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Study of Physiological Water Content of Poultry Reared in the EU (EU Contract number: 30-CE-0460798/00-25)

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Study of physiological water content of poultry reared in the EU



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Objectives

To analyse the physiological water content of chicken raised and slaughtered in the EU (breast fillets/leg cuts)

- Compare results with the 1993 study
- Decide whether the limits of technically unavoidable water uptake (extraneous water), as a result of preparation and cooling of poultrymeat, given in Commission Regulation (EC) No. 543/2008 are still relevant or whether they need to be revised.



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Background

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Legal Basis

- Council Regulation (EC) No 1234/2007 - the consolidated marketing regulation for all agricultural products
- Commission Regulation (EC) No 543/2008 - detailed rules and requirements for poultrymeat placed on the EU market

Commission Regulation (EC) No 543/2008

- Annex VIII of this regulation states the limits of technically unavoidable water uptake (extraneous water) allowed as a result of preparation and cooling as:
 - 2% for air chilling
 - 4% for air spray chilling
 - 6% for immersion chilling
- When physiological water is taken into account the current limits for water in poultry are:
 - Chicken breast without skin: 3.40%
 - Chicken leg cuts with skin: 4.05%, 4.15% and 4.30% for air, air spray and immersion chilling respectively

Legal Basis

Enforcement of these limits ensures that:

- poultrymeat is prepared according to good manufacturing and hygienic practice
- consumers are not being disadvantaged by excess “added water” in the fresh poultrymeat they purchased

Background to current study (1)



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- Limits set in Regulation (EC) No 543/2008 were based on a study published in 1993 which measured the physiological water content of chicken produced in the European Union (EU)
- Poultry production in the EU is an intensive agricultural activity. Developments in respect of breeds, age and weight at slaughter have taken place across the EU since 1993

Background to current study (2)

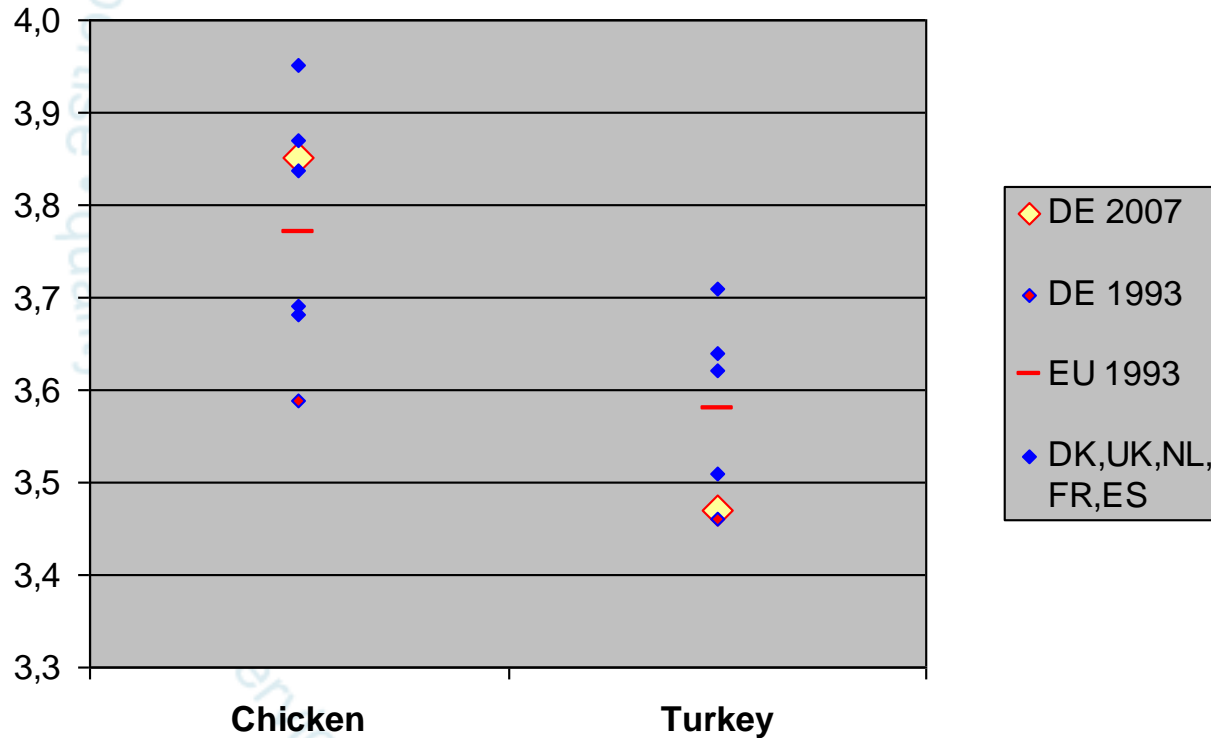


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- A UK study (2000) indicated a lower nitrogen content in chicken leg cuts - around 1% reduction from the previous study (1963)
- A German study (2007) indicated a 7% change in the water/protein ratio of chicken leg cuts within Germany
- Control data submitted to the Commission from NRLs suggested an increase in the failure rate of enforcement samples

Provided a rationale to re-examine W/P ratios across the EU

Data from German study (2007)



