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Analytical Methods

Inter-laboratory validation study of two immunochemical methods for detection of processed ruminant proteins

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ABSTRACT

In order to facilitate safe re-introduction of non-ruminant processed animal proteins (PAPs) in aqua feed, two immunoassays have been tested in an interlaboratory study for their capability to detect ruminant PAPs processed under European conditions. The sensitivity of the MELISA-TEK assay was improved by applying a specific extraction kit. Six approved blank pork and poultry samples were adulterated to produce 15 samples spiked at 0.5%, 1.0% and 2.0% with ruminant material, sterilised at either 133 °C or 137 °C. Fourteen participants investigated the 6 blanks and 15 spiked samples, making 21 samples for the final test. For both assays specificity and sensitivity were at 97% or higher. Concordance and accordance were higher than 95% with one exception. The results indicate that both assays provided correct results at 0.5% and higher for the detecting ruminant PAPs (sterilised at 133 °C) in non-ruminant PAPs. Given the 2% upper limit of ruminant PAPs in non-ruminant PAPs for avoiding an increase in BSE incidents, these methods are fit for monitoring non-ruminant PAPs intended for aqua feed.

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1. Introduction

The consumption of food products of animal origin is an inevitable part of our daily diet. As a result of the production of meat, milk and egg products approximately 17 Million metric tonne of animal by-products are produced in the European Union each year (Woodgate, Margry, Geesink, & Hoven, 2006). These by-products could be a highly valuable source of nutrients, especially proteins. However, consumption by farmed animals is generally prohibited for avoiding mad cow disease in particular and transmissible spongiform encephalopathies in general (European Commission, 2001).

Re-entry of safe animal by-products into the animal production chain would have great advantages for economic valorisation and sustainability. For these reasons, a gradual relaxation of the general ban, as implemented in the European Union, has been started in the past years by lifting minor elements of the ban. Examples are the use of blood meal and blood products in aqua feed, and the use of fishmeal as milk replacer for weaning ruminants (European Commission, 2008). A recent development is the permission to utilise non ruminant processed animal proteins (PAPs) as ingredient in aqua feed, provided the absence of prohibited ruminant PAP (European Commission, 2013). Besides PCR (Cawthraw et al., 2009; Fumière, Marien, Fernanderz Pierna, Baeten, & Berben, 2010; Fumière, Veys, et al., 2009; Ha, Jung, Nam, & Moon, 2006; Prado et al., 2007; Yancy et al., 2009), immunoassays have shown to be promising methods for testing the presence of ruminant PAP (Bremer et al., 2013; Fumière, Baeten, & Berben G., 2009, 2010; Myers et al., 2007). For instance, MELISA-TEK® shows to be capable to detect ruminant PAP sterilized at a temperature of 133 °C in a non-ruminant PAP at a level of 1%. The problems with the high and variable noise levels (background signals) were solved (Fumière, Baeten, et al., 2010). This improvement, together with the application of a dedicated extraction kit, resulted in a higher sensitivity (Bremer et al., 2013).

Considering the need for a sustainable use of non-ruminant PAPs in aqua feed, the need for valorisation of animal by-products, and most importantly a low and acceptable level of risk, an interlaboratory study was carried out thereby using two commercially available immunoassays (Reveal for Ruminant in feed and MELISA-TEK®, the latter combined with the MELISA-TEK high Sensitivity Sample Extraction kit) for screening the presence of ruminant protein in non-ruminant PAPs intended as ingredient for aqua feed. The study aimed at the validation of these two immunoassays rather than on testing the proficiency of the laboratories, which implied that strict study requirements had been set in order to assure the comparability of the lab results. The study

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and the materials used were chosen to comply with conditions as set in European legislation (European Commission, 2011: rendering at 133 °C for 20 min). Full details have been published as study report (van Raamsdonk, Margry, van Kaathoven, & Bremer, 2012).

This paper presents the core of results of this interlaboratory study and provides a framework for future application of immunoassay kits for the detection of ruminant animal proteins. Furthermore, this paper will provide elements for the procedure of organising collaborative studies for qualitative detection methods.

2. Material and methods

The interlaboratory study was organised according to the AOAC guidelines for collaborative studies (AOAC, 2002) as far as applicable to qualitative studies, with additional requirements from IUPAC (Horwitz, 1995), European Union (European Commission, 2002) and DG-SANCO (SANCO, 2009). Result evaluation was carried out with methods dedicated to qualitative tests (Macarthur & von Holst, 2012; van Raamsdonk & van der Voet, 2003; van der Voet & van Raamsdonk, 2004).

