MICROBIOLOGICAL MONITORING OF SILKWORM FARMS IN VENETO REGION: A CASE STUDY

Luca Tassoni[1*], Silvia Cappellozza [2], Antonella Dalle Zotte[1], and Alessio Saviane [2]

[1]Department of Animal Medicine, Production and Health, University of Padova, Italy

[2] Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Centro di Ricerca Agricoltura e Ambiente, Laboratorio di Gelsibachicoltura, 35143 Padua, Italy

*Corresponding author email: luca.tassoni@studenti.unipd.it

I. INTRODUCTION

Silkworm (*Bombyx mori*) has recently been added to the list of insect species intended for the production of feed for farmed animals in Europe [1]. Silkworms are primarily farmed for their silk, and their pupae are the main by-product of the silk reeling process. The pupae are rich in protein and contain a high amount of unsaturated fatty acids [2]. Moreover, silkworm pupae are characterised by the presence of bioactive and antimicrobial peptides [3] that could be an added value for their utilisation in animal nutrition and could partially obviate the use of antibiotics in intensive livestock farming. These factors make silkworm pupae a promising feed ingredient for poultry, swine and aquaculture. However, more research is required to examine the microbiological risk linked to the farming and use of silkworms, which may serve as vectors for common microorganisms with the potential to be transferred to feed and food. This study is an initial effort to evaluate how microbial contamination varies across different stages of insect rearing in both the insects and the actual rearing environment. Finally, we assessed how the dried pupae are affected by the microbial contamination that arose throughout the rearing phases.

II. MATERIALS AND METHODS

Unlike other insect species, silkworms were still farmed seasonally on a modest scale by local farmers and fed solely mulberry leaves. During the spring of 2022, four farms in the Veneto region (Italy) were monitored in the period of silkworm-rearing activities in the framework of a project for rural development (Serinnovation). Environmental samples and whole animals were collected in triplicate at various phases of the rearing cycle, from the first larval instar to the pupal stage, as fresh but even as dried material that might be the starting point for feed production. Samples were tested for a limited number of microorganisms such as mesophiles, coliforms, yeast and molds, which are often employed as indicators of the hygiene quality in food production environments. Values regarding coliforms and mesophiles were reported as raw data in Table 1. Due to their adoption of a good practice production policy, all the participating farmers were aware of the primary sanitary processes to apply in silkworm rearing. Reared larvae belong to a poly-hybrid strain developed at and supplied by the CREA - Sericulture Laboratory of Padova (Italy), which also sanitises the eggs and ensures they are free of specific pathogens, agents of the main silkworm diseases.

III. RESULTS AND DISCUSSION

The analyses reveal that the newly-born larvae are free from coliforms and display only mild mesophile contamination. Throughout the 40-day rearing period, yeast and moulds did not appear to be a problem, but we did observe a general increase in mesophiles and coliforms for the hatching stage. We noticed a great data variability both among and within farmers, suggesting a great variation in rearing conditions even inside the single farm. Fresh pupae were characterised by

mesophile contamination ranging from 1.5×10^4 to 3.6×10^7 CFU/g and coliforms from 7×10^1 to 1.3×10^7 CFU/g. Mulberry leaves, as well as the presence of small animals and other insects in the environment, could represent significant sources of microbiological pollution in the examined farms. The high load of coliforms found, however, suggests the possibility that farmers themselves may be responsible for the contamination of larvae and leaves. Despite the high microbial load observed in fresh pupae the drying process they underwent dramatically reduced the number of mesophiles and coliforms, bringing the level below 1.2×10^5 and 3.8×10^3 CFU/g respectively, indicating that the thermal processing could partially mitigate the microbial risk within certain limits.

Table 1. Data of coliforms and mesophiles expressed as CFU/g.

farmer	coliforms		mesophiles	
	Fp	Dp	Fp	Dp
f1	3.9E+04	0.0E+00	1.8E+05	5.1E+04
	7.0E+01	0.0E+00	1.2E+05	3.1E+02
	3.3E+02	9.0E+01	6.7E+04	8.5E+02
f2	2.5E+05	0.0E+00	6.5E+05	1.1E+04
	2.6E+05	0.0E+00	2.5E+05	2.4E+02
	3.7E+04	0.0E+00	3.7E+05	4.5E+03
f3	6.7E+02	7.9E+02	1.5E+04	3.9E+03
	1.3E+07	3.8E+03	3.6E+07	1.2E+05
	4.1E+04	6.0E+01	1.2E+05	3.2E+02
f4	6.3E+03	5.0E+01	1.6E+05	7.6E+03
	4.5E+04	8.4E+02	1.3E+05	7.0E+03
	1.1E+05	2.0E+01	3.4E+05	1.3E+03

Fp = fresh pupae; DP = Dried pupae.

IV. CONCLUSION

The research emphasises the importance of providing more extensive training on proper hygiene practices to farmers who rear silkworms, particularly if the pupae are intended for feed use. While thermal processing can effectively reduce the risk of microbial contamination, it may not be sufficient if the initial material is heavily contaminated.

ACKNOWLEDGEMENTS

This research was supported by funds of the project “Serinnovation - Dimostrazione d’innovazione, qualità, tracciabilità in gelsibachicoltura per lo sviluppo di fonti integrative per le aziende agricole - Misura 16.1-2 - Programma di sviluppo rurale per il Veneto 2014-2020-DGR 2175 del 23/12/2016”. The authors thank all the farmers involved in the study, belonging to the Serinnovation Operational Group, for their assistance during the monitoring and sample collection.

REFERENCES

1. COMMISSION REGULATION (EU) 2021/1925 of 5 November 2021 "amending certain Annexes to Regulation (EU) No 142/2011 as regards the requirements for placing on the market of certain insect products and the adaptation of a containment method".
2. Wang, W., Xu, L., Zou, Y., Pang, D., Shi, W., Mu, L., Li, E., Lan, D., Wang, Y. & Liao, S. (2020). Comprehensive identification of principal lipid classes and tocochromanols in silkworm (*Antheraea pernyi* and *Bombyx mori*) pupae oils. European Journal of Lipid Science and Technology 122:1900280.
3. Altomare, A. A., Baron, G., Aldini, G., Carini, M. and D'Amato, A. (2020). Silkworm pupae as source of high-value edible proteins and of bioactive peptides. Food Science & Nutrition 8:2652-2661.