Water/Protein ratios in German samples comparing 1993 and 2007 studies¹

¹Effects of sample preparation on the water/protein ratio of poultry cuts in relation to the identification of extraneous water. MRI (Max Rubner Institute), 2009:

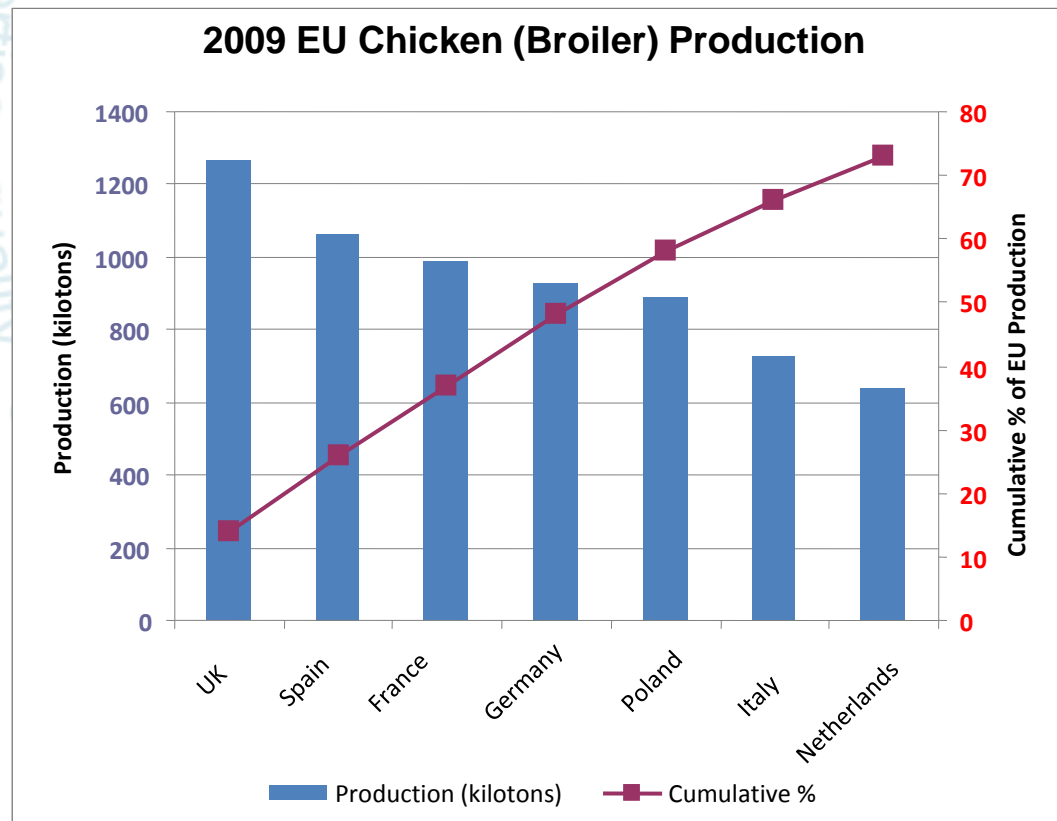
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Key Features of EU Poultry Production



- Most common breeds of poultry produced in the EU
 - Ross
 - Cobb
- Two class of weights, based on age
 - Light (UK approximately 1.3-1.6 kg for 5-5.5 weeks)
 - Heavy (UK approximately 2.5-2.7 kg for 7-8 weeks)
- Gender
 - both males and females important at both weights
 - heavy females may not be available in some Member States as they mature more quickly than males and reach commercial carcass weights earlier
- Most commonly consumed cuts chosen
 - Breast (no skin)
 - Leg with skin

EU Broiler Production



7 countries account for just over 74% of the total production

Sampling protocol – per country



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Full Factorial Balanced Design of the Key Variables Associated with Commercial Poultry Production in the EU

Breed	Ross				Cobb				Total
Flock Number	1		2		3		4		
Weight:	Light	Heavy	Light	Heavy	Light	Heavy	Light	Heavy	
Female:	3	3	3	3	3	3	3	3	24
Resultant Breast:	3	3	3	3	3	3	3	3	24
Resultant Leg:	3	3	3	3	3	3	3	3	24
Male:	3	3	3	3	3	3	3	3	24
Resultant Breast:	3	3	3	3	3	3	3	3	24
Resultant Leg:	3	3	3	3	3	3	3	3	24

Details of samples to be collected in one member state

Sampling protocol – total



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Per Member State:

Total number of birds:	48
Total number of breast samples for analysis:	48
Total number of leg samples for analysis:	48

From 7 Member States:

Total number of birds:	336
Total number of breast samples for analysis:	336
Total number of leg samples for analysis:	336
Total number of samples for analysis:	672



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Samples and Sample Collection

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Issues Encountered – Before Sampling



