

Detection and identification of banned Processed Animal Protein in feedingstuffs by microscopic and PCR methods

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Abstract

The aim of the study was to present the results of comparative evaluation of the usefulness of PCR and microscopic methods for the detection of Processed Animal Protein (PAP) in feedingstuffs. In the validation study, the limit of the detection for PCR was determined on 0.05% for beef, 0.1% for pork and 0.2% for poultry meat and bone meal (MBM). Among 62 doubtful samples of feedingstuffs examined by microscopic method 41 (66.13%) were found as positive. Based on the results obtained with the use of the microscopic and PCR methods it is possible to state that the molecular biology methods can, at present, be used as a supplementary method in PAP detection.

Key words: animal feedingstuffs, meat and bone meal, PCR, PAP

Introduction

It is generally accepted that BSE infection of the cattle is caused by animal feedingstuffs containing processed animal protein (PAP) contaminated with prions. At present, with exceptions for fish meal, processed animal proteins (PAP) are banned to use as feed material for all farmed animals. General rules regarding the safe use of animal by-products are laid down by EC Regulation 1069/2009 (Regulation EC 2009).

In the present work, a specific PCR-based procedure for detection of animal constituents in feedingstuffs was used parallel with microscopic method (Commission Regulation 2009). The sensitivity and usefulness of PCR technique were discussed in the light of the results obtained.

Materials and Methods

Samples used in the study. Samples were divided into three groups: the sixty two doubtful samples of feedingstuffs; different meat and bone meal, samples of compound feedingstuffs spiked of meat and bone meal (MBM) on levels 5%, 2%, 1%, 0.5%, 0.2%, 0.1% and 0.05%.

Microscopic method. Microscopic method is described in the Commission Regulation (EC) No. 152/2009.

PCR protocol. Total DNA was extracted from 200 mg of sample using Wizard Magnetic Purification DNA System for food (Promega), according to manufacturer instructions. Three species-specific primer pairs were designed for the amplification of bovine

Table 1. Number of doubtful samples of feed materials and compound feedingstuffs examined by microscopic and PCR test for the presence of processed animal proteins.

Kind of feedingstuffs	No. of samples examined	No. of positive samples by microscopic method (%)	No. of positive samples by PCR			No. of positive samples by PCR					
			beef (%)	pork (%)	poultry (%)	sediment			flotat		
						beef (%)	pork (%)	poultry (%)	beef (%)	pork (%)	poultry (%)
Feed materials	23	9 (39.13)	1 (4.35)	1 (4.35)	2 (8.70)	1 (4.35)	1 (4.35)	1 (4.35)	1 (4.35)	1 (4.35)	1 (4.35)
for ruminants	17	14 (82.35)	0 (0)	1 (5.88)	2 (11.76)	2 (11.76)	3 (17.65)	7 (41.18)	2 (11.76)	4 (23.53)	3 (17.65)
Compound feedingstuffs											
for poultry	11	9 (81.82)	0 (0)	1 (9.09)	2 (18.18)	0 (0)	1 (9.09)	1 (9.09)	1 (9.09)	1 (9.09)	1 (9.09)
for swine	11	9 (81.82)	2 (18.18)	3 (27.27)	3 (27.27)	2 (18.18)	4 (36.36)	4 (36.36)	3 (27.27)	4 (36.36)	4 (36.36)
Total	62	41 (66.13)	3 (4.84)	6 (9.68)	9 (14.52)	5 (8.06)	9 (14.52)	13 (20.97)	7 (11.29)	11 (17.74)	9 (14.52)

(Martin et al. 2007), pork and poultry (Frezza et al. 2008). Parameters of the amplification were drawn up for individual species. Obtained products of amplification were subjected electrophoresis in 2% agarosis gel in TBE buffer, at permanently voltage 110V.

Results and Discussion

As shown in the Table 1, overall processed animal protein presence was detected by microscopic method in 41 (66.13%) out of 62 examined samples. In the study limit of detection for PCR method was determined 0.05% for beef MBM, 0.2% for poultry MBM and 0.1% for pork MBM. It was also shown that out of the total number 62 samples examined by PCR, beef DNA was detected in 3 (4.84%), poultry DNA in 9 (14.52%), and pig DNA in 6 samples (9.68%) (Table 1). In the sediments beef DNA was detected in 5 (8.06%), poultry DNA in 13 (20.97%) and pork DNA in 9 (14.52%) samples. In the flotat beef DNA was detected in 7 (11.29%), poultry DNA in 9 (14.52%) and pork DNA in 11 (17.74%) samples.

At present in European Community microscopic evaluation is the only accepted method for detection of PAP's in feedingstuffs, and it allows to detect contamination at the requested level of 1 g/kg with negative and positive results. Currently, a range of analytical approaches have been taken for the determining the animal species in wide array of degraded and processed substrates, mainly based on DNA (Martin et al. 2007). In all samples dividing with use tetrachloroethylene

previous results of PCR were confirmed indicating that application of this reagent does not influence results of the examination with PCR technique. Appearing of braking substances can be a cause of this occurrence.

Moreover, it should be mentioned that with the microscopic method, detection of even a little addition of MBM in feedingstuffs is rather easy. The analysis of the results obtained with the help of the microscopic method and PCR technique has shown that methods of the molecular biology at present can be used as supplementing tool (Frezza et al. 2008).

References

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