Methods available for re-entry of PAPs in feed

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Acknowledgements

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Overview of presentation

- Legislation
- Production of reference materials (pilot steriliser)
- Methods for species identification
- Reveal dipstick method for *screening* ruminant *protein*
- PCR technique for DNA detection
  - of ruminant (cattle) material
  - of porcine material
  - of poultry (chicken) material
- Dilemma of feed analysis (allowed products)
- EU SAFEED-PAP project

EU legislation

- **Feedban:** temporary ban on use ruminant PAP in compound feed
- **Animal By-Products Regulation (EC) No 1774/2002**

  (May 1st 2003) *requires*:

  1) **permanent marking of cat. 1 and 2 ABP**

  2) **avoidance of cannibalism**

  (i.e. intra species recycling ban of cat. 3 ABP in feed)

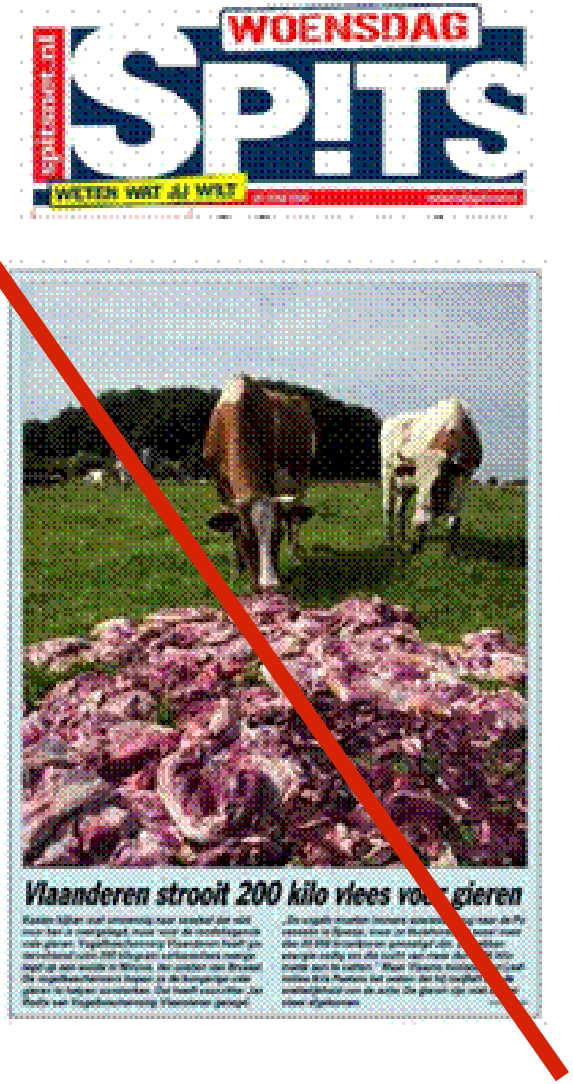
  poultry PAP allowed in feed for pigs

  porcine PAP allowed in feed for poultry

  temporary ban on use animal proteins in ruminant feed continued

  temporary ban on use ruminant MBM in compound feed continued

Ban on animal proteins
in ruminant feed continued



Animal protein ban partly lifted

Today:

- Blood and milk products, gelatine, fat etc. are allowed

Near future:

- Use of poultry /porcine PAP in aquafeed

Future:

- Use of porcine PAP in poultry feed
- Use of poultry PAP in pig feed

Possibly on long term:

- Use of ruminant PAP in non-ruminant feed

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Production of reference materials

CCL (NL) has prepared pure porcine and chicken reference materials (PAPs)

- in a pilot sterilizer (140 L) with a stirring device
- 20 min. 133ºC or 159ºC
- pre-, post- or defatted post-pressure cooking

