IDENTIFICATION OF PROCESSED ANIMAL PROTEINS (PAPS) IN FEEDSTUFFS

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ABSTRACT. Identification of processed animal protein in animal feedstuffs was performed under the feed safety programme to ensure that the products used locally to feed the livestock are safe and properly labeled to prevent unnecessary incidence that will affect both animal and human. A "silica-membrane technology" method was applied based on its fast and effective purification of nucleic acids from various matrices. The silica membranes were optimized for high DNA recovery and low binding efficiency for impurities. Results from 58 with various kinds of samples showed negative of unwanted processed animal protein.

Keywords: processed animal protein (PAPS), animal feedstuff

INTRODUCTION

Today's demanding market requires animal products to be safe and high quality. Species determination plays a central role in the regulation of the genuineness and marketability of a product. It has become increasingly important for the trade of products of animal origin that the species type be correctly declared and on the other hand this can also be verified.

Sensitive method for verification of the species type are required, particularly for already mixed and processed products and where ethical and religious concerns (e.g. halal, kosher or vegetarian) play an increasing role. Adulteration of animal feed products has become a common issue in many countries. Adulteration is the act of intentionally substituting one species for another whereby the animal feed products from one species have been mixed intentionally with either similar substitute ingredient or cheaper ones of another species. For instance, food manufacturers often choose lard as a substitute ingredient for oil because it is relatively cheap and easily available. However, for instance the usage of lard and pork is forbidden in Islam. It is because any food containing ingredients from pig sources are prohibited (*haram*) for Muslims to consume. Due to this restriction, it is crucial to develop scientific methods to detect the presence of lard and pork in halal food products (Nurulhidayah, 2011).

Bovine spongiform encephalopathy is a prion disease of ruminants that was first recognized in 1986 in the United Kingdom. Early in the epidemic, it became obvious that the presence of meat and bone meal in feed rations was a common factor in all bovine spongiform encephalopathy cases. The first ban of derived animal proteins in feed was enforced in Europe in 1994 and implemented by Regulation 999/2001 that prohibited the feeding of animal-derived protein to farm animals. The ban of processed animal proteins (PAPs) in feed for farmed animals led to a significant reduction of the number of bovine spongiform encephalopathy cases (Olivier, 2009).

Genetic ID using polymerase chain reaction (PCR) offers comprehensive tests for detection and identification of animal by-products in animal feed and for the determination of the actual species contained in meat products. These tests are also highly effective to detect adulteration of meat products with tissue from other species.

The objective of this research is to ensure that animal feedstuff produced for animal consumption genuinely represent the content as declared by feed producer delivered to the farmers and most importantly, prevent diseases spread through animal feed such as bovine spongiform encephalitis (BSE).

MATERIALS AND METHOD

The monitoring programme started from June 2014 to June 2016. Samples were obtained from feed millers and farmers throughout Peninsular Malaysia. 58 samples received were either processed animal feedstuffs in raw material, pelleted or powdered form and the species identification was conducted at Veterinary Public Health Laboratory, Salak Tinggi, in Sepang, Selangor. NucleoSpin® isolation technology from Macherey-Nagel GmbH, and GMO experience from GEN-IAL GmbH were combined to provide an optimal lysis and purification system for nearly all types of feed samples. Resulting eluates were ready-to-use for all types of subsequent detection methods, especially for real-time and basic PCR technologies. About 200 mg of each sample was homogenized with a commercial homogenizer.

The resulting powder was transferred to a collection tube (2 mL) and 550 µL buffer CF was added (preheated to 65 °C), mixed carefully (15 s), then 10 µL proteinase K was added and mixed again (2-3 s). The mixture was centrifuged for 10 min (>10,000 ×g) to pellet contaminants and cell debris. Clear supernatant from step 2 was transferred into a microcentrifuge tube capable of holding at least 3 sample volumes. 1 vol buffer C4 and 1 vol ethanol were added and the mixture vortexed for 30 seconds. 700 µL mixture was pipetted onto the column and centrifuged for 1

min at 11,000 ×g. The flow-through was discarded.