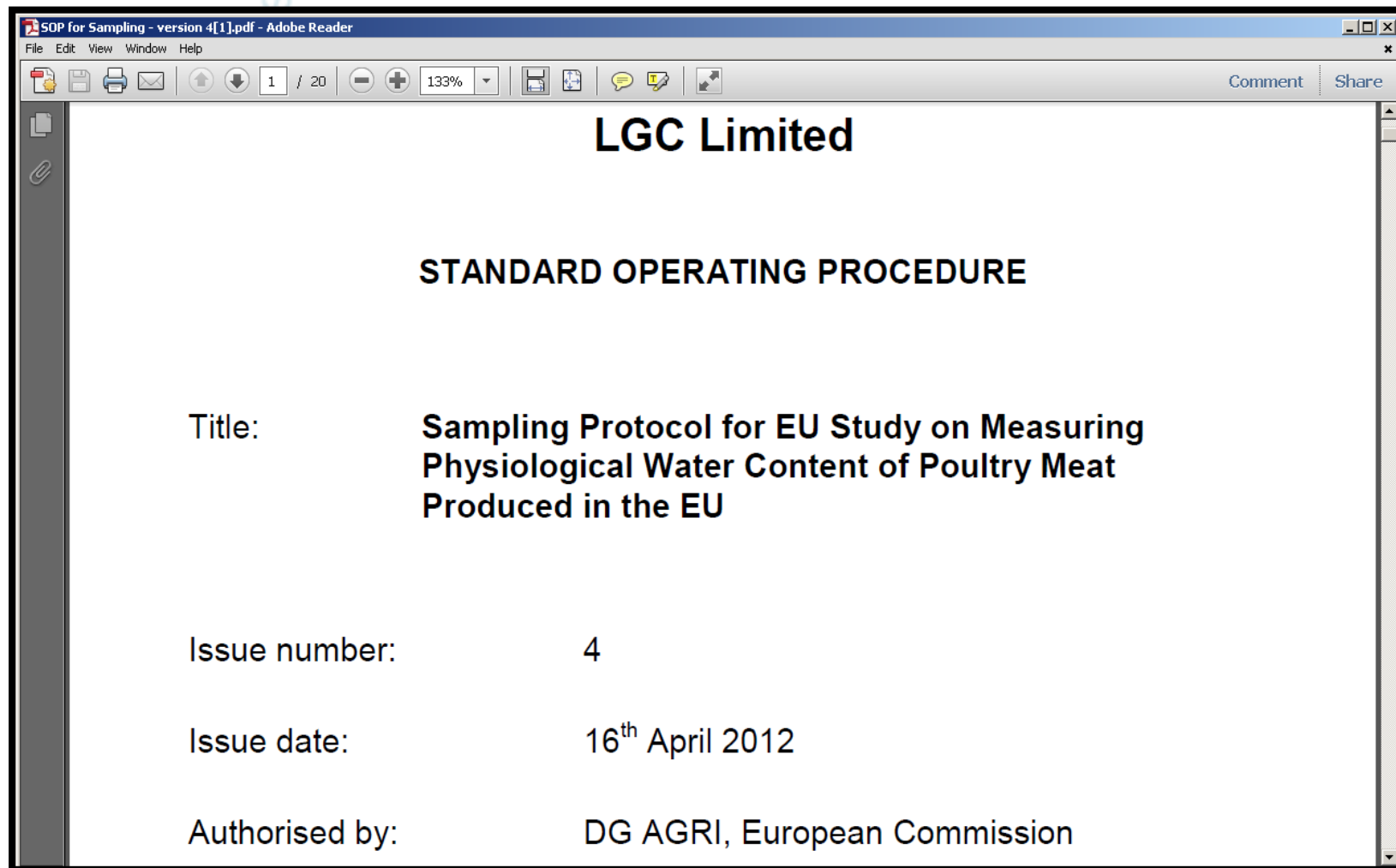
- Breed differences between Member States
- Sub breeds e.g. Ross 308, 708 etc.
- Weight – the weight ranges for light and heavy categories differed between Member States
- Different use of genders
- Slaughterhouse
 - schedules only set a week in advance
 - schedules confirmed around 24h before slaughter
 - difficult to guarantee availability of specified samples
 - some slaughterhouses use only one breed

Final Sampling Plan

- Weight categories as in the Member State but based on UK classification
- Not possible to sample light and heavy birds from the same flock (4 flocks per breed)
- Hubbard (not Cobb) collected in France
- Italy – Ross only sampled, more Male birds, different weight categories (medium 2.4-2.8kg, heavy 3.6-4kg):

Breed:	Ross (308)				Ross (708)				Total
	Flock Number:	1	2	3	4	1	2	3	
Weight:	Medium	Heavy	Medium	Heavy	Medium	Heavy	Medium	Heavy	
Female:	0	0	0	0	6	0	6	0	12
Resultant Breast:	0	0	0	0	6	0	6	0	12
Resultant Leg:	0	0	0	0	6	0	6	0	12
Male:	6	0	6	0	6	6	6	6	36
Resultant Breast:	6	0	6	0	6	6	6	6	36
Resultant Leg:	6	0	6	0	6	6	6	6	36

Sampling SOP



The sampling SOP was agreed with the Commission steering group and local NRLs. It was sent to the slaughterhouses in advance of sampling