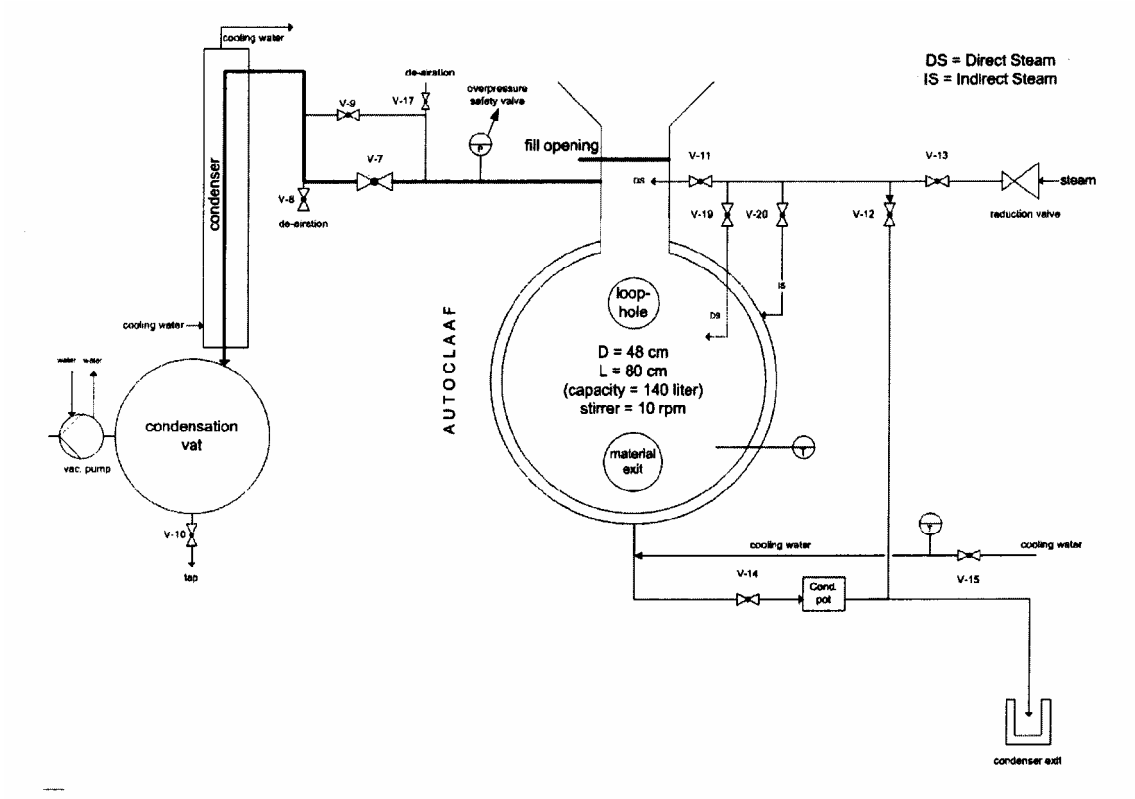
PDM (UK) has prepared pure bovine, ovine, porcine and avian (= chicken) reference materials (PAPs)

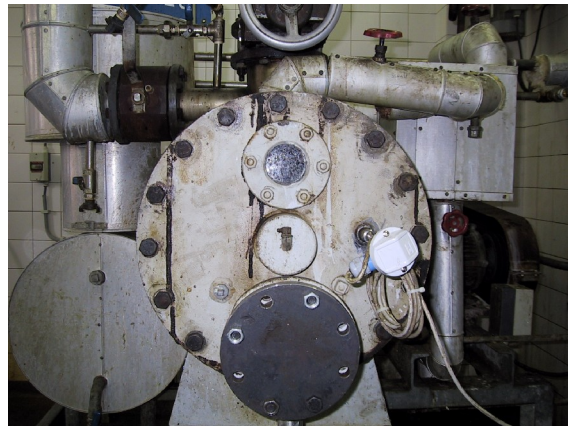
- dedicated pilot plant
- 20 min. 133ºC, 137ºC, 141ºC and 145ºC
- post-pressure cooking

Choice of tissues

Porcine soft	Porcine bone	Chicken soft	Chicken bone
100% large intestines (washed)	5% tails + 95% legs & trotters	100% intestines (not empty)	40% heads + 60% shanks
	 		 

