Silkworm *Bombyx mori L.* (Lepidoptera: Bombycidae) as an effective feed alternative.

Is it environmentally efficient?

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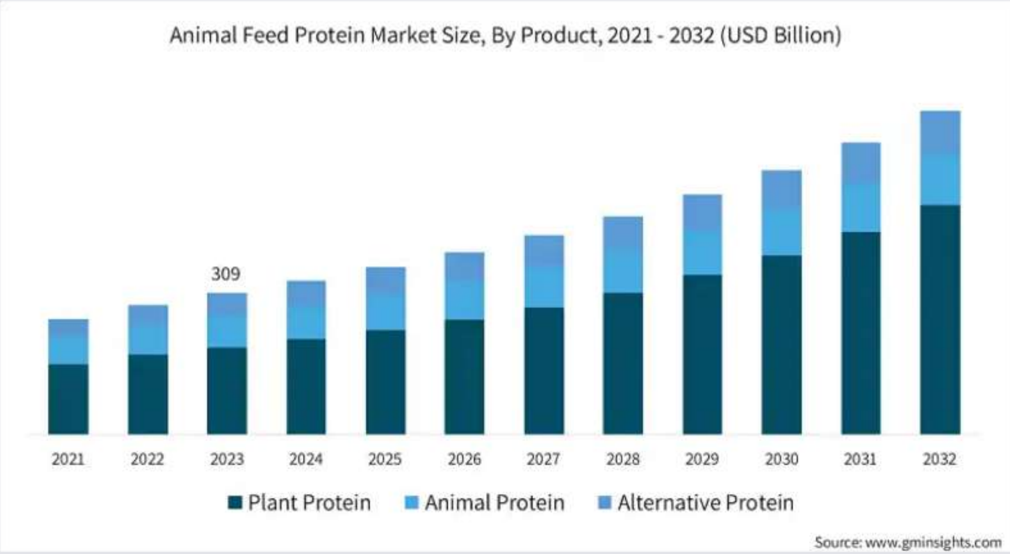
Introduction



- Global population is projected to exceed 10 billion by 2050
- Demand for animal protein to **double**, with pork and poultry consumption rising by over 100%
- Challenge: **Sustainability of traditional protein sources**.
- Insects as alternative protein sources due to:
  - **High protein content**
  - **Lower environmental impact**.

International Feed Industry Federation (IFIF) 2024: https://ifif.org/wp-content/uploads/2024/11/IFIF-Fact-Sheet-Nov-2024-singles.pdf
McLeod, A. (with Organisation des Nations Unies pour l'alimentation et l'agriculture). (2011). *World livestock 2011: Livestock in food security*. FAO

Asimi, O., Bhat, T., Nasir, H., & Irfan, K. (2017). Alternative source of protein "silkworm pupae"(Bombyx mori) in coldwater aquaculture. *Int. J. Poult. Fish. Sci*, *1*, 1–4.

Van Huis, A. (2020). Insects as food and feed, a new emerging agricultural sector: A review. *Journal of Insects as Food and Feed*, *6*(1), Article 1. https://doi.org/10.3920/JIFF2019.0017

Background

- Main protein sources for animal feed in the past years : soybeans, fishmeal, oilseed by-products
- Issues:
    - Environmental costs (land, water, transport)
    - Dependence on imports (European Union)

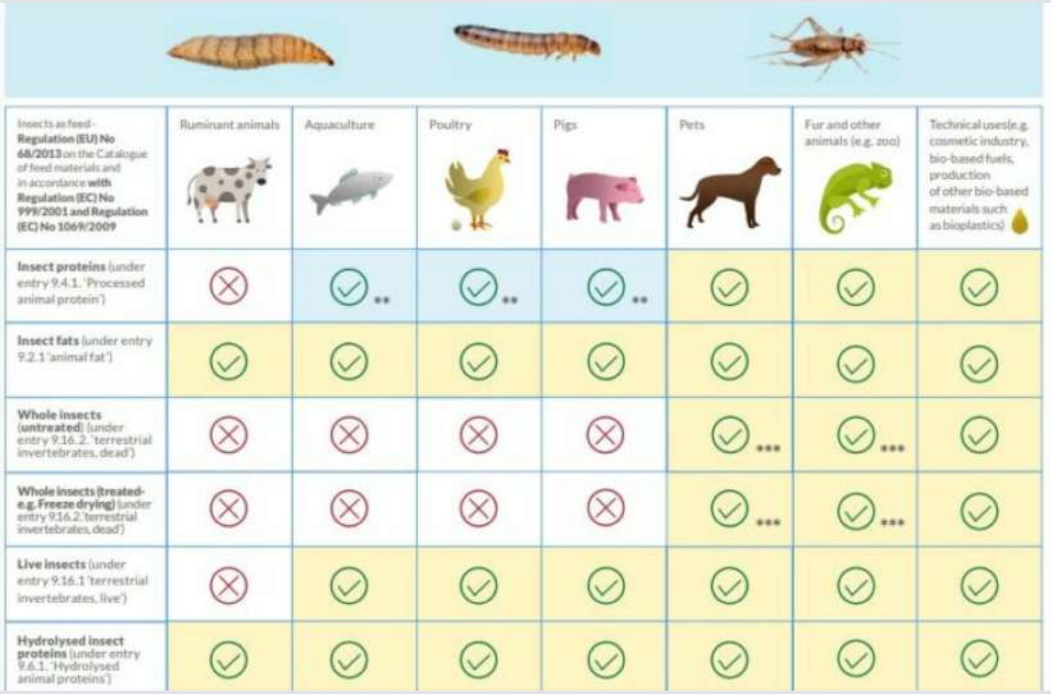
- Insects :
    - Natural protein source for many animals
    - Increasing production with forecasts of 1.2 million tones by 2025

Wiedemann, S. G., McGahan, E. J., & Murphy, C. M. (2016). Environmental impacts and resource use from Australian pork production assessed using life-cycle assessment. 1. Greenhouse gas emissions. *Animal Production Science*, *56*(9), Article 9. https://doi.org/10.1071/AN15881
FECAC (2023) FOOD AND FEED : https://fefac.eu/wp-content/uploads/2024/03/FF_2023.pd

Allegretti, G., Talamini, E., Schmidt, V., Bogorni, P. C., & Ortega, E. (2018). Insect as feed: An emergy assessment of insect meal as a sustainable protein source for the Brazilian poultry industry. *Journal of Cleaner Production*, *171*, 403–412. https://doi.org/10.1016/j.jclepro.2017.09.244