2.1. Materials

Six non-ruminant blank PAPs, verified for not containing ruminant material, were spiked with a ruminant contaminant rendered at two temperature levels (133 and 137 °C) at two spike levels (1% and 2%), each in triplicate.

2.1.1. Blanks

In view of the purpose of the study, i.e. support of the re-entry of non-ruminant animal proteins in aqua feed, both pig and poultry materials have been used as blanks and as matrices for the spiked samples.

Six animal protein materials were selected from commercially produced batches, with the criterion to comply with the requirements of the EU regulations. Materials were selected after repeated confirmation by analysis with PCR cattle, Reveal for ruminant in feed (Neogen) and MELISA-TEK® (ELISA-Technologies) that no cattle or more general no ruminant material was detectable.

The final selection consisted of three processed animal proteins of porcine origin, and three processed animal proteins of poultry origin:

- Pig A: Protein Meal 58%, supplied 1-6-2011 by DAKA, Løsning, Denmark.
- Pig B: Porcine Meal, supplied 17-6-2011 by Oldenburg Fleischmehlfabrik, Friesoythe-Kampe Germany.
- Pig C: Porcine Meal, supplied 22-6-2011 by Ten Kate, TerApelkanaal, The Netherlands
- Poultry D: Poultry Protein, supplied 21-9-2011 by Verlirend, Olen, Belgium.
- Poultry E: Poultry Meal, supplied 22-9-2011 by Avifood SL, Santa Barbara, Spain.
- Poultry F: Poultry Meal, supplied 4-7-2011 by Sonac-Denderleeuw, Denderleeuw, Belgium.

2.1.2. Spiked samples

The six blanks were used for spiking by step-wise dilution using a mixture of bovine and ovine Meat Meal (MM) and Meat and Bone Meal (MBM) (mix of 60% bovine carcase, 20% ovine carcase, 15% bovine muscle and 5% ovine muscle), each sterilised by PDM (UK) at either 133 °C or 137 °C. Spike levels of 1% and 2% were produced. Depending on their use in the different tests, either 44 or 61 jars were produced per sample.

2.1.3. Challenger sample

In addition to the 12 spiked samples, a challenger was produced in triplicate, based on non-ruminant PAPs sterilised at 133 °C and spiked with ruminant material at 0.5%.

2.1.4. Total set

Every individually sample (six blanks, twelve spiked samples, and three challengers) was based on one individual non-ruminant PAP, either pig or poultry. See for details van Raamsdonk et al. (2012) and Fig. 1.

The 21 prepared samples were analysed with MELISA-TEK® (kit Number series MRM 120106-75; kits 10, 19 and 20 of 80) for assessing the homogeneity. Each sample type was extracted and analysed in the same analysis run in fivefold (sampled from five different jars). This represented either 11.4% or 8.2% of the total number of jars produced per sample.

2.2. Experimental design

An experimental design was made according to the principles of Taguchi (Atil & Unver, 2000; Rao, Kumar, Prakasham, & Hobbs, 2008) based on a 2 × 2 (temperature × concentration) lay-out with every combination in triplicate, resulting in 12 spiked samples. The design implied that every combination of the two factors was included in the design. Six blanks were included. Additionally, three samples adulterated at 0.5% (treatment 133 °C) were added as "challengers". Every sample was analysed in duplicate (two different blind jars per sample) by the participants. This resulted in the analysis of 12 samples for every variable. See Fig. 1 for the full design. A constraint for the total design was the maximum number of analyses, which should fit in one series on one 96-wells plate. As a consequence, the 0.5% spike level was not included with a rendering temperature of 137 °C.

The total interlaboratory study was organised in three steps:

1. Training set: every participant analysed two blank and five spiked samples, fully labelled. These seven samples were meant for training purposes. Time frame: February 2012.
2. Entrance test: after showing their capability, every participant analysed a set of seven blind samples. Every set consisted of two blank and five spiked samples. This test was considered a proficiency test. Time frame: March 2012.
3. Validation experiment: after approval of the individual results of step 2, every participant analysed a set of 42 blind samples, with every individual sample of the design included in duplicate, a total of 84 analyses. Time frame: April–May 2012.

For all three tests a set of six kit blanks, negative and positive controls were added in duplicate to the series for the MELISA-TEK® procedure.

Every participant extracted the samples once, and analysed these extracts in duplicate with MELISA-TEK®. The samples were also extracted once with the Reveal extraction buffer and analysed once with a Reveal strip.

Treatments:	Blanks	Challenger	Spike levels	
Temperature:	0%	0.5%	1%	2%
Not applicable	6			
133 °C		3	3	3
137 °C			3	3

Fig. 1. Overview of the experimental design of the study. The total number of samples included in the design is 21. The final validation experiment comprised of 42 samples; every sample in duplicate.

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