Sampling Points



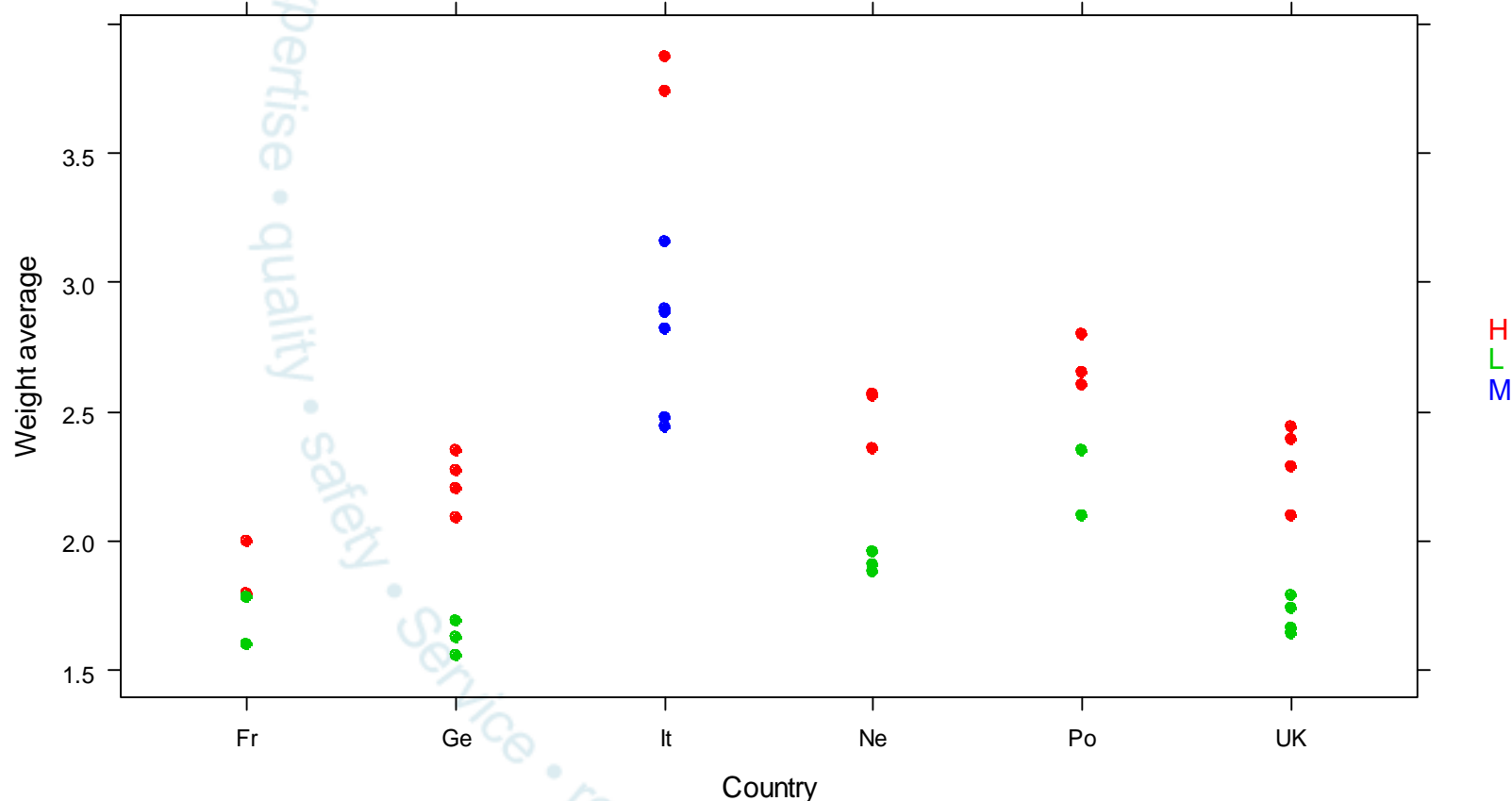
- Samples collected from major poultry producers in each of the 7 Member States to maximise chance of collecting all samples in 1 or 2 days
- In all Member States except Poland:
 - samples were collected by slaughterhouse staff under the supervision of the NRL and UK NRL / UK expert external consultant.
- Poland
 - samples were collected by slaughterhouse staff under the supervision of the UK NRL.
 - A member from the EC project steering group witnessed sampling in Italy

Samples Collected - summary



- Equal numbers of Ross and Cobb (except Italy/France)
- Up to four flocks per breed (defined as per farm/shed)
- Equal numbers of light and heavy birds (except Italy)
- Equal numbers of male and female birds (except Italy)

Weight categories collected



*Spain (not shown) heavy and light birds from same flock but heavy/light carcasses selected in line with UK weight classifications

*France Ross weight's based on average weight of flock estimated from weight range

Samples - cuts

- The samples consisted of:
 - chicken breast fillets without skin
 - chicken legs with bones and skin
- Breasts samples were removed by cutting the skin (without plucking)
- Leg samples were dry plucked by hand.
- **Sampling was performed avoiding contact with water.**



Samples - cuts

- Samples were triple bagged in two individual air-tight bags and a tamper evident bag and sealed.

- Each bag was labeled with an identification number stating:

- Country
- Flock
- Breed
- Weight
- Cut



- Samples were frozen (-18°C or below) for a minimum of 48 hours and sent by overnight courier to LGC.