EU Approved Insects as protein source for feed

Regulation No 2001/999 (Annex IV) amended by the Regulation 2017/893 (Annex X)



Insects as feed - **Regulation (EU) No 68/2013** on the Catalogue of feed materials and in accordance **with Regulation (EC) No 999/2001 and Regulation (EC) No 1069/2009**	Ruminant animals	Aquaculture	Poultry	Pigs	Pets	Fur and other animals (e.g. zoo)	Technical uses(e.g. cosmetic industry, bio-based fuels, production of other bio-based materials such as bioplastics)
Insect proteins (under entry 9.4.1. 'Processed animal protein')	✗	✓ **	✓ **	✓ **	✓	✓	✓
Insect fats (under entry 9.2.1 'animal fat')	✓	✓	✓	✓	✓	✓	✓
Whole insects (untreated) (under entry 9.16.2. 'terrestrial invertebrates, dead')	✗	✗	✗	✗	✓ ***	✓ ***	✓
Whole insects (treated- e.g. Freeze drying) (under entry 9.16.2. 'terrestrial invertebrates, dead')	✗	✗	✗	✗	✓ ***	✓ ***	✓
Live insects (under entry 9.16.1 'terrestrial invertebrates, live')	✗	✓	✓	✓	✓	✓	✓
Hydrolysed insect proteins (under entry 9.6.1. 'Hydrolysed animal proteins')	✓	✓	✓	✓	✓	✓	✓

Black Soldier Fly - *Hermetia illucens* L. (Diptera: Stratiomyidae)

Housefly - *Musca domestica* L. (Diptera: Muscidae)

Yellow Mealworm - *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)

Lesser Mealworm - *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae)

House Cricket - *Acheta domesticus* L. (Orthoptera: Gryllidae)

Banded Cricket - *Gryllodes sigillatus* Walker (Orthoptera: Gryllidae)

Field Cricket - *Gryllus assimilis* (F.) (Orthoptera: Gryllidae)

EU Approved Insects as protein source for feed

Regulation No 2021/1925

Insects as feed - **Regulation (EU) No 68/2013** on the Catalogue of feed materials and in accordance **with Regulation (EC) No 999/2001 and Regulation (EC) No 1069/2009**	Ruminant animals	Aquaculture	Poultry	Pigs	Pets	Fur and other animals (e.g. zoo)	Technical uses (e.g. cosmetic industry, bio-based fuels, production of other bio-based materials such as bioplastics)
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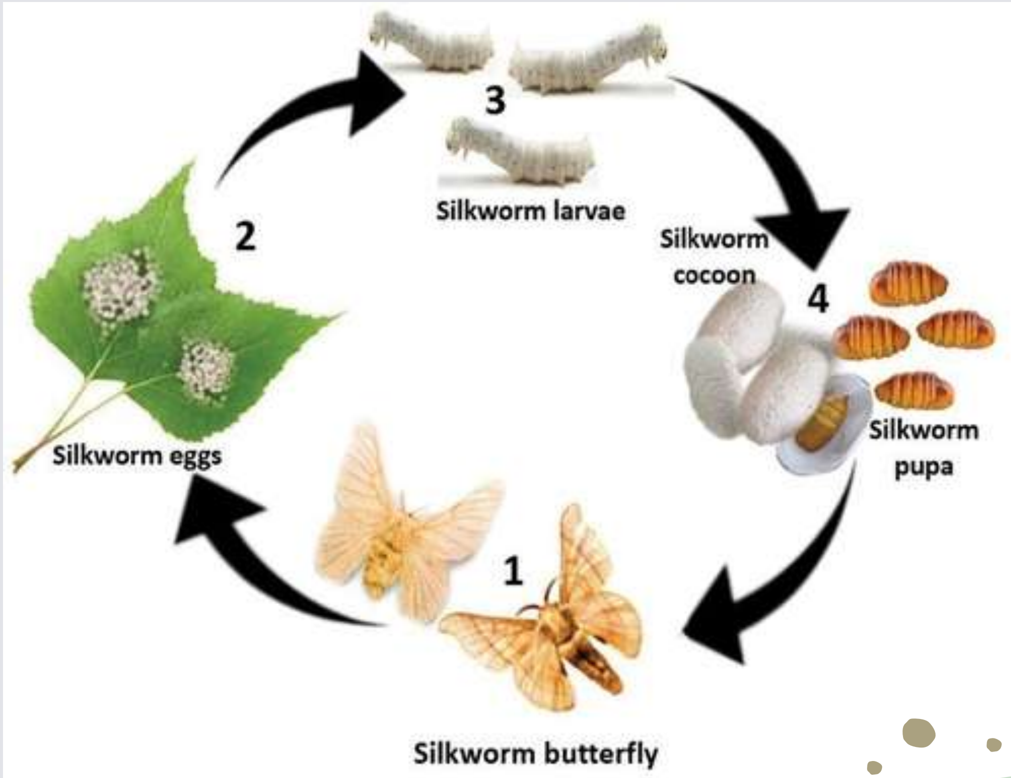
Whole insects (untreated) (under entry 9.16.2. 'terrestrial invertebrates, dead')	✗	✗	✗	✗	✓ ***	✓ ***	✓
Whole insects (treated- e.g. Freeze drying) (under entry 9.16.2 'terrestrial invertebrates, dead')	✗	✗	✗	✗	✓ ***	✓ ***	✓
Live insects (under entry 9.16.1 'terrestrial invertebrates, live')	✗	✓	✓	✓	✓	✓	✓
Hydrolysed insect proteins (under entry 9.6.1. 'Hydrolysed animal proteins')	✓	✓	✓	✓	✓	✓	✓

Silkworm – *Bombyx mori* L. (Lepidoptera: Bombycidae)

