

Detection of ruminant PAPs in non-ruminant PAPs

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Due to growing aquaculture industry the demand for high quality proteins for aquatic feeds is increasing. To partially lift the European feed ban and safely re-introduce non-ruminant processed animal proteins (PAPs) in aqua feed, methods discriminating between ruminant PAPs and non-ruminant PAPs are necessary. The aim of this study was to test the performance of a commercial immunoassay, MELISA-TEK™ Ruminant, for detection of ruminant PAPs in non-ruminant PAPs.

Materials and methods

Samples

A wide range of PAPs of different types and species were tested; 72 non-ruminant PAPs and 149 non-ruminant PAPs with a known content (0.1%, 0.2%, 0.5%, 1%, 2%) of ruminant PAPs (carcase and / or muscle) processed at different temperatures (133°C, 137°C, 141°C, 145°C).

ELISA

All samples were tested with the commercially available MELISA-TEK™ Ruminant kit. To improve sensitivity sample extracts were centrifuged in protein concentrator devices before analysis.

Results

The specificity of this assay was 99% (Table 1), which indicates no cross-reactivity with non-ruminant PAPs. Overall sensitivity was 95% (Table 2). The assay correctly classified all samples (n=95) with ruminant PAPs processed at 133°C (0.1%, 0.2%, 0.5%, 1%, 2%) and 137°C (1 and 2%) and all samples (n=20) with 2% ruminant PAPs of 141°C and 145°C. Only 7 samples with (0.1%, 0.2%, 0.5% or 1%) ruminant carcase material of 141°C or 145°C were falsely classified.

Conclusion

The detection limit of the MELISA-TEK™ Ruminant kit is below 0.5% for ruminant PAPs (133°C) and below 2% for ruminant PAPs (145°C) in non-ruminant PAPs. This makes this assay a suitable candidate for monitoring the safe re-introduction of non-ruminant PAPs in aqua feed.

Table 1. Specificity evaluation of MELISA-TEK™ Ruminant

Non-ruminant PAP samples	# samples tested	TN	FP	Specificity = TN/(FP+TN)
Porcine DCP/TCP	2	2	0	100%
Feather meal	2	2	0	100%
Fish meal	2	2	0	100%
Milk powder	2	2	0	100%
Porcine blood plasma/ haemoglobin	3	3	0	100%
Porcine/poultry PAP mixture	12	12	0	100%
Poultry PAP	22	21	1	95%
Porcine PAP	27	27	0	100%
	72	71	1	99%

TN= True Negatives, FP = False Positives

Table 2. Sensitivity evaluation of MELISA-TEK™ Ruminant

Spike level	# samples tested	TP	FN	Sensitivity = TP/(FN+TP)
0.1%	6	5	1	83%
0.2%	6	5	1	83%
0.5%	28	27	1	96%
1%	68	64	4	96%
2%	41	41	0	100%
	149	142	7	95%

TP= True Positives, FN = False Negatives