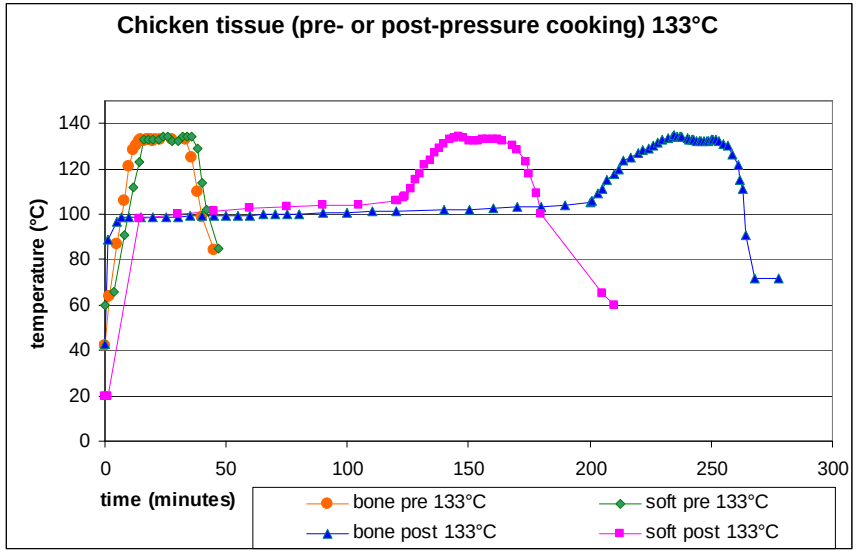
Sterilisation equipment (used by CCL)



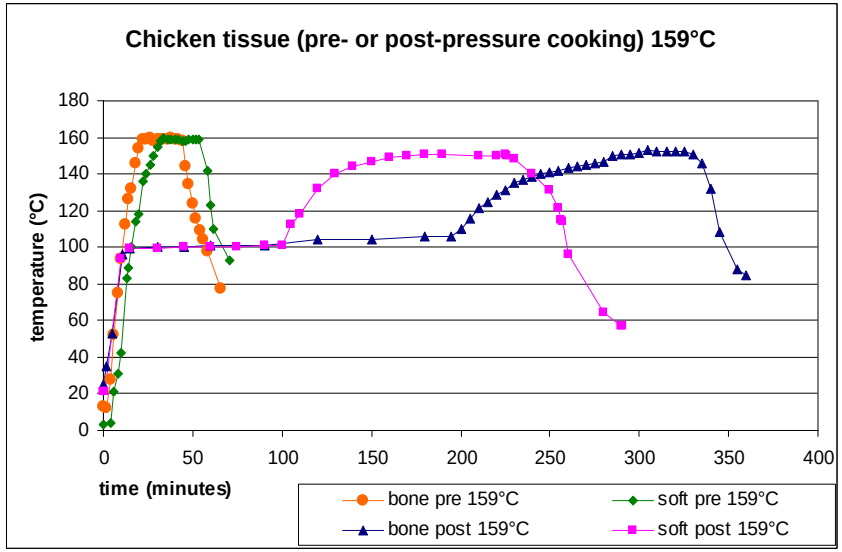


Temperature profiles of reference materials

20 min. 133ºC



20 min. 159ºC



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Official method for control of feedingstuffs: microscopy

Directive 98/88/EC of November 13th 1998

and 126/2003/EC (based on STRATFEED)

Limitations/ disadvantages:

- much experience/ training required
- method is time consuming (2-4 samples/day)
- only detection of particles
- qualitative results (detection limit 0.1%)
- species identification not possible (sometimes indicative)

Methods for species identification

Microscopy (current official method): detection of animal **tissues**

- very hard (not possible) to distinct between species
- much education and experience needed

ELISA: specific **protein** detection

- proteins not present in every tissue cell
- easy to perform

PCR: specific **DNA** detection

- present in every tissue cell
- experience needed

Promising techniques

Commercially available:

Reveal for Ruminant	**Neogen**	**(USA)**
MELISA-TEK Ruminant	**ELISA Technologies**	**(USA)**
FoodExpert ID	BioMérieux	(F)
SureFood Animal ID	Congen	(USA)
FeedChek	SDI	(USA)

In development:

Real-time PCR	**CRA-W**	**(B)**
Real-time PCR	**TNO**	**(NL)**
PCR	**UCM Madrid**	**(ES)**
ELISA	AntibodyShop	(DK)
T-RFLP	Fraunhofer-IME	(D)
PCR	Fraunhofer-IME	(D)
PCR	Impetus Bioscience	(D)
PCR	VLA	(GB)
PCR	UAB Barcelona	(ES)

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Reveal equipment & interpretation