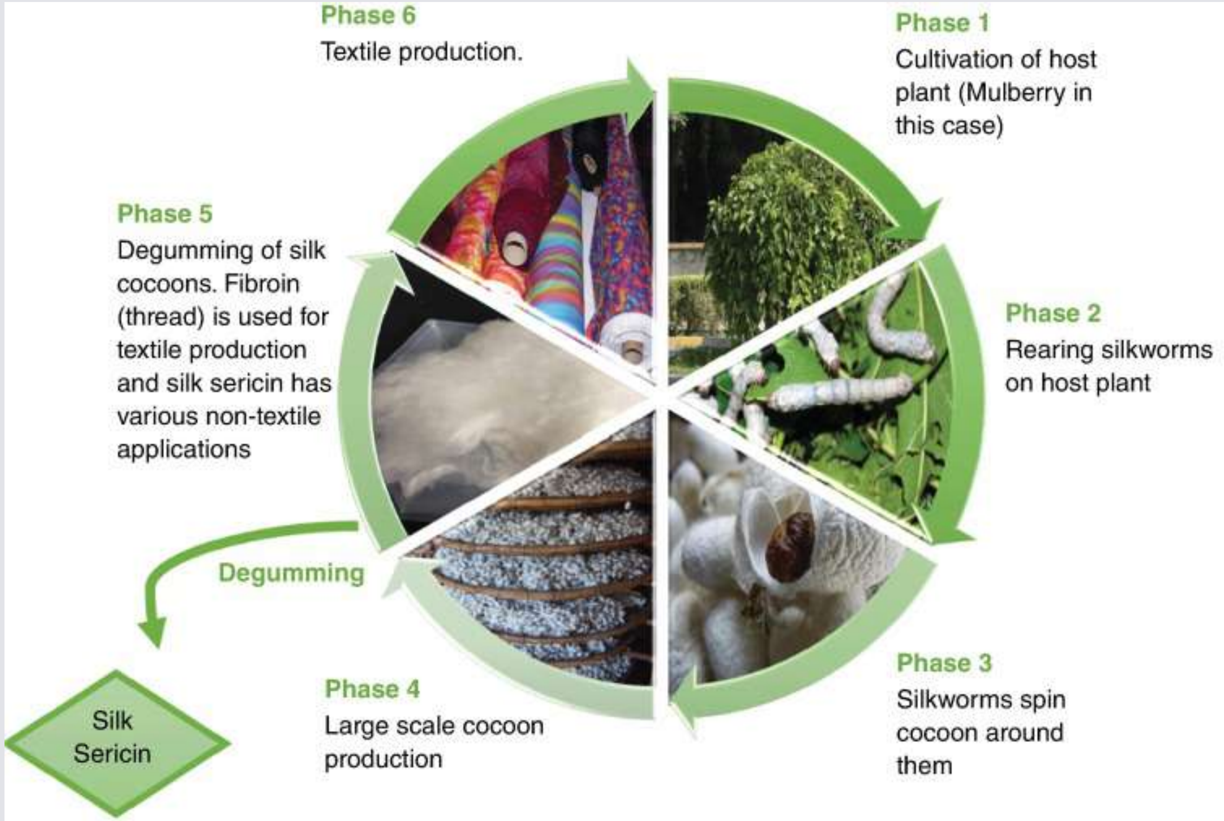
Bombyx mori **L.**

•Economically significant: **99% of global silk production**.



Silk production

- Mulberry cultivation
- Larva feeding
- Cocoon spinning
- Cocoon harvesting
- Stoving
- Sorting
- Reeling
- Free chrysalis



Bombyx mori L.

- Diet: Exclusively **mulberry leaves**
- Leaves are collected after pruning and provided to larvae instantly to remain tender
- Trees need irrigation and fertilization

Processing the feed:

- 1st – 3rd instar: Leaves are **chopped** for easier consumption
- 4th instar: whole leaves
- 5th instar: Whole stem with leaves

Feed consumption for 1 'box' of eggs (20k eggs)

- 650kg fresh leaves
- 0,06ha

Dedos, S. (2023). Sericulture. 330. https://doi.org/10.57713/KALLIPOS-207

Bombyx mori L.

- 65% of working hours → larva feeding
- 35% for cocoon preparation and harvesting

Dedos, S. (2023). Sericulture. 330. https://doi.org/10.57713/KALLIPOS-207

Aim of the Study

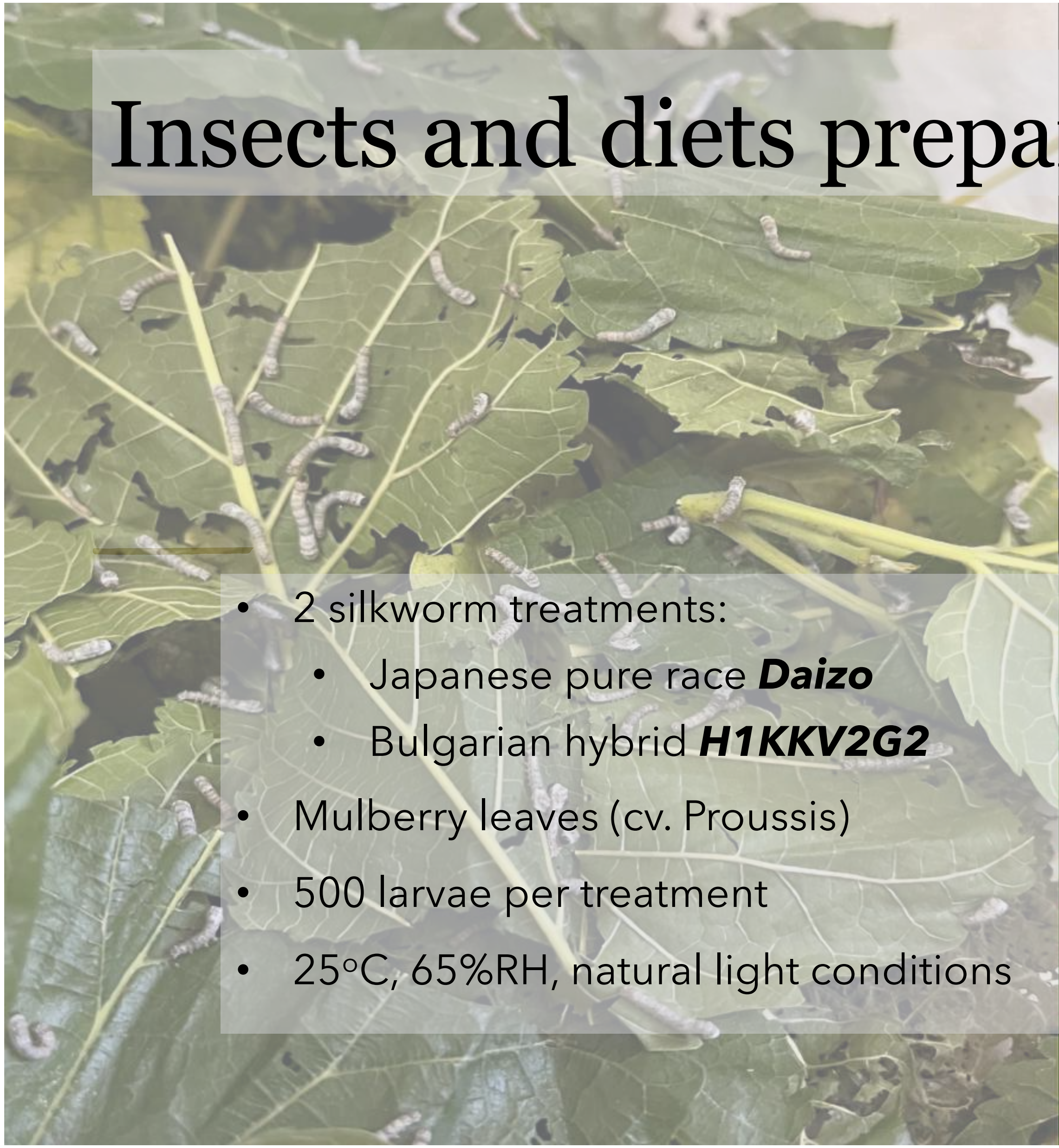
•Assess the **nutritional composition** and **conversion efficiencies** of:

•***Bombyx mori*** larvae and pupae

•Examine the **environmental efficiency** of silkworm-based feeds.

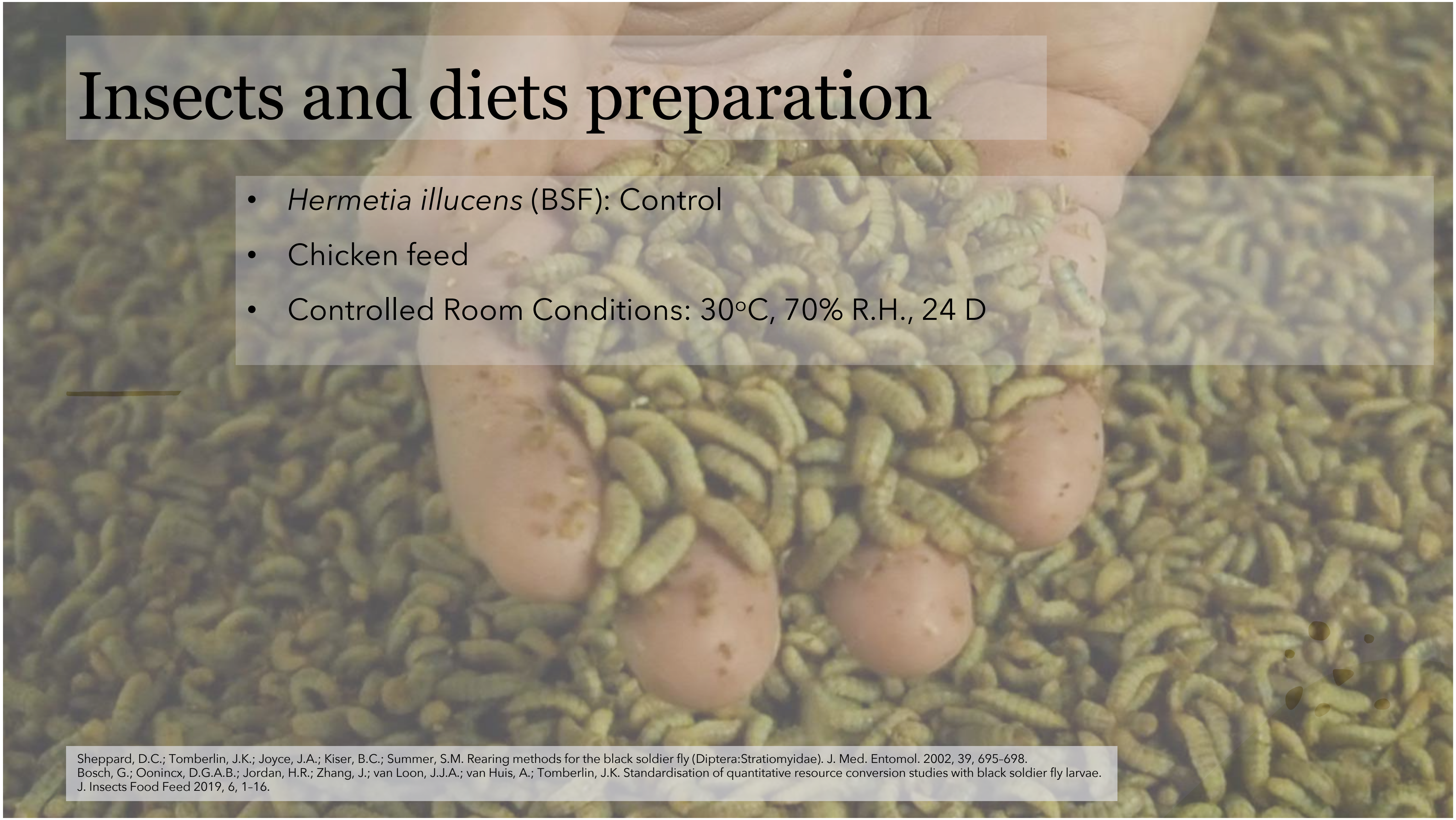
Materials and Methods

Insects and diets preparation



- 2 silkworm treatments:
  - Japanese pure race ***Daizo***
  - Bulgarian hybrid ***H1KKV2G2***
- Mulberry leaves (cv. Proussis)
- 500 larvae per treatment
- 25°C, 65%RH, natural light conditions

Insects and diets preparation



- *Hermetia illucens* (BSF): Control
- Chicken feed
- Controlled Room Conditions: 30^{o}C, 70% R.H., 24 D

Sheppard, D.C.; Tomberlin, J.K.; Joyce, J.A.; Kiser, B.C.; Summer, S.M. Rearing methods for the black soldier fly (Diptera:Stratiomyidae). J. Med. Entomol. 2002, 39, 695–698.
Bosch, G.; Oonincx, D.G.A.B.; Jordan, H.R.; Zhang, J.; van Loon, J.J.A.; van Huis, A.; Tomberlin, J.K. Standardisation of quantitative resource conversion studies with black soldier fly larvae. J. Insects Food Feed 2019, 6, 1–16.

