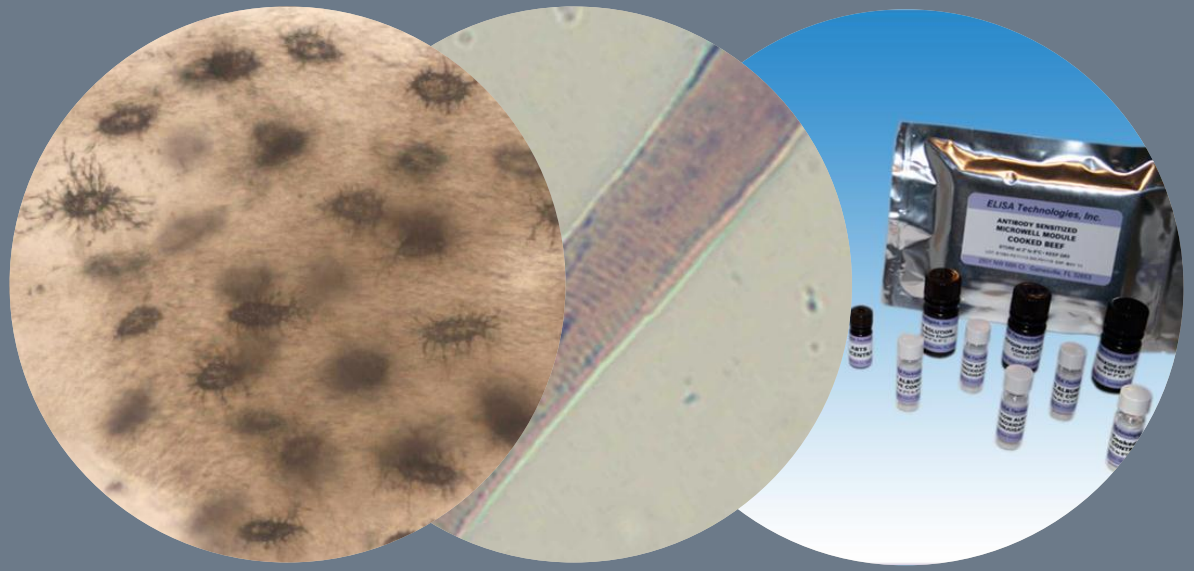
Immunoassay validation for monitoring ruminant proteins in aquafeed

L.W.D. van Raamsdonk, M. Bremer, RIKILT Wageningen

R. Margry, R. van Kaathoven, CCL, Veghel



RIKILT
WAGENINGEN UR

Animal proteins monitoring

- Microscopy: screening; visible particles
- PCR: identification; DNA
- Near Infrared spectroscopy / microscopy
- Immunoassays: screening or confirmation; proteins
- Other

Animal proteins in aquafeed

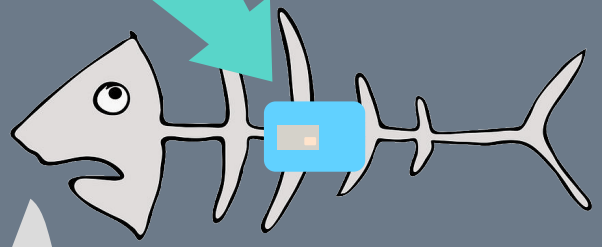
Aquafeed

Non-ruminant PAP

25%

2%

Fishmeal



10% gut content

Feed for weaning ruminants

5%

RIKILT
WAGENINGEN UR

Animal proteins in aquafeed

EFSA critical limit: **2**% ruminant-PAP in *non*-ruminant PAP (EFSA, 2011)

Concentration of PAP in ruminant feed:
0.25 * 0.1 * 0.05 = 0.00125%

LOD microscopy: 0.0025 % (Veys et al., 2009)

