IAG ring test feed composition 2014

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RIKILT Wageningen UR
Wageningen, November 2014

RIKILT report 2014.010

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Raamsdonk, L.W.D. van, V.G.Z. Pinckaers, J.J.M. Vliege, 2014. *IAG ring test composition 2014.* Wageningen, RIKILT Wageningen UR (University & Research centre), RIKILT report 2014.010. 24 blz.; 3 tab.: 2 fig.; 7 ref.

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RIKILT report 2014.010

Distribution list:

- International Association for Feeding stuff Analysis (IAG), Section Feeding stuff Microscopy (I. Paradies-Severin, J. Vancutsem, G. Frick, R. Weiss)
- European Union Reference Laboratory, Animal Proteins (CRA-W; G. Berben, P. Veys, V. Baeten)
- Joint Research Centre, Geel (IRMM-JRC; C. von Holst, A. Boix-Sanfeliu)
- All participants of the ring test
- Competent authorities of EU member states

Contents

Summary

A ring test was organized for the microscopic determination of composition in animal feed in the framework of the annual ring tests of the IAG - International Association for Feeding stuff Analysis, Section Feeding stuff Microscopy. The organizer of the ring test was RIKILT - Wageningen UR, The Netherlands. The aim of the ring study was to provide the participants information on the performance of the local implementation of the method for composition analysis of feed.
The sample was based on a chicken feed produced at a pilot plant dedicated to produce animal protein free test feeds. The sample was contaminated with 1% of insect meal (*Locusta*) and offered with an incorrect declaration. All participants were requested to confirm or reject the declaration and report the correct composition. The results were analysed using the IAG model for uncertainty limits. Shares of ingredients in the feed formulation outside the limits of the model were indicated as “wrong”.
A total of 24 sets of results were returned. Seven participants made one error and two participants made more than one error. One lab reported up to four wrong results. Wheat meal at a share of 45.5% in the formulation was underestimated four times, and corn meal with a share of 10.9% was overestimated four times. Results of IAG ring tests in previous years revealed in general underestimation for higher shares and overestimation for lower shares, which is consistent with the current results.
The analysis of composition in terms of ingredients is important for detecting economic fraud and for monitoring feed safety. Composition analysis and label control of feed is regulated in Regulation (EC) 767/2009. In a broader view, composition analysis in the entire food chain can improve the effect of monitoring actions. The new legislation on food labelling (Regulation (EC) 1169/2011), effective from December 13th 2014, obliges to provide more detailed information to customers on composition and related topics.
The current results indicate that feed ingredients can be identified and shares can be estimated successfully. Besides a proper method, maintenance and dissemination of expertise of technicians are vital for a good performance. An evaluation of the IAG uncertainty model can help to improve its application.

1 Introduction

The analysis of composition of feeds by means of microscopic methods has a long history. It has been a major activity of the IAG section Microscopy from its existence in 1959 (www.iag-micro.org). In 1998 a protocol on the microscopic identification of ingredients in feed was established in German, and translations to English and French were decided to be prepared (http://www.iag-micro.org/files/39_wien98.pdf?10,12).
The legal basis for this examination is the obligatory label declaration of feeds, regulated for years by EU legislation and currently part of Regulation (EC) 767/2009. The main objective might be the transparency of trade activities, with emphasis on the prevention of economic fraud and a sufficient monitoring of feed safety.
Besides the availability of a protocol, the current practices are heavily based on the existing skills of the technicians. In the view of a process of improvement of monitoring programs, which was recently established for food in Regulation (EC) 1169/2011, the maintenance and dissemination of these skills needs priority.

In this report the ring test for composition 2014 is presented, which was organised by RIKILT on behalf of the IAG Section Feeding stuff Microscopy.