Insects and diets preparation

Diet Nutritional Value

	D.M	Total N (%DM)	C. Protein (%DM)	C.Fat (%DM)	Ash (%DM)
Mulberry leaves	92.53	2.89	18.11	7.25	16.88
BSF diet	91.40	15.23	95.18	2.46	5.47

Insect Nutrients

Insects Samples:

- *B. mori* 5th instar larvae
- *B. mori* pupae
- BSF 6th instar larvae
- Dried
- Ground
- Total nitrogen content: Kjeldahl method
- Non-protein nitrogen (NPN): trichloroacetic acid (TCA) method
- Crude fat (CF): Soxhlet method
- Ash: Ashing samples at 550°C for 4 hours

International Organization for Standardization. (1999). *Animal Feeding Stuffs: Determination of Fat Content*. International Organization for Standardization.
International Organization for Standardization. (2002). *Animal Feeding Stuffs: Determination of Crude Ash*. International Organization for Standardization.
International Organization for Standardization. (2005). *Animal Feeding Stuffs: Determination of Nitrogen Content and Calculation of Crude Protein Content*. International Organization for Standardization.
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Biological & Conversion Parameters

- 15 first-instar silkworms were selected
- Daily measurements:
  - Viability
  - Body weight
  - Feces weight
  - Feed provided
  - Feed consumption
  - BCR
  - PrCR

Dedos, S. (2023). *Sericulture*. 330. https://doi.org/10.57713/KALLIPOS-207

Biological & Conversion Parameters

$$Bioconversion\ rate\ \% \quad = \frac{Weight\ gain\ (g)}{Feed\ mass\ (g)}\ x\ 100$$

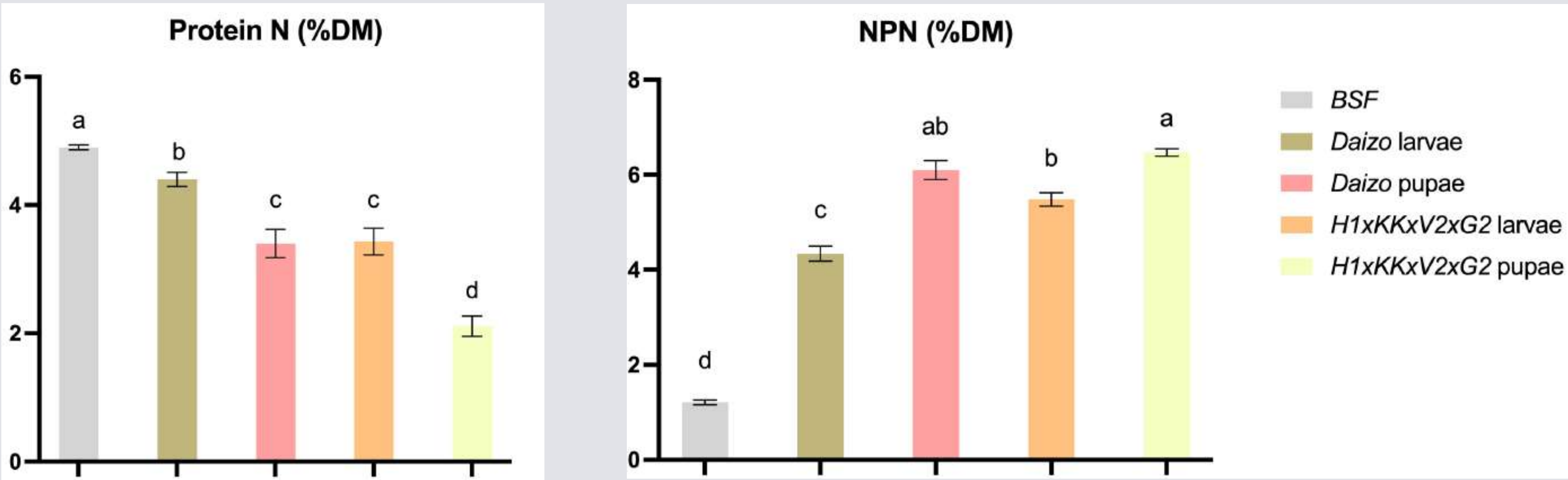
$$Protein\ Conversion\ Ratio\ \% \quad = \frac{Protein\ gain\ (g)}{Protein\ in\ feed\ mass\ (g)}\ x\ 100$$

Statistical Analyses

- ANOVA (a<0.05)
- Data transformation [y=log(x+1)]
- Multiple comparisons (Tukey HSD)
- JMP v 18.0 (SAS Statistics)
- Prism (GraphPad)