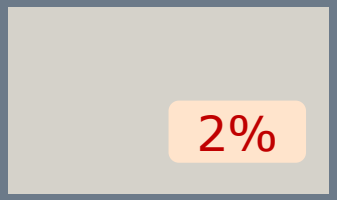
EFSA critical limit: **0.1**% AP in feed (EFSA, 2011)

Current scenario: 0.000025% of ruminant PAP in ruminant feed

Animal proteins in aquafeed

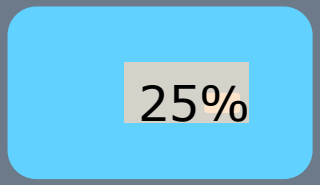
monitoring

Non-ruminant PAP

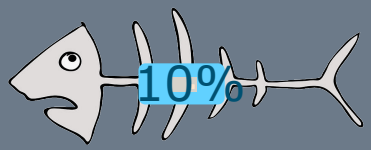


< 2%:
Certification for aquafeed

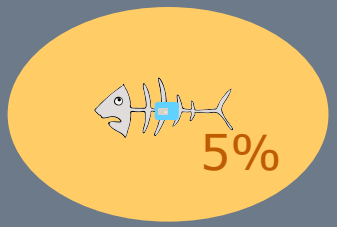
aquafeed



fishmeal



Ruminant feed



< 0.1%:
Approved as feed

Immunoassays

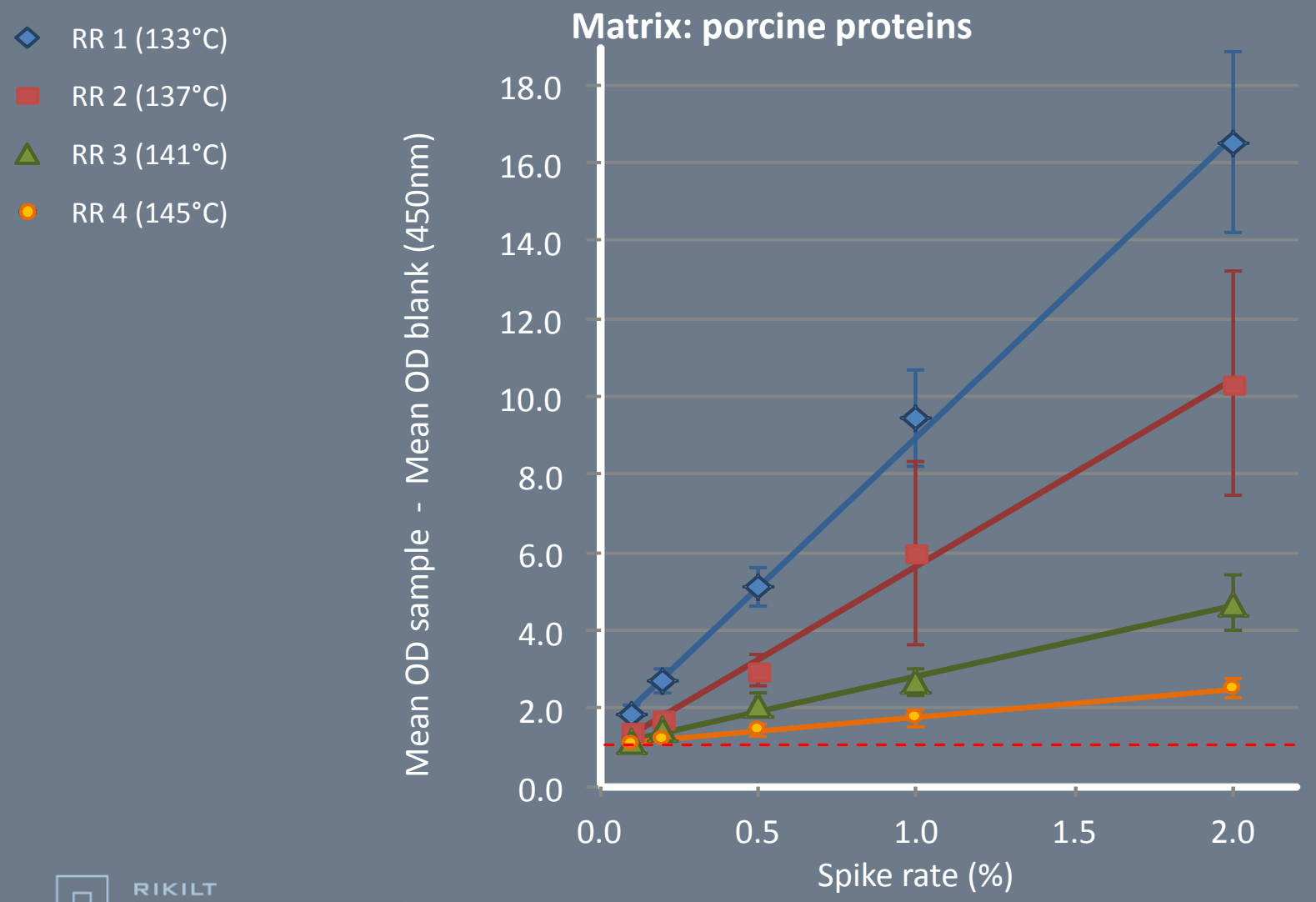
	Immunoassay	PCR
Analytical features		
Official method	no	no
Samples/day	100-200	10-20
Analytical time/sample	1 h – 1 day [1]	1½-2 days
Sampling	~ 10 g	≥ 0.1 g
Transferability	high	medium to high [3]
LOD for detection of PAP in PAP	~ 2 % or more	< 0.5%
Quantitative possibilities	no data	not considered
Interfering features		
Other authorized products	yes/no [11]	yes
Fat	sometimes	sometimes
Particle size influence	no	no
Miscellaneous		
Fully validated (inter-laboratory study)	no [12]	yes
Existing facilities	yes	yes
Experience of operator required	low to moderate	moderate to high
Equipment costs	low to moderate [1]	high

