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.

Deeulte

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 carcase 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.

Assay 1 Assay 2 Assay 3 Assay 4 Assay 4 Cattle Ruminant Bovine Ovine Ovine Rumi Reference samples Cattle Ruminant Bovine Ovine Ovine Numinant Bovine Ovine Rumi Pork soft, pre-pres cook 133°C - <	Results									
Cattle Ruminant Bovine Ovine Rumi Reference samples -<				Assay 1	Assay 2	Assay 3		Assay 4		Assay 5*
Reference samples Park soft, pre-pres cook 133°C - <t< th=""><th></th><th></th><th></th><th>Cattle</th><th>Ruminant</th><th>Bovine</th><th>Ovine</th><th>Bovine</th><th>Ovine</th><th>Ruminant</th></t<>				Cattle	Ruminant	Bovine	Ovine	Bovine	Ovine	Ruminant
Pork soft, pre-pres cook 133°C - <td< td=""><td>Reference samp</td><td>les</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>	Reference samp	les								
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Chicken hones, pre-pres cook 133°C -	Chicken soft, pre-	pres cook	133°C			-				-
Bovine carcase 145°C +	Chicken bones, pre-pres cook 133°C			-	-				+	
Owne carcase 133°C - +	Bovine carcase 145°C			+	+			+		+
Avian carcase 133°C -	Ovine carcase 133°C		-	+				+	+	
Avian muscle 133°C -	Avian carcase 133°C		-		-	-			-	
Commercial available products Feather meal 1	Avian muscle 133°C				-	-			-	
Feather meal 1 2 2 3 3 Feather meal 2 -<	Commercial avai	lable products								
Feather meal 2 -	Feather meal 1			-	±	-	-			-
Mixed samples (softbones pre 133°C)	Feather meal 2					-				-
Chicken 95% + Pork 5% 2 2 4 4 Chicken 95% + Pork 25% -	Mixed samples (soft/bones pre 133	°C)							
Chicken 98% + Pork 2% - <td< td=""><td colspan="3">Chicken 95% + Pork 5%</td><td></td><td></td><td></td><td></td><td></td><td>1.1</td><td>-</td></td<>	Chicken 95% + Pork 5%								1.1	-
Chicken 99 5% + Pork 0.5% Chicken % Chicken % Chicken % Chicken % 0 0 100 -	Chicken 98% + Pork 2%								-	
Pig feed % Bovine % Chicken % . . <td colspan="3">Chicken 99.5% + Pork 0.5%</td> <td>-</td> <td></td> <td></td> <td></td> <td></td> <td>-</td> <td>+</td>	Chicken 99.5% + Pork 0.5%			-					-	+
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S O S S C	0	0.1	99.9	+ (trace)	+	-				+
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* This assay is not intended for use with meat and bone meal.	* This assay is no	t intended for use w	ith meat and bone r	neal.						
Correct result Minht be influenced		Correct result		Might be inf	luenced					

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.

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