

## Effect of a Purification Step and the Type of Internal Standard Used on Fatty Acid Determination of Grass and Maize Silages

SUSANA P. ALVES,<sup>†,‡</sup> ANA R. J. CABRITA,<sup>§</sup> ANTÓNIO J. M. FONSECA,<sup>‡</sup> AND  
RUI J. B. BESSA<sup>\*,†,||</sup>

<sup>†</sup>INRB - Instituto Nacional dos Recursos Biológicos, Unidade de Produção Animal, Fonte-Boa, 2005-048 Vale de Santarém, Portugal, <sup>‡</sup>REQUIMTE, ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua Padre Armando Quintas, 4485-661 Vairão VC, Portugal, <sup>§</sup>REQUIMTE, SAECA, Faculdade de Ciências, Universidade do Porto, Rua Padre Armando Quintas, 4485-661 Vairão VC, Portugal, and <sup>||</sup>Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, CIISA, Pólo Universitário do Alto da Ajuda, Av. da Universidade Técnica, 1300-477 Lisboa, Portugal

The fatty acid (FA) analysis of grass and maize silages was studied by application of a direct transesterification method (DT) followed by purification by solid-phase extraction (SPE). The choice of the internal standard (IS) for quantification of FA by gas–liquid chromatography (GLC) was also studied. The acidic DT method applied to grass silage samples produced a high amount of non-fatty acid methyl ester compounds (non-FAME) compared with those formed in maize silages. The application of the SPE cleanup step reduced significantly the amount of non-FAME compounds in both samples. Five FAs were tested as IS; among them, 3 were naturally present in all silages, however their use as IS did not affect quantification of total FA composition. Nevertheless, some minor FAs present in silages were significantly affected by the IS used. Additionally, application of corrections to the GLC peak areas did not significantly influence quantification of total FA composition of silages.

**KEYWORDS:** Silage; fatty acid methyl esters; internal standard; gas–liquid chromatography; solid-phase extraction

\*Corresponding author. Tel: (+351) 213652871. Fax: (+351) 213652884. E-mail: rjbbessa@fmv.utl.pt.