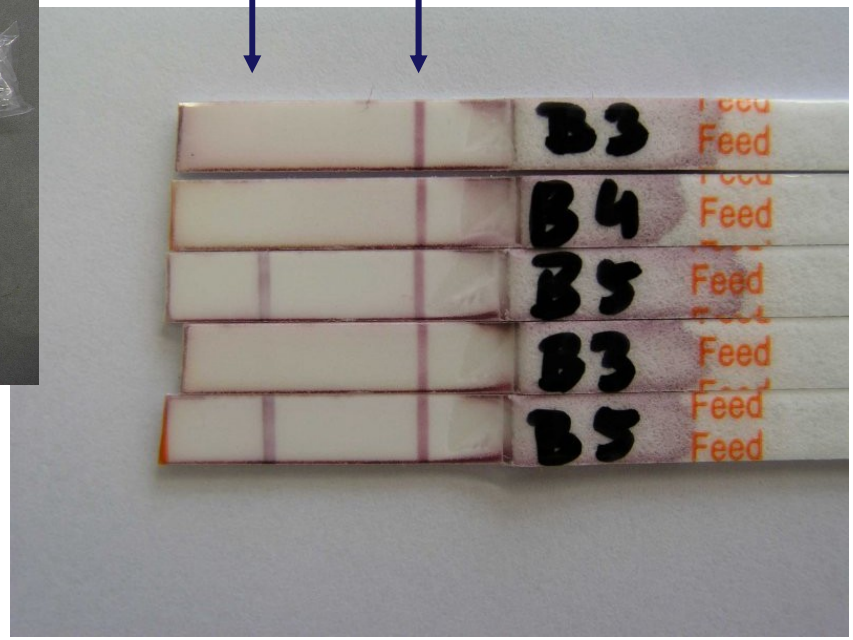
Conical flasks (e.g. 300 ml)

Water bath 100ºC

Timer

sample zone: line = positive

control zone: line = correct



Results Reveal with pure PAPs

			Reveal
Pork soft	133°C	pre-pressure cooking	
	159°C		
	133°C	post-pressure cooking	
	159°C		
	133°C	defatted post-pressure cooking	
	159°C		
Pork bones	133°C	pre-pressure cooking	
	159°C		
	133°C	post-pressure cooking	
	159°C		
	133°C	defatted post-pressure cooking	
	159°C		
Chicken soft	133°C	pre-pressure cooking	
	159°C		
	133°C	post-pressure cooking	
	159°C		
	133°C	defatted post-pressure cooking	
	159°C		
Chicken bones	133°C	pre-pressure cooking	+
	159°C		
	133°C	post-pressure cooking	+
	159°C		
	133°C	defatted post-pressure cooking	
	159°C		

right
wrong

		Reveal
Bovine carcase	133°C	
	137°C	
	141°C	
	145°C	
Ovine carcase	133°C	
	137°C	
	141°C	
	145°C	
Porcine carcase	133°C	
	137°C	
	141°C	
	145°C	
Avian carcase	133°C	
	137°C	
	141°C	
	145°C	
Bovine muscle	133°C	
	137°C	
	141°C	
	145°C	
Ovine muscle	133°C	
	137°C	
	141°C	
	145°C	
Porcine muscle	133°C	
	137°C	
	141°C	
	145°C	
Avian muscle	133°C	
	137°C	
	141°C	
	145°C	

Results Reveal for control of PAPs for aquafeed

Non ruminant PAP for aquafeed	Inclusion level; % Ruminant PAP "spike"					
	Zero	0,1	0,5	1	2	5
Porcine PAP a		-				
Porcine PAP b		-	-			
Porcine PAP c		-				
Avian PAP a						
Avian PAP b		-	-			
Avian PAP c		-				
Feather PAP a		-				
Feather PAP b		-				
Fishmeal PAP		-				

Advantages Reveal dipsticks

In general:

- rapid and easy to use
- commercial available
- detects muscle protein (is advantage as screeningstest for feed, because allowed milk and blood products are not detected)
- approved by AOAC

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Most promising PCR technique for detection cattle/ ruminant tissue (DNA)

Community Reference Laboratory (CRA-W, Gembloux, B):

PCR test for **cattle**

- Testkit operational at CCL
- In future commercial available

TNO (Zeist, NL):