2 Methods

2.1 Materials and procedure

The sample was based on a chicken feed produced in the framework of the European project STRATFEED. The sample was also part of the IAG ring test on the detection of animal proteins 2014 (van Raamsdonk *et al.*, 2014). The chicken feed consisted of wheat meal (46%), soybean products, partly extracted (28%), corn meal (11%), rapeseed and rapeseed meal (7%), vegetable fat (5%), mix of minerals and vitamins (3%).
The insect meal (grasshoppers; *Locusta*) was bought at internet as entire animals. This material was ground and degreased before being used as animal protein.
The feed and insect meal were checked on purity (absence of any contamination) and identity, and were all found to be fit for application (Table 1).
In order to avoid any cross contamination, the samples were produced in a strict order. This production process was fully presented in van Raamsdonk *et al.* (2014).
The final sample was analysed in fivefold (Table 1). The microscopy research group did not participate in the further laboratory analysis of this ring trial.

Table 1
Results of the homogeneity study. Sediment amounts are based on 10 grams. Microscopy: five replicates. Green cells indicate the correct positive findings.

	Sediment amount	Microscopy		
Sample		MBM	fish	insect
Locusta meal		Neg	Neg	Pos
Locusta meal, degreased		Neg	Neg	Pos
Chicken feed		Neg	Neg	Neg
2014-C 1.0% insect n=5	18.2 – 19.8 mg/g	Neg	Neg	Pos

2.2 Organization of the ring trial

All IAG members, all NRLs, participants of former ring tests and a series of putative interesting laboratories were informed about the ring test for 2014. In all cases an invitation letter(see Annex 1), a participation form and an invoice were distributed. Until the beginning of March a total of 29 participants for the microscopic composition analysis were listed. The samples with an accompanying letter were sent to all participants on Tuesday 4th of March 2014. On Wednesday March 5th an E-mail message was sent to all participants, together with a file containing a sheet with instructions (see Annex 2) and the electronic report forms (see Annex 3 and 4), and the request to confirm the receipt of the package.
The sample was intended to be analysed according to IAG method 2: “Method for the Identification and Estimation of Constituents in Animal Feedingstuff” (IAG, s.n.). Further instructions to the participants were enclosed in the box with samples, which are reproduced in Annex 5.
The closing date for reporting results was fixed at April 1st . Several requests were received to extend the period for analysis with two weeks. This request was granted and the closing date was set at April 15th. In several cases participants appeared not to be able to submit their results even within the extended period. A total of 24 sets of results were received late March or during April. Since the analysis of the results was carried out during May, all these results were considered valid and taken into consideration. All sets received after May 1st were ignored.
The draft report was finalised at June 2nd .

2.3 Participants

The 24 participants, which successfully submitted their microscopic results, originated from 10 countries: 9 member states of the European Union, and one other country. The list of participants is presented in Annex 6. More than half of the participants originated from Germany (14).

2.4 Analysis of results

The results are analysed according to the IAG scheme of uncertainty limits as approved during the 2006 meeting in Rostock. These limits are presented in Table 2. The model is graphically presented in Figure 1. Shares of ingredients in the feed formulation outside the limits of the model were indicated as “wrong”.

Table 2

IAG model for uncertainty analysis of the composition of a compound feed.

Actual amount in %	Accepted uncertainty limits
< 2%	“traces “
2.0 – 5.0%	+/- 100% relative
5.01 – 10.0%	+/- 5% absolute
10.01 – 20.0%	+/- 50% relative
– 50.0%	+/- 10% absolute
> 50%	+/- 20% relative

