002/mnfr.201200735.
11. Liu, Changqi, et al. "Growth Performance and Nutrient Composition of Mealworms (Tenebrio molitor) Fed on Fresh Plant Materials-Supplemented Diets." *Foods*, vol. 9, 5 Feb 2020, doi:10.3390/foods9020151
12. Van Broekhoven S, et al. "Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by- products." *J Insect Physiol*, vol. 73, 7 Jan 2015, pp. 1-10, doi:10.1016/j.jinsphys.2014.12.005
13. Oonincx DG, et al. "Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products." *PLoS ONE*, 23 December 2015, doi:10.1371/journal.pone.0144601.
14. Czarniewskab E, et al. "The long-term immunological effects of alloferon and its analogues in the mealworm Tenebrio molitor." *Insect Sci*, vol. 25, no. 3, 1 June 2018, pp. 429-438, doi:10.1111/1744-7917.12427
15. Lee N, et al. "Inhibition of lytic reactivation of Kaposi's sarcoma-associated herpesvirus by alloferon." *Antivir Ther*, vol 16, no.3, Jan 2011, pp, 17-26, doi:10.3851/IMP1788
16. Agarwal, Garima, and Reema, Gabrani. "Antiviral and antitumor peptides from insects." *PNAS*, vol. 27, no. 1, 18 May 2020, pp. 149-168, doi:10.1007/s10989-020-10072-0
17. Chernysh S, et al. "Anti-tumor activity of immunomodulatory peptide alloferon-1 in mouse tumor transplantation model." *International immunopharmacology*, vol. 12, no. 1, 1 Jan 2012, pp. 312-314 doi:10.1016/j.intimp.2011.10.016.
18. Ershov FI, et al. "Allokine-α effect on the course of chronic genital herpes relapses." *Materia Medica*, vol. 40, Jan 2003, pp. 103–11
19. Lee NE, et al. "Antiviral effect of Alloferon on Kaposi's sarcoma-associated herpesvirus." *FASEB J*, vol. 22, 1 March 2008, pp. 859.19, doi: 10.1096/fasebj.22.1_supplement.859.19
20. Ryu MJ, et al. "Activation of NF-kB by alloferon through down-regulation of antioxidant proteins and IkBα." *Mol Cell Biochem*, vol. 313, no. 1-2, 1 June 2008, pp. 91-102, doi:10.1007/s11010-008-9746-0.
21. Lee SK, et al. "The anti-inflammatory effect of Alloferon on UVB irradiated human skin keratinocyte cell line, HaCaT via the regulation of pro-inflammatory cytokine production." *Immunology letters*, vol. 149, no. 1-2, 1 Jan 2013, pp. 110-8, doi:10.1016/j.imlet.2012.09.005.

22. R Payne, C. L., et al. “Are edible insects more or less 'healthy' than commonly consumed meats? A comparison using two nutrient profiling models developed to combat over- and undernutrition.” *Eur J Clin Nutr tion*, vol 70, 16 Mar 2016, pp. 285–291, https://doi.org/10.1038/ejcn.2015.149
23. Jajić I, et al. “Fatty and amino acid profile of mealworm larvae.” *Biotechnology in Animal Husbandry*, vol. 36, 7 June 2020, pp. 167-180, doi: 10.2298/BAH2002167J
24. Kim HS, et al. “Clinical application of invalid foods using mealworms and evaluation of nutrition status and immune function: a study protocol for a randomized, double-blind, placebo-controlled trial.” *BMC Nutrition*, vol. 5, no. 44, 18 Nov 2019, doi:10.1186/s40795-019-0307-6.
25. Bai PP, et al. “Phenoloxidase and its zymogen are required for the larval-pulpal transition in Bactrocera dorsalis (Diptera: Tephritidae).” *Journal of insect physiology*, vol. 71, Dec 2014, pp. 137-46, doi:10.1016/j.jinsphys.2014.10.013
26. Cortes Ortiz J, et al. “Insect mass production technologies. In Insects as Sustainable Food Ingredients.” *Elsevier*, Dec 2016, pp.153-201, doi: 10.1016/B978-0-12-802856-8.00006-5
27. Oonincx DG, et al. “Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products.” *PLoS ONE*, 23 Dec 2015, doi: 10.1371/journal.pone.0222043