Results

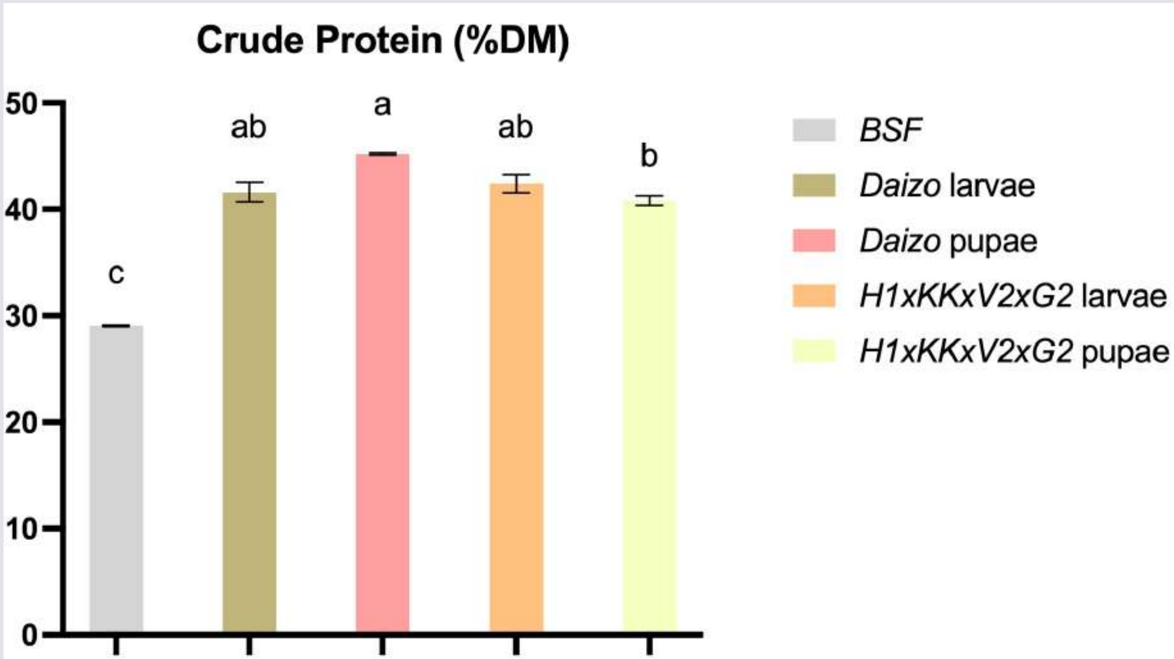
Protein and Non-Protein nitrogen



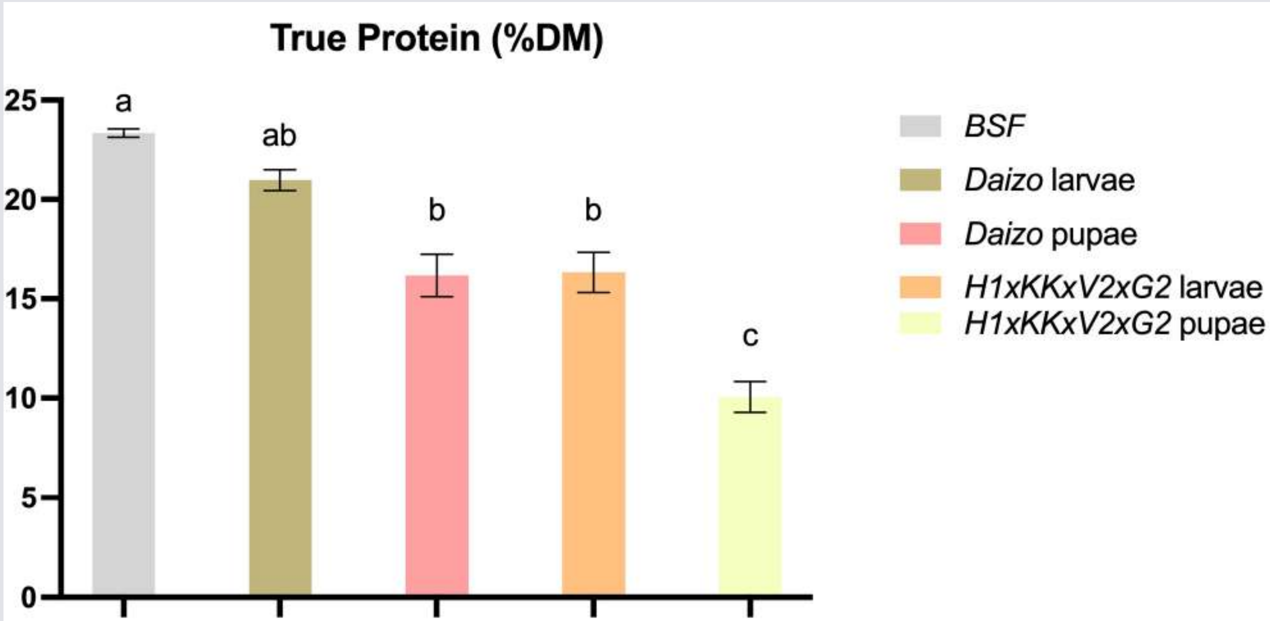
Protein and non-protein nitrogen (% of DM) for BSF and *Bombyx mori* Daizo race and H1x KKx V2x V2 hybrid (mean ± standard error). Columns without common letters indicate significant differences.

Crude Protein

Crude Protein (% of DM) for BSF and *Bombyx mori* Daizo race and H1x KKx V2x V2 hybrid (mean ± standard error). Columns without common letters indicate significant differences



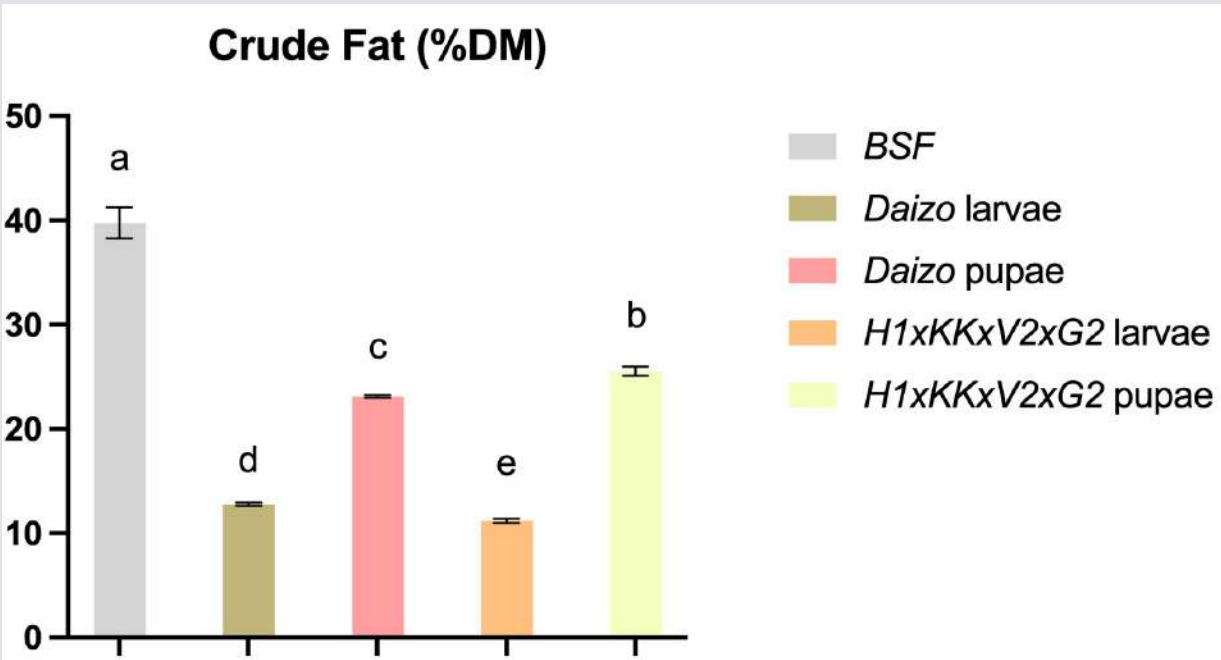
True Protein



True Protein (% of DM) for BSF and *Bombyx mori* Daizo race and H1x KKx V2x V2 hybrid (mean ± standard error). Columns without common letters indicate significant differences