Sampling in Spain





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Sample Homogenisation

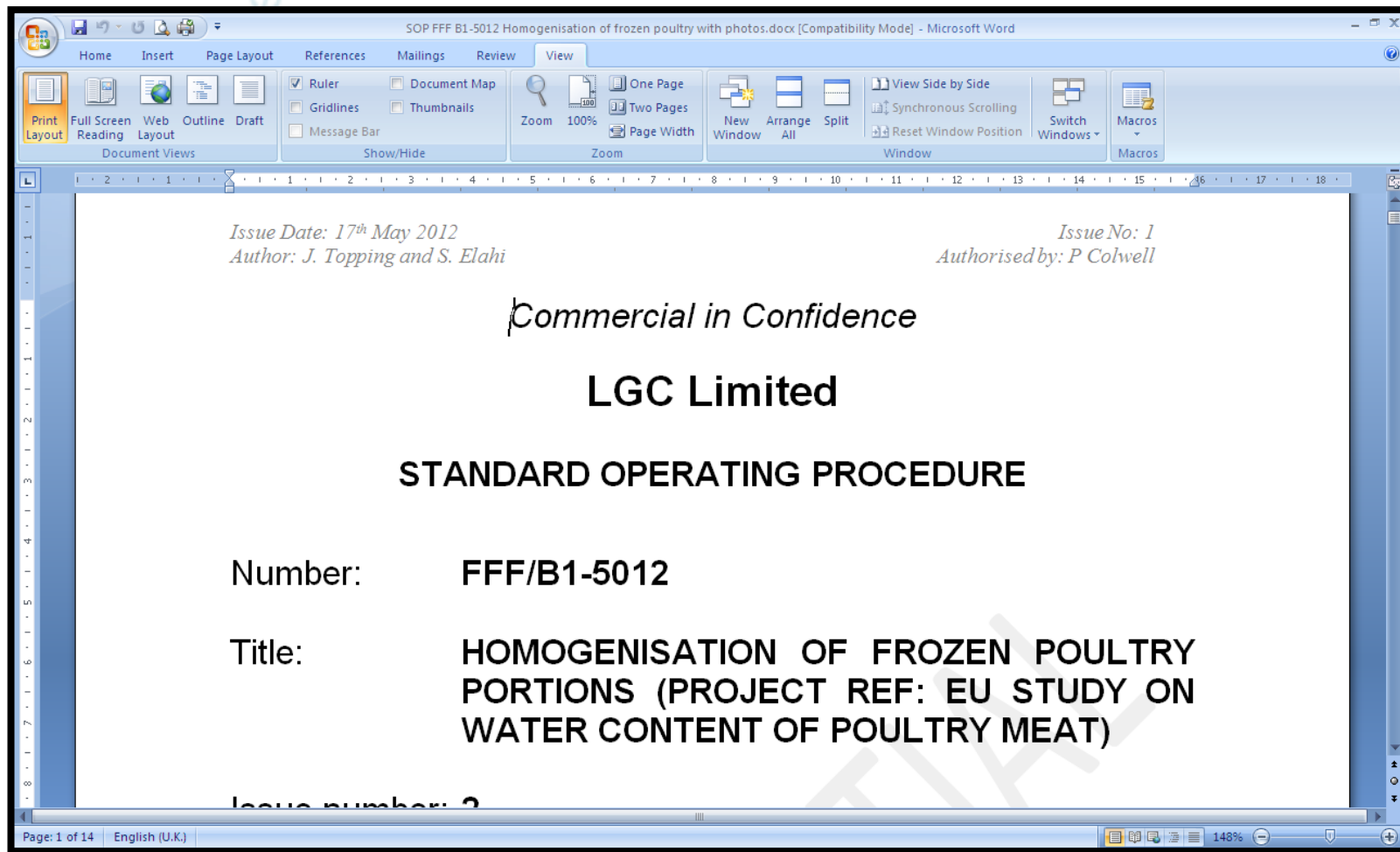
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Sample Homogenisation



- Sample preparation requirements according to Regulation (EC) 543/2008:
 - Within 1 hour after the sample was taken from freezer
 - Meat cleaver – used saw and Delta clamp
 - Mincer with 4mm bore
 - Further homogenisation if required
- Samples from one county only homogenised at any one time