PCR test for **ruminants**

- Analysis by TNO
- Test kit not (yet) commercial available

Cattle/ruminant detection in pure reference materials (PAPs)

			PCR CRA-W	PCR TNO
Pork soft	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			
	133°C	defatted post-pressure cooking		
	159°C			
Pork bones	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			
	133°C	defatted post-pressure cooking		
	159°C			
Chicken soft	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			
	133°C	defatted post-pressure cooking		
	159°C			
Chicken bones	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			
	133°C	defatted post-pressure cooking		
	159°C			

right
wrong

		PCR CRA-W	PCR TNO
Bovine carcase	133°C		
	137°C		
	141°C		
	145°C		
Ovine carcase	133°C		
	137°C		
	141°C		
	145°C		
Porcine carcase	133°C		
	137°C		
	141°C		
	145°C		+
Avian carcase	133°C		
	137°C		
	141°C		
	145°C		
Bovine muscle	133°C		
	137°C		
	141°C		
	145°C		
Ovine muscle	133°C		
	137°C		
	141°C		
	145°C		
Porcine muscle	133°C		
	137°C		
	141°C		
	145°C		
Avian muscle	133°C		
	137°C		
	141°C		
	145°C		

Most promising PCR technique for detection ruminant/ cattle tissue

PCR method of	Detects DNA of	Detection limit (%)	% false-positives	% false-negatives
CRL (Gembloux)	cattle	< 0.1 – 0.5	< 0.9	8.3
TNO (Zeist)	ruminants	< 0.1	7.5	3.5

Porcine detection in pure reference materials (PAPs)

			PCR CRA-W	PCR TNO
Pork soft	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			
	133°C	defatted post-pressure cooking		
	159°C			-
Pork bones	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C		-	-
	133°C	defatted post-pressure cooking		
	159°C		-	-
Chicken soft	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			
	133°C	defatted post-pressure cooking		
	159°C			
Chicken bones	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			
	133°C	defatted post-pressure cooking		
	159°C			

right
wrong

		PCR CRA-W	PCR TNO
Bovine carcase	133°C		
	137°C		
	141°C		
	145°C		
Ovine carcase	133°C		
	137°C		
	141°C		
	145°C		
Porcine carcase	133°C		-
	137°C		-
	141°C		-
	145°C		-
Avian carcase	133°C		
	137°C		
	141°C		
	145°C		
Bovine muscle	133°C		
	137°C		
	141°C		
	145°C		
Ovine muscle	133°C		
	137°C		
	141°C		
	145°C		
Porcine muscle	133°C		-
	137°C		-
	141°C		-
	145°C	-	-
Avian muscle	133°C		
	137°C		
	141°C		
	145°C		

Most promising PCR techniques for detection porcine tissue

PCR method	detection limit	% false-positives	% false-negatives
CRL (Gembloux)	≤ 0.5%	12.1	6.7
TNO (Zeist)	≤ 0.5%	6.8	18.0

Chicken detection in pure reference materials (PAPs)

			PCR CRA-W	PCR TNO
Pork soft	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			
	133°C	defatted post-pressure cooking	+	
	159°C			
Pork bones	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			
	133°C	defatted post-pressure cooking	+	
	159°C			
Chicken soft	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			
	133°C	defatted post-pressure cooking		-
	159°C		-	-
Chicken bones	133°C	pre-pressure cooking		
	159°C			
	133°C	post-pressure cooking		
	159°C			-
	133°C	defatted post-pressure cooking		
	159°C		-	-

right
wrong

		PCR CRA-W	PCR TNO
Bovine carcase	133°C		
	137°C		
	141°C		
	145°C		
Ovine carcase	133°C		
	137°C		
	141°C		
	145°C		
Porcine carcase	133°C		
	137°C		
	141°C		
	145°C		
Avian carcase	133°C		
	137°C		
	141°C	-	-
	145°C	-	-
Bovine muscle	133°C		
	137°C		
	141°C		
	145°C		
Ovine muscle	133°C		
	137°C		
	141°C		
	145°C		
Porcine muscle	133°C		
	137°C		
	141°C		
	145°C		
Avian muscle	133°C	-	-
	137°C	-	-
	141°C	-	-
	145°C	-	-