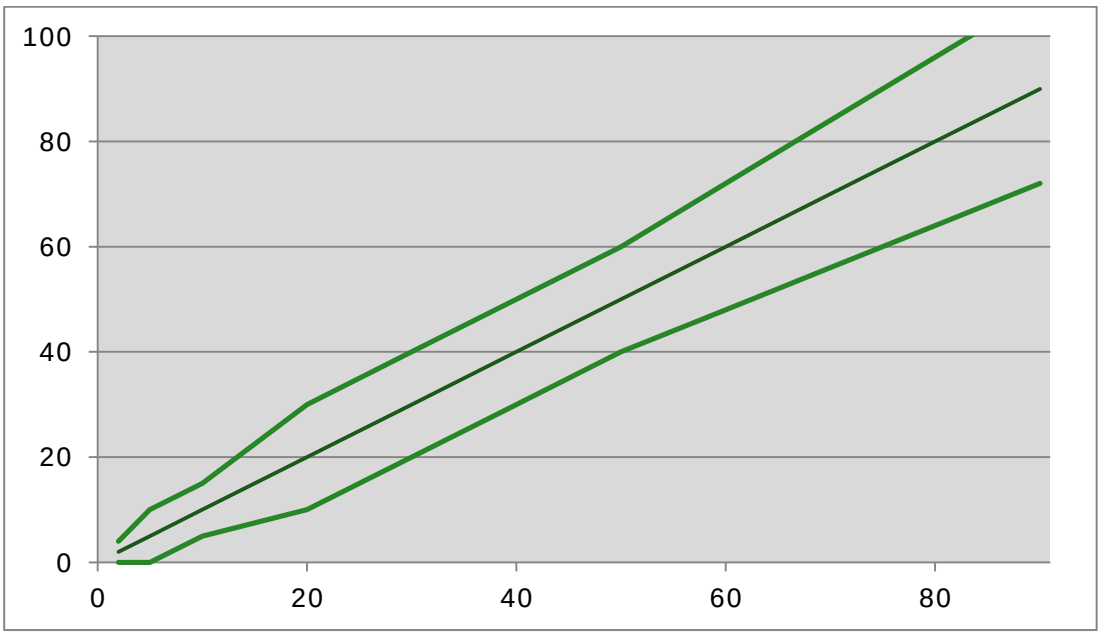

Figure 1 *IAG model for estimating uncertainty. X-axis: correct portion of ingredient in%, Y-axis: estimated portion of ingredient in%. Inner line: correct estimation, outer lines: limits for uncertainty interval at a given percentage.*

3 Results

Twenty-nine samples were sent to all participants, of which 24 were returned. All results were received by E-mail, in most cases by means of a scan and the original report file. Not in all cases a scan as pdf-file was submitted although this was clearly requested. In all those cases that a participant send in several versions of the report sheet the most recent version was used. All reports were included.

The procedure for the analysis of the composition is described in IAG method A2 (IAG, s.n.). This method is familiar to most participants as members of IAG section Microscopy. This method was applied by most participants or a method based on IAG method A2 was used. Only a few participants reported to have applied an internal or other method.
The results of the 24 participants are fully presented in Annex 7 and summarised in Table 3.

Table 3
overview of the main ingredients of the analysed sample, the (wrong) declaration, the correct composition, the uncertainty range, and the numbers of participants that under- or overestimated the share of the ingredients. N = 24.

	declared	correct	range:	# (%) under est.	# (%) over est.
wheat meal	34.0%	45.5%	35.5-55.5%	4 (16.7%)	0 (0%)
soyabean products, partly extracted	28.0%	27.7%	17.7-37.7%	0 (0%)	0 (0%)
beet pulp	12.0%	0.0%	0.0%	0 (0%)	3 (12.5%)
corn meal	11.0%	10.9%	5.4-16.3%	0 (0%)	4 (16.7%)
rapeseed and rapeseed meal	7.0%	6.9%	1.9-11.9%	0 (0%)	2 (8.3%)
vegetable fat	5.0%	5.0%	0.0-9.9%	-	-
minerals and vitamins	3.0%	3.0%	0.0-5.9%	0 (0%)	0 (0%)

The estimated amounts were correct in most cases. Nine out of 24 participants made one error (7 participants) or more than one error (2 participants). One lab reported up to 4 wrong results. There is no clear correlation with the method applied. Out of the nine participants with at least one wrong result six applied IAG method A2, one a modified version of this method, and two an internal or other method.
The underestimations for wheat meal (4 participants) were just below the uncertainty limit (Figure 2). The overestimations of corn meal (4 participants) showed a larger difference: maximum estimation 19% vs. upper uncertainty limit 16.3%.
Fat is not visible under a light microscope and most participants took the declared amount unaltered as part of their report. Three participants reported the (incorrect) presence of beet pulp at 1%, 5% or 7%, respectively. The two labs with a substantial presence of beet pulp (> 5%) underestimated wheat meal.

