



Detection of Ruminant Meals in Feed by Real-time PCR

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INTRODUCTION

Within the European Union the use of processed animal proteins (PAPs) in animal nutrition is subject to various restrictions in order to avoid the spread of a number of diseases such as BSE. An important criterion is the ban of intraspecies recycling, requiring that PAPs must not feed to animals from the same species (Regulation (EC) No 1774/2002). Enforcing these regulations requires analytical methods capable to allow species-specific identification. The lack of such methods led to the introduction of an extended feed ban for all farmed animals. The availability of analytical methods to correctly differentiate animal species is one of the conditions to re-introduce the use of non-ruminant mammalian proteins to feeds for non-ruminant species. Classical microscopy is the only official method within the EU to detect the presence of constituents of animal origin, however the method is not species specific. PCR-based methods seem a promising solution for the detection and identification of animal species in feed.

PURPOSE OF THE STUDY

The objective of this prevalidation study was to evaluate the performance characteristics of four PCR methods developed by national expert laboratories to detect and correctly identify the presence of cattle and/or ruminant material in different animal feeds.

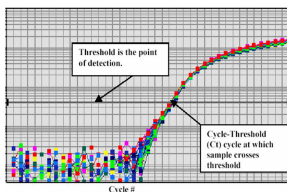
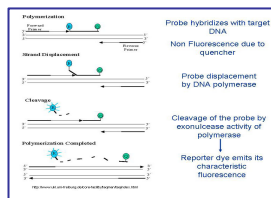
METHODOLOGY

Test materials: 15 real world compound feeds:

- Cattle meat and bone meal (MBM) at 0.1% (target level)
- Other species MBM 5% (specificity)
- Fortified with
 - Other species MBM 5% + 0.1% cattle MBM (sensitivity)
 - Heat treated cattle/pig MBM (133°C/3 bar/20 min)

PCR methods:

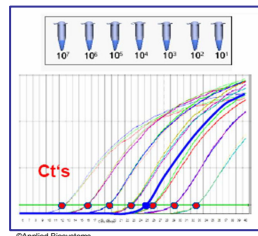
Use of real-time PCR and hybridization probes.



Real-time PCR: expression of the results as numerical values

Threshold Cycle (Ct): cycle number at which the fluorescence emission exceeds the chosen threshold.

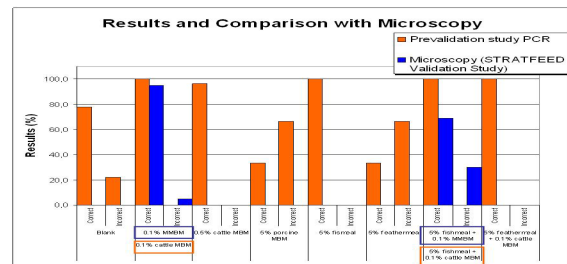
The Ct value: inversely related to the starting copy number of the target DNA sequence.



DATA REPORTED BY LABS:

- Ct values obtained for each material
- Specific cut-off Ct value to distinguish between positive and negative samples

RESULTS



Sensitivity: 100% in samples containing 0.1% cattle MBM.

Specificity: a) 100% in cattle/chicken feeds blanks or with fishmeals
b) **False Positives:** pigfeeds blanks and pigfeeds + feather meal + 5% porcine MBM in cattle or pigfeed for two labs

Evaluation

Higher Ct values than samples containing 0.1% of cattle MBM

Animal fat: possible source of DNA in compound feeds.

CONCLUSIONS

- All tests gained a very high sensitivity with respect to the Intercomparison study carried out in Stratfeed.
- All test showed capability to detect heat treated PAPs.
- A carefully set of the Cut off levels is necessary to have enough sensitivity without losing specificity.
- Some ingredients of feeds such as animal fat can be a source of false positive results, due to the presence of target DNA traces.

REFERENCE

- Prado et al., 2007 *J. Agric. Food Chem.* 55, 7495-7501

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