Sample Homogenisation SOP

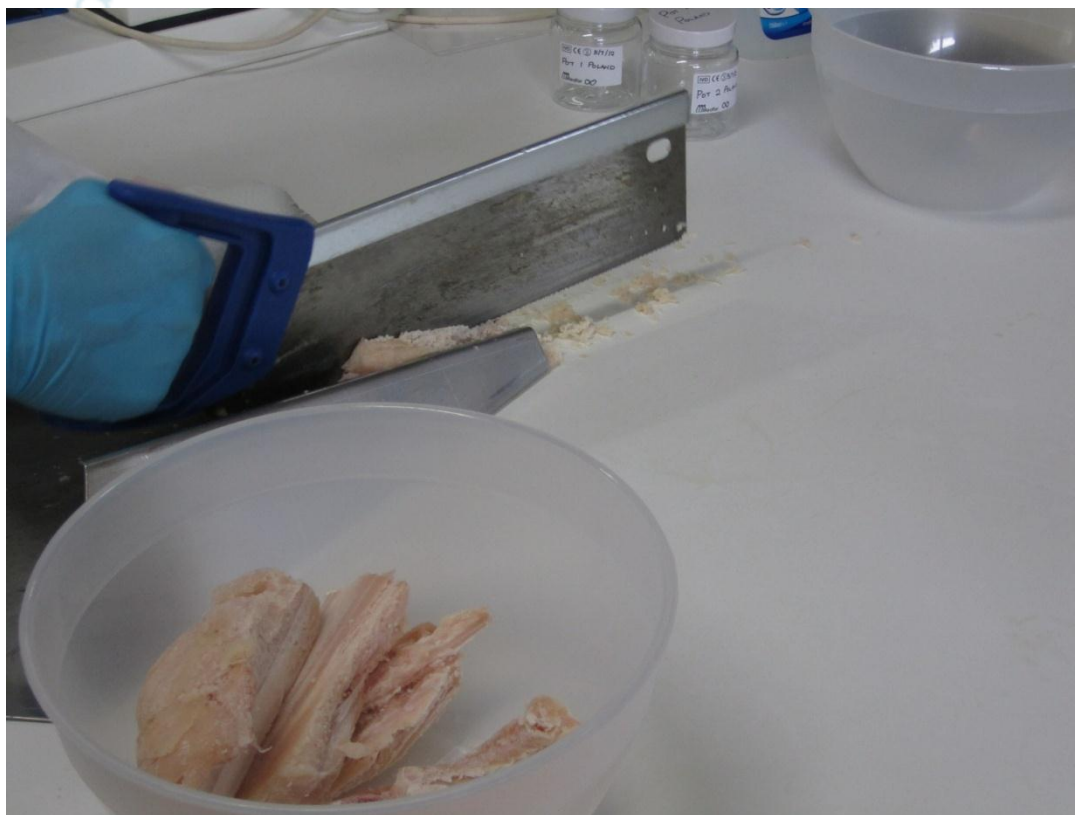


The SOP was agreed with the Commission steering group

Sample Homogenisation



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A saw was used to cut the sample into strips of no greater than 5.5 cm in width taking care to avoid as much 'splatter' of the sample as possible.

Sample Homogenisation



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The sample was collected in a plastic bowl ready for transfer to the mincer. Care was taken to scrape off and include as much sample residue from the Delta clamp and surrounding bench coat as possible.

Sample Homogenisation



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The sample was added to a mincer, with a 4mm hole disc, to produce the first mince.

Breast Samples



Double minced

Leg Samples



Minced once and then further homogenised

Sample Storage



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Homogenised samples stored at -18°C

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Sample Analysis

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Participating Laboratories



NRLS:

Denmark

France

Germany

Ireland

Italy

The Netherlands

Spain

UK

Note: Countries are listed alphabetically and order does not necessarily relate to assigned laboratory number

Stratified Sampling Plan



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- 672 samples to be analysed in 8 different laboratories
- A stratified sampling plan was constructed based on
 - Country of origin of chicken
 - Breed (Ross/Cobb/Hubbard)
 - portion (leg/breast)
- Samples were grouped according to these variables and each lab sent at least three samples from each group.
- Gender and weight category were randomly distributed

Stratified Sampling Plan



- Why a stratified sampling plan?
- Prevents 'confounding' of laboratory and country effects
- Allows separate tests for significance of between-country differences in test items independently of laboratory effects
- Allows any anomalous laboratory effects to be identified

Analysis - protocol



Each laboratory was sent:

- 84 frozen homogenised samples on dry ice by overnight courier
- CRM - ERM®-BB501a Processed Meat (pork)
- Analytical protocol with acceptance criteria
- Reporting spreadsheet

Analysis - protocol



- Analytical Protocol was provided to each laboratory
- Water (moisture) by the oven drying method:
 - ISO 1442:1997, Meat and meat products - Determination of moisture content (Reference method) or equivalent.
- Nitrogen determined by the Kjeldahl:
 - ISO 937, Meat and meat products - for the determination of protein content or equivalent.

Analysis – acceptability criteria



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- a) Each sample to be analysed in triplicate
- b) Agreement required between the three replicates based on repeatability in standard methods
- c) CRM to be analysed in every analytical batch and assess performance against the limits supplied
- d) Instructions for repeats to be performed if b and/or c above result in failures

Analysis – acceptability criteria



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The following acceptance criteria were set for the CRM and the repeatability for triplicate results per sample.

Analyte	Quality control limits for CRM	Repeatability criteria for samples (triplicate results, 99% confidence)
Water	61.8 ± 2.7 g /100g	<0.4 g /100g
Nitrogen	2.30 ± 0.16 g /100g	<0.08 g /100g

Methods by laboratory

Lab no.	Water		Nitrogen	
	Sample weight (g)	Method	Sample weight (g)	Method
1	4	Manual by weighing	0.8 to 1	Kjeldahl
2	5	Manual by weighing	0.2 to 0.4	Leco model No FP323 (Dumas)
3	4.7 to 7.8 (mean 5.2)	Manual by weighing	1.1 to 1.6 (mean 1.4)	Kjedahl
4	5	Manual by weighing	1	Kjeldahl
5	5 to 6	Manual by weighing	2 ± 0.2	Kjeldahl
6	5	Manual by weighing	1.4 to 1.5	Kjeldahl
7	5.0 to 5.1	Manual by weighing	2 to 2.1	Kjeldahl
8	5	Manual by weighing	1	Leco model No CNS2000 (Dumas)

Repeat Analysis

- Acceptance criteria were set for triplicates of the same sample
- Samples that failed on the first analysis were retested
- Samples which failed on the second analysis were not tested further
- The number of samples which failed on the first analysis were investigated by:
 - analytical test (water/nitrogen)
 - cut (breast/leg)

Repeat analysis – data assessment

Cut	No. of Repeats		% Repeats	
	Water	Nitrogen	Water	Nitrogen
Breast	5	5	1.5%	1.5%
Leg	43	4	12.8%	1.2%
Total (Breast and Leg)	48	9	7.1%	1.3%
Total number of samples:	672	672	-	-

- Breast repeats <2% for both analysis – acceptable
- Leg repeats 13% -for nitrogen is this acceptable/fit for purpose?