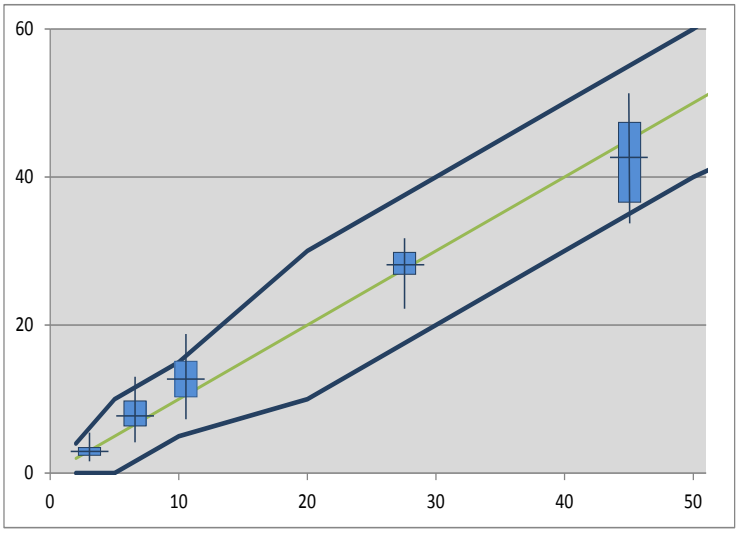

Figure 2 *The results of the IAG ring test composition 2014 projected on the uncertainty limits of the IAG model. Bars: P_{25} – P_{75} percentile interval, vertical line: minimum – maximum range, horizontal line: average.*

In general the ingredients with a high share are underestimated in those cases that the reported results felt outside the range, and an underestimation was reported for ingredients at low or lower share in the composition. The amount of minerals and vitamins is generally not estimated, but established by making a sediment.
A total of 11 participants reported the presence of animal material. These results are discussed further in the report by van Raamsdonk *et al.* (2014).

4 Discussion

4.1 Method application

The method IAG-A2 is based on a procedure of sieving the sample and applying several embedding and staining methods. Examinations are to be carried out both a binocular microscope (up to 70 x magnification) and a compound microscope (100 – 400 x magnification; IAG, s.n.). Since a lot of ingredients contain starch or traces thereof, starch identification is an important part of it. At the final stages the share of the different ingredients are summed up over the different sieve fractions. The methods relies on identification of the ingredients supported by handbooks or reference material (IAG, s.n.). The identification of ingredients which are selected from a large range of legal ingredients (Feed catalogue: Regulation (EC) 242/2010) is a complicated procedure and assumes a high level of skill of the technician.

In the view of this complicated procedure and compared to the established limits (Figure 2) the current results are good. Results of IAG ring tests on composition of compound feeds in previous years (unpublished results) revealed an identical trend in the sense that higher shares are underestimated and lower shares are overestimated. The same was also found for the relatively low level of contamination of animal proteins: contamination levels between 0.02% and 1.5% were all overestimated (Veys and Baeten, 2006; van Raamsdonk *et al.*, 2012). This is a problem for a detection method which is expected to be accurate at least at a level of 0.1%, but in the framework of the IAG uncertainty limits all reported levels could be reported as “traces” (Table 2). The IAG model for uncertainty limits was agreed upon in 2006 after an extensive evaluation of alternatives. Nevertheless, in the range of 5 – 10% share of an ingredient in the formulation of a feed (Table 2) absolute limits were used in the model. This is also the range where overestimations are to be expected. A further analysis of unpublished results of past IAG ring tests for composition could provide data for improving the model.

More than half of the participants originated from Germany. It can be assumed that the evaluation of the composition of compounds feeds is still well established in this country.

4.2 Justification for establishing composition

European legislation requires that feeds and feed materials are labelled according to a range of requirements, including composition. It has been stated that labelling serves enforcement, traceability and control purposes (Regulation (EC) 767/2009, pre-ambule 17). Feed materials should be mentioned in order of decreasing share, and additional information on composition should be available on request with uncertainty limits of +/- 15% (Regulation (EC) 767/2009, Article 17). It is not stated if this is a relative or absolute range. Annex IV of Regulation (EC) 767/2009 presents requirements for the labelling of basic parameters such as crude proteins, crude fibres, sugars, starch, oils and fats, minerals, moisture, crude ash and related parameters with a mix of absolute and relative ranges. Monitoring of the correct declaration of the amount of the feed materials used in a compound feed (or other feed) is necessary for two reasons.