The procedure was repeated to load the remaining sample. 400 µL buffer CQW was pipetted onto the NucleoSpin® food column and centrifuged for 1 min at 11,000 ×g. The flow-through was discarded.

In the second wash, 700 µL buffer C5 was pipetted onto the NucleoSpin® food column and centrifuged for 1 min at 11,000 ×g. The flow-through was discarded.

In the third wash, another 200 µL buffer C5 was pipetted onto the NucleoSpin® food column and centrifuged for 2 min at 11,000 ×g in order to remove buffer C5 completely. The NucleoSpin® food column was placed in a new 1.5 mL microcentrifuge tube. 100 µL elution buffer CE (preheated to 70 °C) was pipetted onto the membrane, incubated for 5 min at room temperature (18-25 °C) and then centrifuged for 1 min at 11,000 ×g to elute the DNA.

Finally, PCR (Takaka, Advance) was run for 20 minutes using the DNA eluates prior to reading the result.

RESULTS

Results were analysed according to types of sample (e.g. plants, pellet, ash, feed additives, water and tissues) and species

Table 1. Results of 58 processed animal protein (PAPs) in feedstuffs received since June 2014 until June 2016.

No.	Type Of Samples	Quantity	Species ID Tested	Result
1	Palm Oil Powder	3	Bovine,Porcine, Caprine, Avian	Neg
2	Prawn Feed	15	Porcine	Neg
3	Pellet	4	Porcine And Caprine	Neg
4	Water	1	Porcine	Neg
5	Meat And Bone Meal	1	Bovine, Porcine, Caprine, Avian	Neg
6	Sheep Pellet	7	Sheep	Neg
7	Decanter Cake	3	Bovine	Neg
8	Broiler Ash	3	Bovine	Neg
9	Cattle Pellet	4	Bovine	Neg
10	Dairy Cattle Pellet	4	Bovine	Neg
11	Oil Palm Fronds 95%	4	Bovine	Neg
12	Oil Palm Fronds + Palm Kernel Cake	4	Bovine	Neg
13	Feed Additive	1	Bovine,Porcine, Caprine, Avian	Neg
14	Fish Meal	4	Bovine,Porcine, Caprine, Avian	Neg

animal ID required (e.g. porcine, bovine, caprine). From the total of 58 samples received for species identification, all results were negatives (Table 1). A total of three samples of palm oil powder, four samples of fishmeal, one sample of meat and bone meal and one sample of feed additive were tested negative for presence the presence of bovine, caprine, porcine and avian DNA. Fifteen samples of prawn feed and one sample of water were also negative for porcine DNA. Four samples of pelleted feeds analysed for bovine and caprine DNA were also found negative. There are also seven samples of sheep pellet that were negative of sheep's DNA. Last but not least, three samples of decanter cake, four samples of broiler ash, 4 samples of cattle pellet, four samples of dairy cattle pellet, four samples of oil palm fronds and four samples of oil palm fronds with palm kernel cake were tested negative for bovine DNA

These results indicate that there are no presence of unwanted processed animal proteins (PAPs) in all samples conducted for this test.

DISCUSSION

Based on the testing of the processed animal protein (PAPs) of the samples received for this feed safety programme, all samples showed negative results on targeted species identification of bovine, caprine, porcine and avian. The other main concern was to increase the number of meat and bone meal (MBM) samples for further research on risk assessment of bovine spongiform encephalitis (BSE) diseases in this country from the aspect of animal feedstuffs usage. Identification of processed animal protein is a crucial tool in making sure animal feedstuffs is safe to consume, prevent spread of disease carried by feed itself, properly labelled and to tackle issues of feed adulteration.

CONCLUSION

Feed safety programmes conducted by this laboratory is one the important programmes under the DVS, Malaysia for the monitoring of adulteration of animal feedstuffs in Malaysia. This is to ensure that all the relevant products could be consistently monitored for their authenticity and are safe for both humans and animals, preventing the spread of unwanted diseases in Malaysia.

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