Crude Fat

Crude Fat (% of DM) for BSF and *Bombyx mori* Daizo race and H1x KKx V2x V2 hybrid (mean ± standard error). Columns without common letters indicate significant differences



Insect Nutrients

Treatments	Total N (%DM)	Protein N (%DM)	NPN (%DM)	C. Protein (%DM)	True Protein (%DM)	Ash (%DM)	Crude Fat (%DM)
BSF	6.11 ± 0,02 [c]	4.9 ± 0,04 [a]	1.21 ± 0,05 [d]	29.07 ± 0,08 [c]	23.32 ± 0,21 [a]	17.43 ± 5,56 [a]	39.76 ± 1,49 [a]
Daizo L	8.74 ± 0,19 [ab]	4.40 ± 0,11 [b]	4.34 ± 0,16 [c]	41.62 ± 0,9 [ab]	20.97 ± 0,52 [ab]	11.18 ± 0,27 [a]	12.76 ± 0,17 [d]
HKVG L	8.91 ± 0,18 [ab]	3.43 ± 0,21 [c]	5.48 ± 0,14 [b]	42.42 ± 0,86 [ab]	16.32 ± 1,0 [b]	10.12 ± 0,15 [a]	11.19 ± 0,20 [e]
Daizo P	9.50 ± 0,02 [a]	3.40 ± 0,22 [c]	6.10 ± 0,20 [ab]	45.20 ± 0,10 [a]	16.17 ± 1,06 [b]	5.49 ± 0,02 [b]	23.12 ± 0,14 [c]
HKVG P	8.58 ± 0,10 [b]	2.11 ± 0,16 [d]	6.47 ± 0,08 [a]	40.84 ± 0,46 [b]	10.06 ± 0,77 [c]	4.89 ± 0,06 [b]	25.53 ± 0,44 [b]

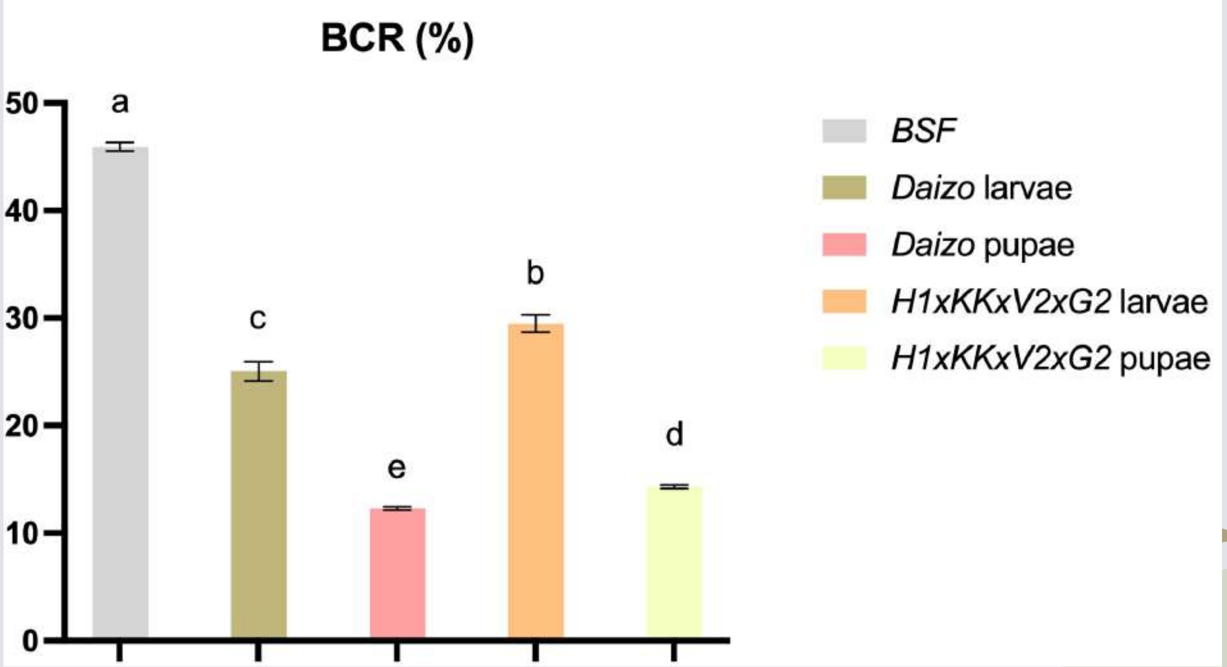
Not common letters within each column indicate significant differences (ANOVA, a<0.05, Tukey HSD)

Biological Parameters

Insects	Development (d)	Survival Rate	Wet mass per larva (g)	Yield per larva (g in DM)
BSF	11.0	100	0.178	0.057
Daizo larvae	35.0	100	4.83	1.18
HKVG Larvae	35.0	100	4.55	1.14
Daizo Pupae	45.0	100	2.39	0.58077
HKVG Pupae	45.0	100	2.28	0.56544

