

Suitability of ruminant identification assays for use in processed animal proteins and animal feed



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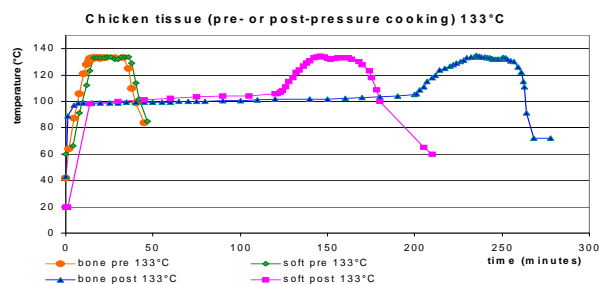
As a preventive measure to avoid the spread of Bovine Spongiform Encephalopathy (BSE) a feed ban has been introduced by the European Union (EC Regulation 999/2001). The detection and identification of animal tissues in feed has therefore gained great interest, especially of ruminant species. At present, microscopy is still the only official method for detection of PAP's in feed in the European Union. Besides microscopy, there is a need for a technique which can discriminate between species and is less time consuming.

The suitability of several ruminant identification assays, is tested with different reference materials.

Reference materials

Some of the reference materials are processed by CCL (Veghel, NL) in a dedicated steriliser of 140 litre under strict conditions (133°C and 159°C during 20 minutes; both pre-pressure and post-pressure cooked):

- Porcine soft material (100% large intestines)
- Porcine 'bone' material (5% tails + 95% hind-legs)
- Porcine bone material (100% bone)
- Chicken soft material (100% digestive system)
- Chicken 'bone' material (40% heads + 60% shanks)



PDM Ltd (Doncaster, UK) processed bovine, ovine, porcine and avian carcass and muscle material, heated at 133°C, 137°C, 141°C and 145°C.

Furthermore, some 'pure' commercial available products are used as reference samples.

With these reference materials several mixtures are prepared (in PAP and feed).

Methods

Several PCR assays and one commercial immunological test, which claim to be able to identify ruminant species, are tested with the reference materials.

1. Real-time PCR CRA-W (Gembloux, B)
2. Real-time PCR TNO (Zeist, NL)
3. PCR of an anonymous institute
4. Real-time PCR UCM (Madrid, ES)
5. Reveal for Ruminant in Feed Neogen (Lansing, USA)

Assay number 1 and 5 are carried out by CCL. The other three assays are carried out by the institute, which developed them. Results of these tests are shown in the following table.

Acknowledgements

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Results

	Assay 1 Cattle	Assay 2 Ruminant	Assay 3 Bovine / Ovine	Assay 4 Bovine / Ovine	Assay 5* Ruminant
Reference samples					
Pork soft, pre-pres cook	133°C	-	-	-	-
Chicken soft, pre-pres cook	133°C	-	-	-	-
Chicken bones, pre-pres cook	133°C	-	-	-	+
Bovine carcass	145°C	+	+	+	+
Ovine carcass	133°C	-	+	+	+
Avian carcass	133°C	-	-	-	-
Avian muscle	133°C	-	-	-	-
Commercial available products					
Feather meal 1	-	+	+	+	-
Feather meal 2	-	-	-	-	-
Mixed samples (soft/bones pre 133°C)					
Chicken 95% + Pork 5%	-	-	-	-	-
Chicken 98% + Pork 2%	-	-	-	-	-
Chicken 99.5% + Pork 0.5%	-	-	-	-	+
Pig feed % Bovine % Chicken %					
0	0	100	-	-	+
0	0.2	99.8	+	+	+
0	0.1	99.9	+	+	+
95	0	5	-	-	-
99	0	1	-	-	-
100	0	0	-	-	-
95	5	0	+	+	+
99	1	0	+	+	+
99.5	0.5	0	+	+	+
99.7	0.3	0	+	+	+
99.8	0.2	0	+	+	+
99.9	0.1	0	+	+	+
Pig feed % Ovine % Chicken %					
0	0.2	99.8	-	-	+
0	0.1	99.9	-	-	+
99	1	0	+	+	+
99.9	0.1	0	+	+	+
Chicken feed % Ovine % Pork %					
90	5	5	-	-	+
94	1	5	-	-	+
94.5	0.5	5	-	-	+
94.7	0.3	5	-	-	+
94.8	0.2	5	-	-	+
94.9	0.1	5	-	-	+
Feathermeal % Bovine carcass %					
99.5	0.5	-	+	+	+
99.9	0.1	-	+	+	+
Feathermeal % Ovine carcass %					
99.5	0.5	-	+	+	+
99.9	0.1	-	+	+	+

* This assay is not intended for use with meat and bone meal.

Correct result (Green), Wrong result (Red), Might be influenced (Yellow), Inconclusive result (Orange)

Conclusions

The tested assays seem to be suitable for identification of ruminant species, even after heating for twenty minutes at the highest temperatures. However, assay number 3 and 5 do not always reach the preferred detection limit of 0.1% and assay number 5 sometimes gives false positive results.