

# Bypassing the rumen using lipid nanoparticles

João Albuquerque<sup>1,2</sup>  
Ingrid V. Dorpe<sup>3</sup>, Ana R. J. Cabrita<sup>4</sup>, Salette Reis<sup>1</sup>, Susana Casal<sup>5</sup>, Ana R. Neves<sup>1</sup>

<sup>1</sup> UCIBIO, REQUIMTE, Department of Chemical Sciences, FFUP, Rua Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal

<sup>2</sup> Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto (UP), Rua Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal

<sup>3</sup> PREMIX-Especialidades Agrícolas e Pecuárias. Lda, Parque Industrial II – Neiva, 4935-232, Viana do Castelo, Portugal

<sup>4</sup> LAQV, REQUIMTE, ICBAS, UP, Rua Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal

<sup>5</sup> LAQV, REQUIMTE, Laboratory of Bromatology and Hydrology, FFUP, Rua Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal

Joao.albuquerque.costa@gmail.com

Feed digestion in ruminant animals largely rely on rumen microbial fermentation. The microbiota of the rumen extensively degrades dietary protein, the resulting products being incorporated into their own microbial proteins, while lipids pass through relatively unscathed. Since microbial protein digested in the small intestine is not sufficient to meet the amino acids requirement of high producing animals, an effective protection of feed N from rumen fermentation is needed. In the present study, we propose a new approach that takes advantage of nanotechnology to bypass the microbial fermentation in the rumen, but also to increase the absorption of the nutrients into the bloodstream, relying on lipid nanoparticles (NPs).

A wide variety of different formulations were proposed and tested, consisting of solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and multiple lipid nanoparticles (MLN). All these formulations were characterized in terms of size, polydispersity index and zeta potential and their stability in rumen inoculum assessed.

Results showed that only SLN formulations composed of stearic acid and arachidic acid could resist digestion in the rumen inoculum, maintaining their size range of 300-500 nm after incubation and with a highly negative surface charge of around -35 mV. Transmission electron microscopy images confirmed that both SLN could resist digestion and maintain both their size and spherical morphology. The NPs were loaded with lysine and an adequate quantification method, based on high performance liquid chromatography (HPLC) with fluorescence detection of dansylated derivatives, was developed. Using this method, the encapsulation efficiency of

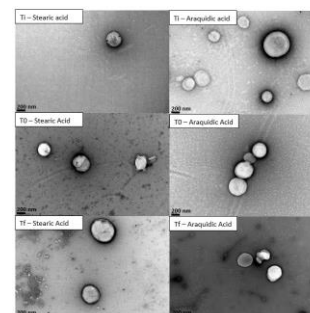
the formulations was determined, rendering values of up-to 40%.

To conclude, this preliminary study showed that SLN could be synthesized, with adequate physical properties, and with the ability to resist digestion in the rumen, retaining their size, zeta potential and morphology. They were also capable of being loaded with a highly hydrophilic molecule with reasonable efficiency. The proposed formulations are, therefore, promising candidates for future rumen-bypass applications in ruminant nutrition and may help to surpass the current limitations of the existing technologies and products.

## Acknowledgments:

This work received financial support from FCT (Fundação para a Ciência e a Tecnologia) and FEDER funds under Program PT2020 (project 007728 - UID/QUI/04378/2013). JA thanks FCT, SANFEED Doctoral Programme and PREMIX® for his PhD grant ref. PD/BDE/114426/2016. ARN also thanks her Post-Doc grant under the project NORTE-01-0145-FEDER-000011.

## Figures



**Figure 1.** Transmission electron micrographs of NPs after synthesis (Ti), after contact with rumen inoculum (TO) and after 24h incubation at 39 °C in rumen inoculum (Tf).