Most promising techniques for detection chicken tissue

PCR method	detection limit	% false-positives	% false-negatives
CRL (Gembloux)	≤ 0.5%	11.8	12.3
TNO (Zeist)	≤ 0.5%	20.2	20.0

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Techniques available today for detection of prohibited species in single species PAPs

	prohibited species tissues	control technique
ruminant MBM	not allowed in feed for farmed animals	control not necessary
porcine or poultry PAPs for aquafeed	ruminant tissues	Reveal dipstick; PCR for confirmation
porcine PAPs for farmed animals	ruminant / poultry tissues	Reveal for ruminant tissues PCR for poultry tissues
poultry PAPs for farmed animals	ruminant / porcine tissues	PCR for ruminant tissues PCR for porcine tissues

Techniques for detection of (prohibited) species in compound feed for farmed animals

	prohibited species tissues	Control technique
ruminant feed	**all animal proteins**	**microscopy**
pig feed	**porcine / ruminant tissues** *(except **allowed products**)*	**PCR for porcine / Reveal for ruminant tissues**
poultry feed	**poultry / ruminant tissues** *(except **allowed products**)*	**PCR for poultry / Reveal for ruminant tissues**

Leads to dilemma

Dilemma

Some animal proteins are already allowed in compound feed,

e.g. hydrolysed proteins, milk, egg, fat, DCP, TCP

gelatine and bloodproducts

How to discriminate between prohibited and allowed products in feed?

Solution for this dilemma

Use analytical methods for control of raw materials (PAPs)

and not for control of feed.

combined with administrative monitoring of compound

feed (tracking and tracing).

Conclusion:
Methods are available today

- Raw materials for aquafeed:

  screening for absence of ruminant material with Reveal dipstick and.

  confirmation with PCR.

  lifting this ban is in discussion with DG-Sanco.

- Raw materials for feed for farmed animals:

  screening for absence of ruminant material with Reveal dipstick and confirmation with PCR.

  but for lifting this ban, the next methods have to be further validated:

  1) Test for poultry material (for acceptance porcine PAP in poultry feed)

  2) Test for porcine material (for acceptance poultry PAP in pig feed)

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EU SAFEEDPAP project

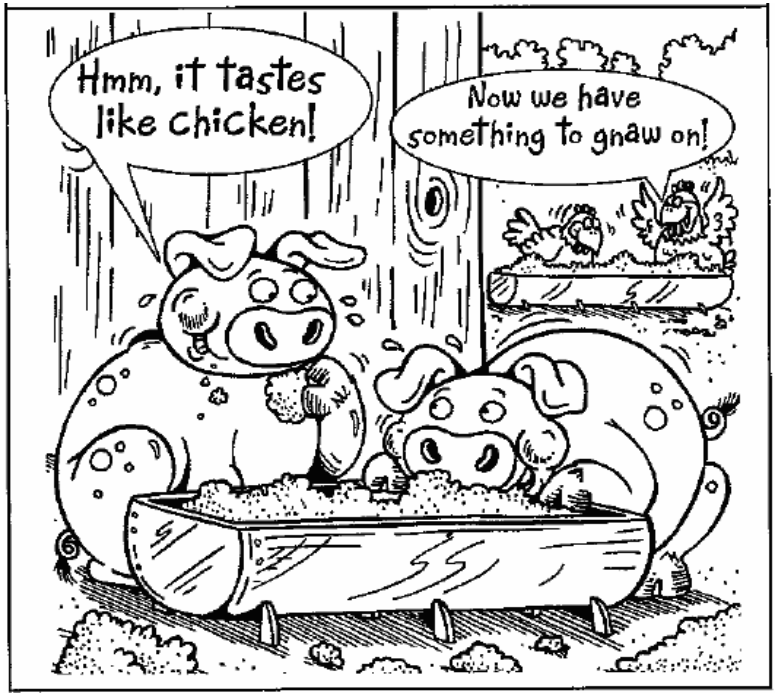
Aim:

To obtain various validated methods (NIR microscopy, PCR, immunochemical assays, LC-MS-MS) for species (ruminant, porcine and poultry) identification.

CCL is **partner** in this project, especially for:

- preparation of reference materials.
- participation in inter-laboratory ring trials for validation of the methods (PCR and Reveal).

Thank you for your attention



Source: Feed Tech 11.5 2007
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