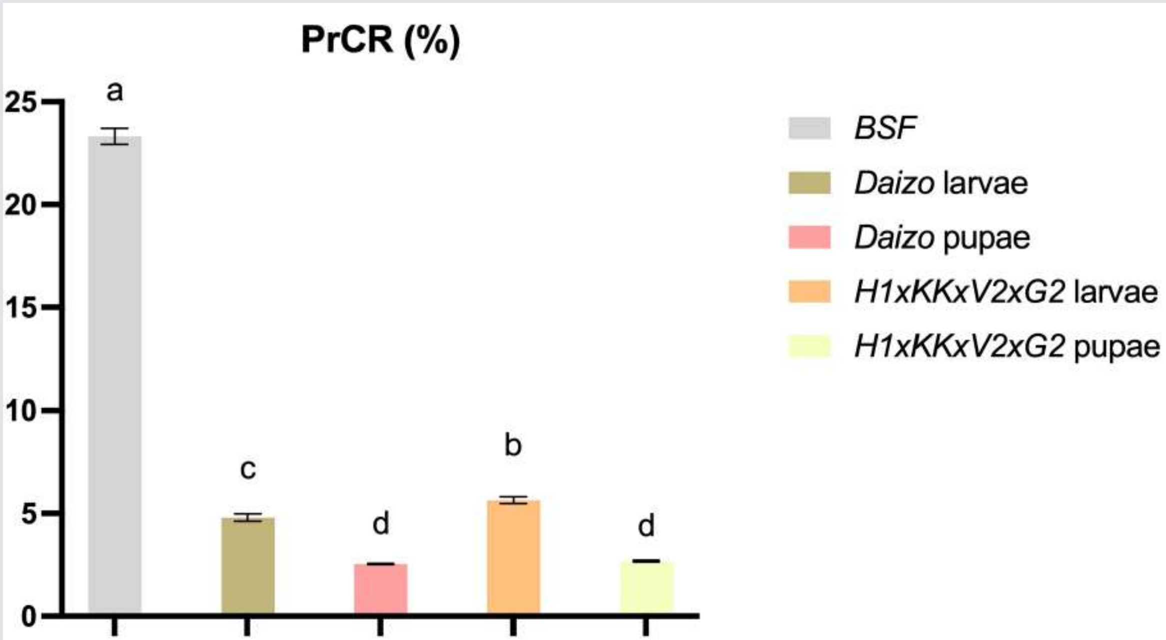
BCR (%)

Bioconversion ratio (%) for BSF and *Bombyx mori* Daizo race and H1x KKx V2x V2 hybrid (mean ± standard error). Columns without common letters indicate significant differences



PrCR (%)

Protein Conversion Rate (%) for BSF and *Bombyx mori* Daizo race and H1x KKx V2x V2 hybrid (mean ± standard error). Columns without common letters indicate significant differences



Conversion Parameters

Insects	PrCR	BCR
BSF	23.31 ± 0,39 [a]	45.91 ± 0,41 [a]
Daizo larvae	4,8 ± 0,18 [c]	25.04 ± 0,92 [c]
HKVG Larvae	5.65 ± 0,16 [b]	29.49 ± 0,81 [b]
Daizo Pupae	2.56 ± 0,03 [d]	12.29 ± 0,15 [e]
HKVG Pupae	2.69 ± 0,03 [d]	14.3 ± 0,16 [d]

Not common letters within each column indicate significant differences (ANOVA, a<0.05, Tukey HSD)

Discussion

Comparisons in nutritional value

- BSF:
  - Higher Protein levels
  - Much higher Fat levels
- Silkworm:
  - From high to decent Protein
  - From decent to low fat
  - In Daizo: Larvae protein not different from pupae
  - Crude fat: Higher in pupae

Conversion

- BSF:
  - Higher PrCR and BCR
  - Ability to process diverse organic waste efficiently
- Silkworm:
  - Efficient utilization of **mulberry protein**.
  - Monophagous: High levels of true protein
  - Larvae: Higher BCR and PrCR than pupae

Environmental impact

- BSF:
  - Less land demand
  - Smaller water footprint

- Silkworm:
  - Fresh mulberry leaves
  - Need for land
  - Low water requirements
  - Mulberry trees: can sequester up to 81.65 t. CO_2 / ha / year
  - Unlike soy: Do not rely on deforestation

Giacomin, A. M., Garcia, J. B., Zonatti, W. F., Silva-Santos, M. C., Laktim, M. C., & Baruque-Ramos, J. (2017). Silk industry and carbon footprint mitigation. *IOP Conference Series: Materials Science and Engineering*, *254*, 192008. https://doi.org/10.1088/1757-899X/254/19/192008

Dai, Y., Zhu, H., Du, H., Zhang, J., & Wen, B. (2009). An evaluation on economic value and ecological function of mulberry resources. *Protection Forest Science and Technology*, *1*, 78–80.

Liu, Y., Li, Y., Peng, Y., He, J., Xiao, D., Chen, C., Li, F., Huang, R., & Yin, Y. (2019). Dietary mulberry leaf powder affects growth performance, carcass traits and meat quality in finishing pigs. *Journal of Animal Physiology and Animal Nutrition*, *103*(6), 1934–1945. https://doi.org/10.1111/jpn.13203

Wang, C., Yang, F., Wang, Q., Zhou, X., Xie, M., Kang, P., Wang, Y., & Peng, X. (2017). Nutritive Value of Mulberry Leaf Meal and its Effect on the Performance of 35-70-Day-Old Geese. *The Journal of Poultry Science*, *54*(1), 41–46. https://doi.org/10.2141/jpsa.0160070

Rahmathulla, V. K., Tilak, R., & Rajan, R. K. (2006). Influence of Moisture Content of Mulberry Leaf on Growth and Silk Production in Bombyx mori L. *Caspian Journal of Environmental Sciences*, *4*(1), 25–30.

Role in Circular Economy

- BSF:
  - Process agricultural by-products, food waste, helping to close the nutrient cycle and reduce food waste disposal issues.
  - >50% of food waste: Transformed into protein and fat
  - Frass: organic fertilizer
  - Variety of waste streams: Adaptable for large-scale waste management
- Silkworm:
  - Larvae: very low impact
  - Pupae: Sericulture by-product
  - No need for feeding
  - Collection of other value-added by-product (sericin)

Giacomin, A. M., Garcia, J. B., Zonatti, W. F., Silva-Santos, M. C., Laktim, M. C., & Baruque-Ramos, J. (2017). Silk industry and carbon footprint mitigation. *IOP Conference Series: Materials Science and Engineering*, *254*, 192008. https://doi.org/10.1088/1757-899X/254/19/192008

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Conclusions

- Silkworm is a promising alternative feed, due to the true protein content and digestibility
- Compared to BSF, silkworm pupae have less true protein content, fat and adaptability to diverse feedstocks
- Their role in the circular economy is significant only as pupae (by-product)
- Economically viable after harvesting silk, potentially sericin

Conclusions

Silkworm pupae stand out as a sustainable, high-protein feed alternative, offering environmental, economic, and nutritional advantages.

Their integration into circular economy models enhances resource efficiency while reducing waste from sericulture.

Despite challenges in scalability and market adoption, their potential in livestock, aquaculture, and bioproduct industries makes them a promising candidate for future sustainable feed solutions.

Thank you for your attention!!