At first economic fraud can be based on the replacement of an expensive ingredient by a cheaper one. Secondly, certain compositions can give direction to look for specific unwanted contaminants. The fractionation of a sample in a sediment and a flotate can help to pinpoint the presence of contaminants and might improve their traceability. In the framework of the current report these opportunities apply to feed analysis. In a broader view, composition analysis in the entire food chain can improve the effect of monitoring actions. The new legislation on food labelling (Regulation (EC) 1169/2011), effective from December 13th 2014, obliges to provide more detailed information to customers on composition and related topics.

5 General conclusions and recommendations

5.1 Conclusions

The current results indicate that feed ingredients can be identified and shares can be estimated successfully. Besides a proper method, well developed skills of technicians are vital for a good performance.

5.2 Recommendations

- Tools for maintenance and dissemination of expertise are important for future performance.
- An evaluation of the IAG uncertainty model can help to improve its application.

Acknowledgements

The board of IAG section Feeding Stuff Microscopy (dr. I. Paradies-Severin (LUFA, Hameln), dr. G. Frick (ALP, Posieux) and ir. J. Vancutsem (FAVV, Tervuren), dr. R. Weiss (AGES, Vienna)) supported this study as advisory board for communication with the scientists and laboratories working in this research field, and in the final report activities. Their contributions are greatly acknowledged.

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Annex 1 Invitation letter

Dear colleague, Dear IAG member,

The IAG section Feeding stuff Microscopy organizes annually several ring tests for the evaluation of composition or detection of prohibited constituents in animal feed. The presidium of the IAG section Feeding stuff Microscopy and RIKILT have agreed to organize together the 2014 ring test for the following situations:

- Test IAG-2014-A. Detection of the presence of animal proteins in a set of four samples. This test was organised by RIKILT in previous years. Targeted protocol: Regulation (EC) 152/2009, consolidated version of February 12, 2013.
- Test IAG-2014-B. Declaration of the composition of a compound feed (one sample). This test was organised in previous years by a colleague institute. RIKILT will take over the organisation for the year 2014. Targeted protocol: IAG method A2.
- Test IAG-2014-C. Detection of undesired botanical substances in two samples of bird feed. Seeds of *Ambrosia* will be part of the test, combined with one other botanical substance as listed in Directive 2002/32/EC. Targeted protocol: IAG method A5.

The costs for participating in the animal protein test will be €220, and for the undesired botanical substances test will be €100. The composition test is free of charge. The single sample for the composition test will be part of the animal protein test. RIKILT will encourage you to subscribe to both these tests (A and B), although this is not mandatory. On behalf of the IAG section Feeding stuff Microscopy, RIKILT will invite you for participation in these ring tests.

The samples for test IAG-2014-A and IAG-2014-B will be sent around late February or early March 2014. Also a questionnaire will be sent by E-mail, together with instructions and relevant documentation on protocols. A time slot of four weeks is planned for the analyses of the samples by every participant. This means that late March or early April all results are expected to be returned to RIKILT. The samples of test IAG-2014-C will be sent mid-March and results needs to be reported mid-April. All results are intended to be reported at the annual meeting of the IAG working group Microscopy in Posieux (Switzerland) in June 2014. The final reports will be published later in 2014. All communications of the evaluation will be fully anonymous.

If you are interested to participate in one or more ring tests, please return the application form and make a payment of the appropriate amount to RIKILT. You will receive an invoice after receipt of your application form. Make sure that the reference number, your name and your institute's name is mentioned. This information is necessary to avoid loss of payments that cannot be linked to participating institutes.

We are looking forward to have a nice cooperation for the next ring tests and to have results which will support your laboratory quality system.
On behalf of the IAG section Microscopy and the RIKILT organizing team,

L. van Raamsdonk

Annex 2 Basic instructions for the test procedure

IAG ring test 2014 composition

Instructions for the IAG ring test

1 You have received a box with an introduction letter and four vials containing 30 grams of possibly contaminated animal feed. Please report the receipt of your package as soon as possible by E-mail to the address mentioned below.

2 The sample meant for analysis of composition is indicated on the letter enclosed in the package. Analysis for composition is preferably carried out using method A2 of the IAG section Microscopy. Other methods, however, are allowed. Take care to homogenise the content of each vial before taking the amount for analysis.

