

## **PROGRAM BOOK**

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## Methods to analyze total endogenous protein losses in pigs

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In protein evaluation, current systems like the Digestible Indispensable Amino Acid Score (DIAAS), correct apparent amino acid digestibility for basal endogenous protein losses (bEPL), ignoring the potential influence of the diet on these losses. The quantification of total endogenous protein losses (tEPL) poses a challenge. We evaluated different methods for quantifying tEPL and bEPL, and to assess their potential in discriminating between tEPL originating from bacteria and the host. Using an incomplete Youden square design, twelve ileal cannulated pigs received ten different protein sources, and a nitrogen-free (NF) diet. Ileal bEPL were assessed with an NF diet (NF method) and whey protein isolate (WPI method). Ileal tEPL were estimated using 16S- + 18S gene copy qPCR, diaminopimelic acid (DAPA)+18S, and separation based on differential AA profiles in digesta, endogenous losses and bacteria. Ileal bEPL based on the NF and WPI methods were highly correlated (r=0.70, P<0.05), but using the WPI method resulted in greater bEPL, probably caused by an underestimation of bEPL by the NF method. In pigs fed the WPI diet, bEPL based on the WPI method and tEPL based on the AA profile method were highly correlated (r=0.88, P<0.01). Overall, tEPL based on the AA profile method were moderately correlated with the 16S+18S method (r=0.58, P<0.001), and DAPA+18S method (r=0.57, P<0.001). No correlations were observed between bacterial EPL based on the AA profile method and 16S or DAPA. Host EPL based on the AA profile method and 18S were weakly correlated (r=0.39, P<0.001). In conclusion, the AA profile method seems promising for tEPL quantification, while caution is advised with the NF method for bEPL. Despite the challenges in distinguishing between bacterial and host EPL in this study, it is evident that bacterial proteins substantially (28-72%, depending on diet and method) contribute to the tEPL.