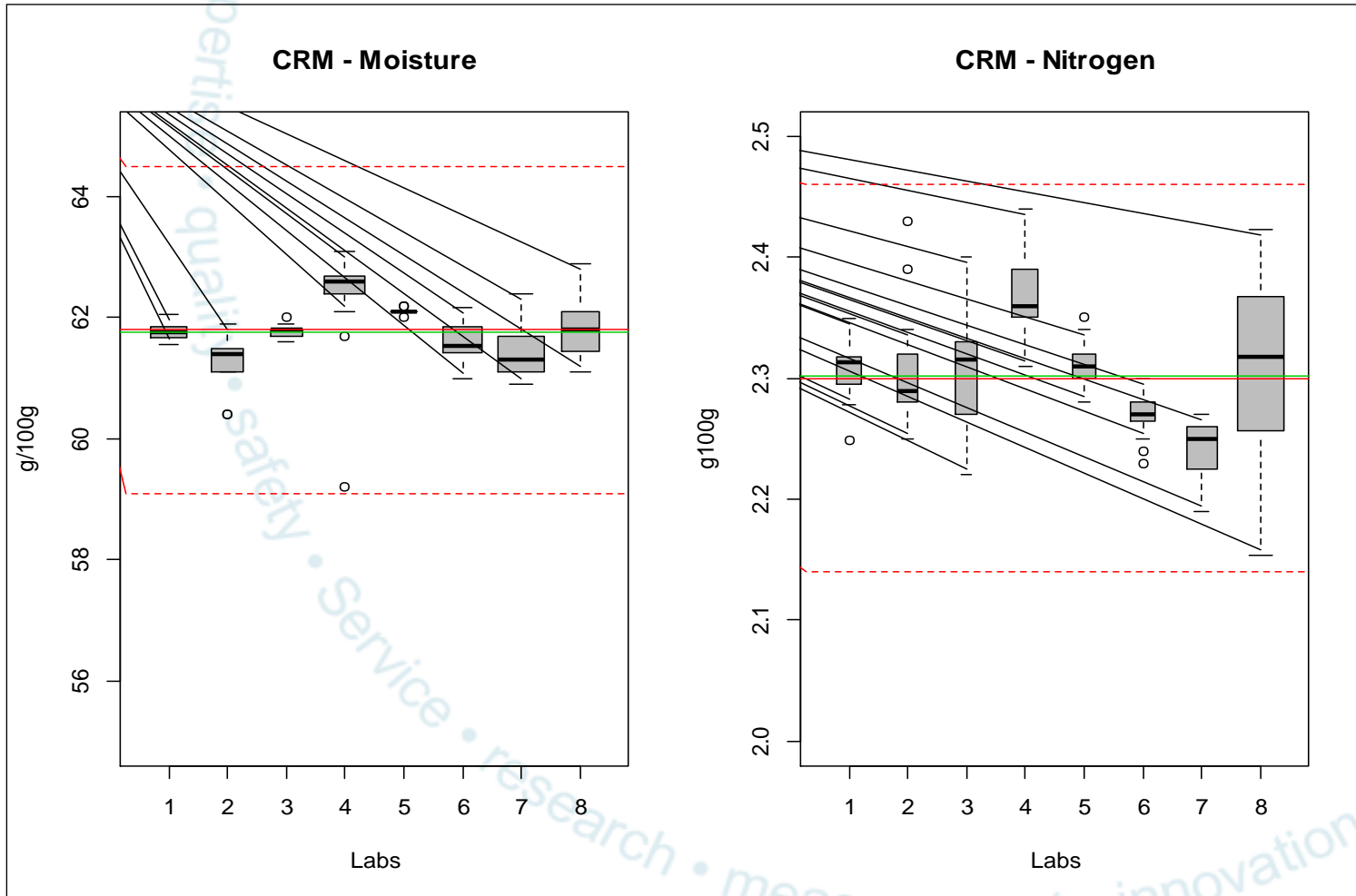
Small pieces of bone and cartilage were observed in homogenised leg samples – possibility of improving homogenisation procedure?

