





## OCCURRENCE OF METALS AND ANTIBIOTICS IN PROCESSEI Animal Protein in ANIMAL PROTEINS (PAPS) FOR ANIMAL NUTRITION

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#### Introduction

Following the bovine spongiform encephalopathy (BSE) crisis, in 2001 processed animal proteins (PAPs) were banned as feed ingredients in livestock in the European Union (EU). PAPs are an important protein source with a high nutritional value and low costs. Such material should be treated according to ruled rendering processes, with defined temperature and pressure conditions, to be eventually used as a feed ingredient [1]. Over the years, a relaxation of feed bans reintroduced non-ruminant PAPs in fish feed first and later allowed pig and poultry PAPs in poultry and pig feed, avoiding cannibalism [2].

In 2015, EFSA evaluated insect species suitable for feed purpose, and assessed the relationship between production methods, substrate and species of insects, and their biological and chemical hazard; in 2017 the European Commission approved the introduction of insects in aquaculture and in 2021 also in pig and poultry feed [3].

Legislative framework describes microbiological quality assessment, while potential chemical hazards are poorly investigated. This study aimed to investigate chemicals in PAPs providing information on essential and nonessential elements (cobalt, nickel, chromium, copper, zinc, iron, and manganese; arsenic, cadmium, lead and mercury) as well as antimicrobial (tetracyclines, TCLs).

To assure quality control and to avoid crosscontamination during production and transport PAPs of different origin, isolation and detection of insect PAPs utilized in feed has also been performed.

### Materials and methods

- a) Metals determination was performed in <u>55 PAPs</u> produced in different EU plant (Italy, France, Spain, and the United Kingdom) according to current European regulations and treated with different rendering processes. Samples were weighted, homogenized and subjected to acid mineralization before metals quantification by ICP-MS (Co, Cr, Cu, Fe, Mn, Ni, Zn), GF-AAS (As, Cd, Pb) and Direct Mercury Analyzer (Hg).
- b) TCLs in 55 PAPs samples were determined by a sensitive LC-ESIMS/MS method developed for this purpose, after an in vitro acidic digestion (Figure 1).
- c) PAPs-insects analysis: <u>48 samples</u> were collected (8 samples of breeding substrate made up by former food), 7 samples of 4th-instar larvae, 15 samples of protein meal of larvae produced according to the current regulation and 18 samples of spent breeding substrate, collected at the end of the productive cycle). Metals analysis (12 trace elements) was performed by ICP MS as previously described.

#### References

[1] EU Council Regulation 2011/142/EC of 25 February 2011 Off. J. Eur. Union 2011, L54,1–254

- [2] Commission Regulation (EU) 2021/1372 C/2021/6012. OJ L 295, 18.8.2021, p. 1–17.
- [3] Commission Regulation (EU) 1372/2021. Of. J. Eur. L 295, p. 1-17

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#### Results

- a. Essential elements were found in the following decreasing order: Fe>Zn>Cu>Mn>Cr>Ni>Co, significative differences among PAPs categories for manganese, cobalt and nickel. Between the nonessential elements, negligible values were found for As, Cd and Hg while in poultry PAPs a Pb maximum value of 13 mg/kg was determined (not compliant).
- 40 out of 55 samples were shown to contain more than 25 μg/kg of TCLs, ranging from 26 to 457 μg/kg) and residual antimicrobial activity was demonstrated in few samples.
- c. Essential elements were found in the following order: Fe>Zn>Cu>Ni>Se>Cr. Non-essential element concentrations were found lower than the limits set by UE Union Regulations.

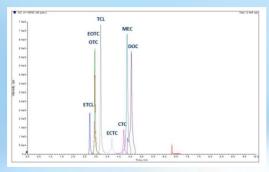


Figure 1 Exracted ion chromatogram (XIC) of TCLs in a sample fortified at 250 µg/kg.

#### **Discussion and Conclusion**

PAPs could be a useful supplement for animal diet due to their natural content of essential elements. A careful monitoring of chemical elements should be required and eventually guidelines have to be drafted for a correct use of PAPs to ensure a safe and sustainable feed production. Insect farming for feed production could at compensate the growing demand for protein sources.

However, further studies are necessary to investigate the level of contamination of TCLs, other antibiotics and their metabolites, and their possible involvement of the onset of multidrug resistance among pathogenic bacteria in PAPs.



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