The sample meant for analysis of composition is also part of the sample set for the detection of animal proteins. Perform at first the test for composition and keep the material for the analysis of animal proteins. Making a sediment is an obvious part of the estimation of the composition. It is recommended to use 10 grams of the sample for making this sediment, which suits the need for the method for detection of animal proteins.

Link to IAG method A2

3 The results need to be reported as percentual estimations on the tab "Results". The organiser will apply the uncertainty intervals to your estimations as part of the evaluation. Reporting consists of the following steps:

3a Please fill in the questionnaire on the page "Procedure".
Most of the cells contain a drop-down list. These lists can be used to select an answer as follows. When clicking on a cell, the cursor changes into a hand. A second click will open the drop-down list.

Your unique lab number is mentioned in the introduction letter.
All the fields with a drop-down list have to be completed.

3b Please enter your results in the fields at page "Results". Your unique lab number automatically shows up after your have entered it at the page Procedure. Enter yourself the unique label of the vial.

4 After completing the two forms "Procedure" and "Results", they have to be sent to the organisers in two ways:

4a Save the Excel file by using "Save as …", add your unique lab code to the end of name (replace the ## signs with your lab number). The forms have to be sent by E-mail as Excel file and as a scan (preferably *.PDF) to leo.vanraamsdonk@wur.nl.

4b Results will be included in the final evaluation and report only if both forms are sent in by electronic mail, and after the proper receipt of the requested fee.

5 Direct any questions to leo.vanraamsdonk@wur.nl

6 Closing date is April 1st, 2014.

RIKILT Institute of food safety, Wageningen, the Netherlands

Annex 3 Report form for procedure details

Please complete at least all the pink cells with a drop down list that apply to your procedure	select your choice from a drop down list	type in your answer if necessary
IAG ring test 2014 composition		
Please select your unique lab number	**-- select --**	
Have you read the ring test instructions?	-- select --	
Which detection method do you use?	-- select --	

Annex 4 Report form

Please complete all the pink cells of the declared ingredients and/or of the sediment. If an ingredient is not found enter zero.

IAG ring test 2014 composition

lab number		
sample number		
	declaration	estimated
wheat meal	34.0%	
soyabean products, partly extracted	28.0%	
beet pulp	12.0%	
corn meal	11.0%	
rapeseed and rapeseed meal	7.0%	
vegetable fat	5.0%	
minerals and vitamins	3.0%	
other, please specify:		
sediment amount in mg		
Total:	100.0%	0.0
Final conclusion on declaration:		-- select --
Comment if necessary		

Signature:

Date: 2-6-2014

Annex 5 Additional instructions

Test 2014-B: botanic composition of sample: [ ]

The sample with the number indicated here ↑ is meant for the analysis of the botanic composition. Take care to homogenise the content of the vial before taking the amount for analysis. This sample will be used for two purposes: detection of animal proteins, and analysis of botanic composition.

The current test is designed as “label control”. The report form contains the label information, which can be either correct or wrong. Your results can be entered in a second column.

All results can be entered in the report form with “composition” in the name.

Annex 6 List of participants

Austrian Agency for Health and Food Safety-AGES	Austria
FLVVT	Belgium
Danish Veterinary and Food Administration	Denmark
IPL Atlantique	France
IDAC	France
Bayerisches Landesamt fur Gesundheit und Lebensmittelsicherheit	Germany
CVUA-RRW	Germany
LTZ Augustenberg	Germany
SGS Germany GmbH	Germany
LLFG Landesanstalt für Landwirtschaft	Germany
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft, GB6-Labore Landwirtschaft / LUFA, FB62	Germany
Agri Q-service GmbH	Germany
Landesbetrieb Hessisches Landeslabor, Landwirtschaft und Umwelt	Germany
Futtermittelinstitut Stade (LAVES)	Germany
Landeslabor Berlin-Brandenburg	Germany
LUFA-Speyer	Germany
LUFA Nord-West	Germany
Thüringer Landesanstalt für Landwirtschaft	Germany
Universität Hohenheim, LA Chemie (710)	Germany
MGSZH ÉTBI TAKARMÁNYVIZSGÁLÓ NEMZETI LABORATÓRIUM	Hungary
MasterlabBV	Netherlands
Instytut Zootechniki PIB, Pracownia w Szczecinie	Poland
Trouw nutrition Espana	Spain
Agroscope (ALP), Swiss Research Station	Switzerland