(EFSA, 2011)

11. depends on the target used, e.g. if troponin is the antigen there is no interaction with milk or milk powder

RIKILT WAGENINGEN UR

Immunoassay validation; in house validation MELISA-TEK



RIKILT
WAGENINGEN UR

L.W.D. van Raamsdonk et al., Beijing, Feedsafety conference, 12-9-2012

Immunoassay validation; in house validation MELISA-TEK

- Specificity and robustness

	TN	FP
Porcine PAP	27	0
Poultry PAP	21	1
Other material *	9	0
Milk powder	2	0

Threshold: mean OD ratio + 3 * SD

*: blood plasma, porcine DCP/TCP, feather meal, fish meal, gelatine

Immunoassay validation; interlaboratory study

- MELISA-TEK and Reveal
- 16 participants
- Three parts: training samples, entrance test, final study
- After entrance test: 14 participants
- Period: January to May 2012
- AOAC guidelines, *as far as applicable*

Immunoassay validation; results Melisa-Tek

- Accuracy (n=84)

	133 °C	137 °C
Blank	98.8%	
0.5%	100%	
1.0%	100%	100%
2.0%	100%	100%

Immunoassay validation; results Reveal

- Accuracy (n=84)

	133 °C	137 °C
Blank	97.0%	
0.5%	100%	
1.0%	98.8%	98.8%
2.0%	100%	100%

Immunoassay; future strategy

- Current situation: performance limit (0.5% < 2.0%) approved
- Future target: performance limit: 0.1%
- Option: concentration experiments for target (= troponine)

Immunoassay; conclusions

- Immunoassays (Melisa-Tek and Reveal) are validated at 0.5% and higher with PAP as matrix
- A strategy with immunoassays will avoid the presence of positive signals for legally applied ingredients
- The design of the study can be used as guideline for future studies with qualitative results
- Application of immunoassays after muscle concentration could be feasible to reach the 0.1% performance limit

THANK YOU

RIKILT
WAGENINGEN UR