Annex 7 Results: presence of animal proteins, microscopic detection

	declared	correct	range:	lab:	2	3	5	9	10	11	12	17	18	19	22	24
wheat meal	34.0%	45.5%	35.5%	- 55.5%	45%	50%	42.5%	46%	52.0%	40%	45.0%	35%	36%	37%	38%	44%
soyabean products, partly extracted	28.0%	27.7%	17.7%	- 37.7%	27%	26%	28.1%	27%	29.0%	22%	30.0%	29%	30%	31%	28%	27%
beet pulp	12.0%	0.0%	0.0%	- 0.0%	0%	0%	0.0%	0%	0.0%	0%	0.0%	0%	0%	0%	0%	0%
corn meal	11.0%	10.9%	5.4%	- 16.3%	11%	16%	14.8%	9%	7.0%	18%	10.0%	15%	13%	19%	15%	11%
rapeseed and rapeseed meal	7.0%	6.9%	1.9%	- 11.9%	6%	5%	6.6%	8%	6.5%	7%	7.5%	7%	7%	6%	10%	10%
vegetable fat	5.0%	5.0%	0.0%	- 9.9%	5%	ND	5.0%	5%	4.0%	5%	5.0%	5%	5%	5%	5%	ND
minerals and vitamins	3.0%	3.0%	0.0%	- 5.9%	3%	3%	2.5%	3%	1.5%	5%	2.0%	3%	3%	2%	3%	2%
Deviations basic composition					0	0	0	0	0	1	0	1	0	1	0	0
insect / shrimp / lobster / chitin	0.0%	1.0%	0.0%	- 2.0%	1%	ND	0.5%	2%	ND	3%	ND	6%	ND	ND	ND	ND
weed seeds										traces						traces
other					2% [a]					traces [a]						traces [b]
sunflower																
bycatch flour													6%			
animal proteins											0.5% [c]			traces [d]	1% [e]	present [f]

	declared	correct	range:	lab:	26	27	30	31	38	42	43	44	46	49	51	52
wheat meal	34.0%	45.5%	35.5%	- 55.5%	34%	39.6%	35%	48%	51.0%	48.0%	47%	45%	46%	50%	35%	49.5%
soyabean products, partly extracted	28.0%	27.7%	17.7%	- 37.7%	27%	29.8%	30%	28%	26.0%	30.0%	32%	28%	22%	28%	30%	29.3%
beet pulp	12.0%	0.0%	0.0%	- 0.0%	5%	0.0%	0%	0%	0.0%	0.0%	0%	1%	0%	0%	7%	0.0%
corn meal	11.0%	10.9%	5.4%	- 16.3%	18%	10.3%	15%	17%	8.5%	11.0%	12%	10%	12%	9%	13%	10.2%
rapeseed and rapeseed meal	7.0%	6.9%	1.9%	- 11.9%	13%	9.5%	10%	4%	6.8%	7.0%	4%	8%	13%	5%	7%	7.1%
vegetable fat	5.0%	5.0%	0.0%	- 9.9%	0%	3.4%	5%	0%		0.0%	1%	5%	5%	5%	5%	ND
minerals and vitamins	3.0%	3.0%	0.0%	- 5.9%	3%	3.7%	3%	3%	2.0%	3.0%	3%	3%	2%	3%	3%	2.1%
Deviations basic composition					4	0	1	1	0	0	0	1	1	0	2	0
insect / shrimp / lobster / chitin		1.0%	0.0%	- 2.0%	ND	3.6%	ND	present	0.5%	1.0%	1%	ND	ND	ND	ND	1.6%
weed seeds						0.1%										0.2%
bycatch flour																
animal proteins							1% [f]									

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RIKILT report 2014.010

RIKILT Wageningen UR is part of the international knowledge organisation Wageningen University & Research centre. RIKILT conducts independent research into the safety and reliability of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

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