Methods & Repeat Rates



- Water
 - All laboratories used the gravimetric method
 - All laboratories used similar sample weight ~5g
 - Repeats attributed to inhomogeneity of leg samples
- Nitrogen
 - 6 laboratories used the Kjeldahl method
 - 2 laboratories used the Dumas method
 - Sample weights used varied from 0.2 – 2.2g
- No correlation between sample weight used and the number of repeats

Laboratory Performance (CRM)



Certified content: Moisture = 61.8 +/- 2.7 g/100g

Nitrogen = 2.30 +/- 0.16 g/100g



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Statistical Analysis and Results

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Statistical Analysis



Results analysed by LGC's statistics team to detect differences between:

- Breed
- Age/Weight
- Gender
- Country
- Cut
- Laboratory effects

Statistical Analysis



- a) outlier identification methods of ISO 5725 and additional techniques as appropriate
- b) Multi-way analysis of variance to test for the significance of the above effects compared to measurement variance
- c) Variance component extraction to establish the individual variance contributions
- d) Estimation of the mean water content, protein content and W/P ratio
- e) Calculation of confidence limits appropriate for the sample size used in current regulations
- f) Comparison of results with the results of the 1993 study
- g) Evaluate whether the limits Regulation (EC) No. 543/2008 are still relevant

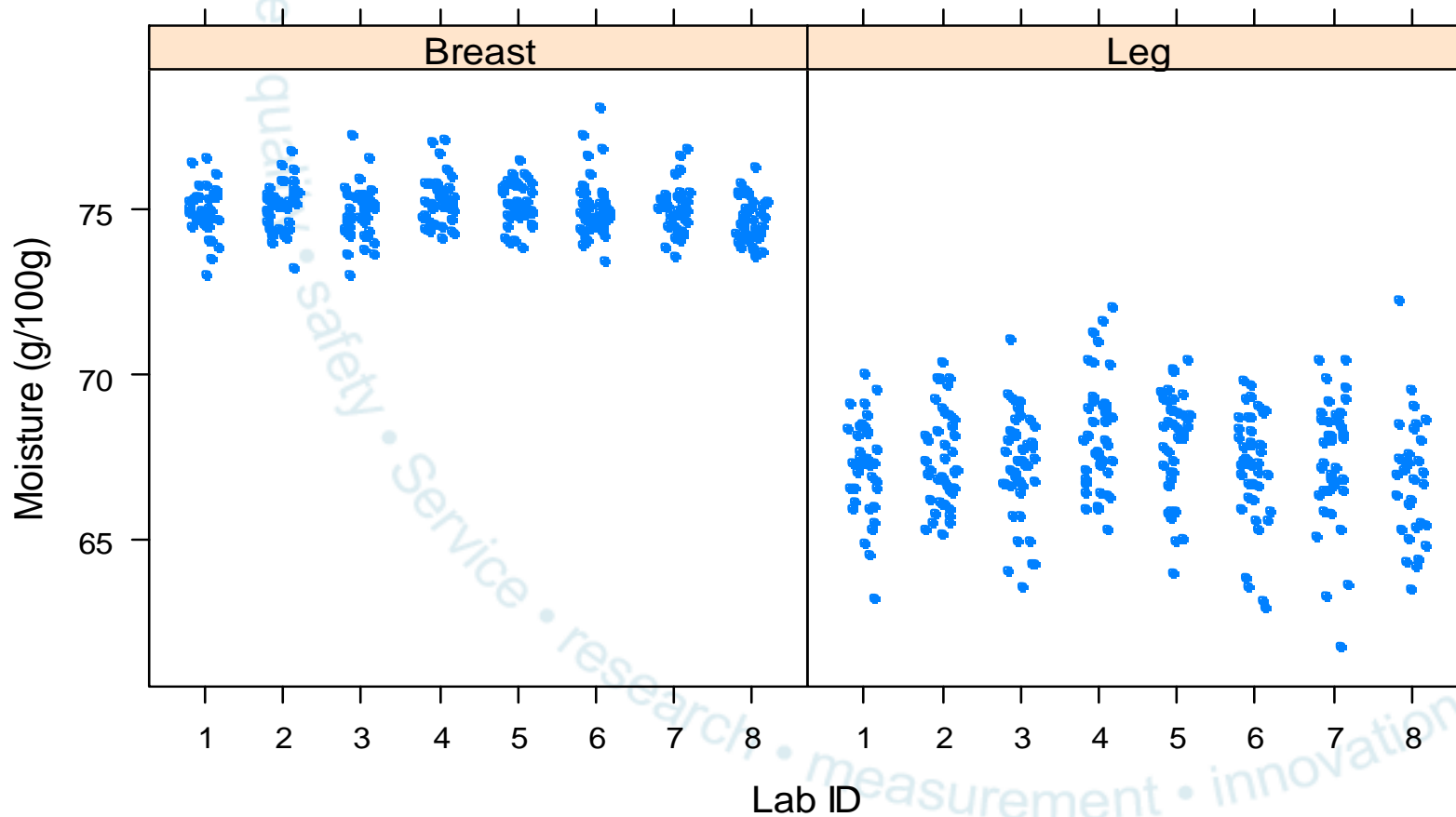
Results – data set

Breed*	C						H						R								
	F			M			F			M			F			M					
	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M	H	L	M			
Fr							11	11					11	11		12	10		12	10	
Ge	10	11			12	12										11	12			11	12
It																		24	23		46
Ne	12	12			12	12										12	10			11	12
Po	12	11			12	12										12	12			12	11
Sp	12	12			12	12										11	11			12	11
UK	12	11			11	12										12	12			11	11

- Data for samples which had failed either water or nitrogen analysis on two occasions were not used (< 4% entire data set)
- Unbalanced data set – due to modifications to sampling plan to take into account breeds and weight categories used in France & Italy

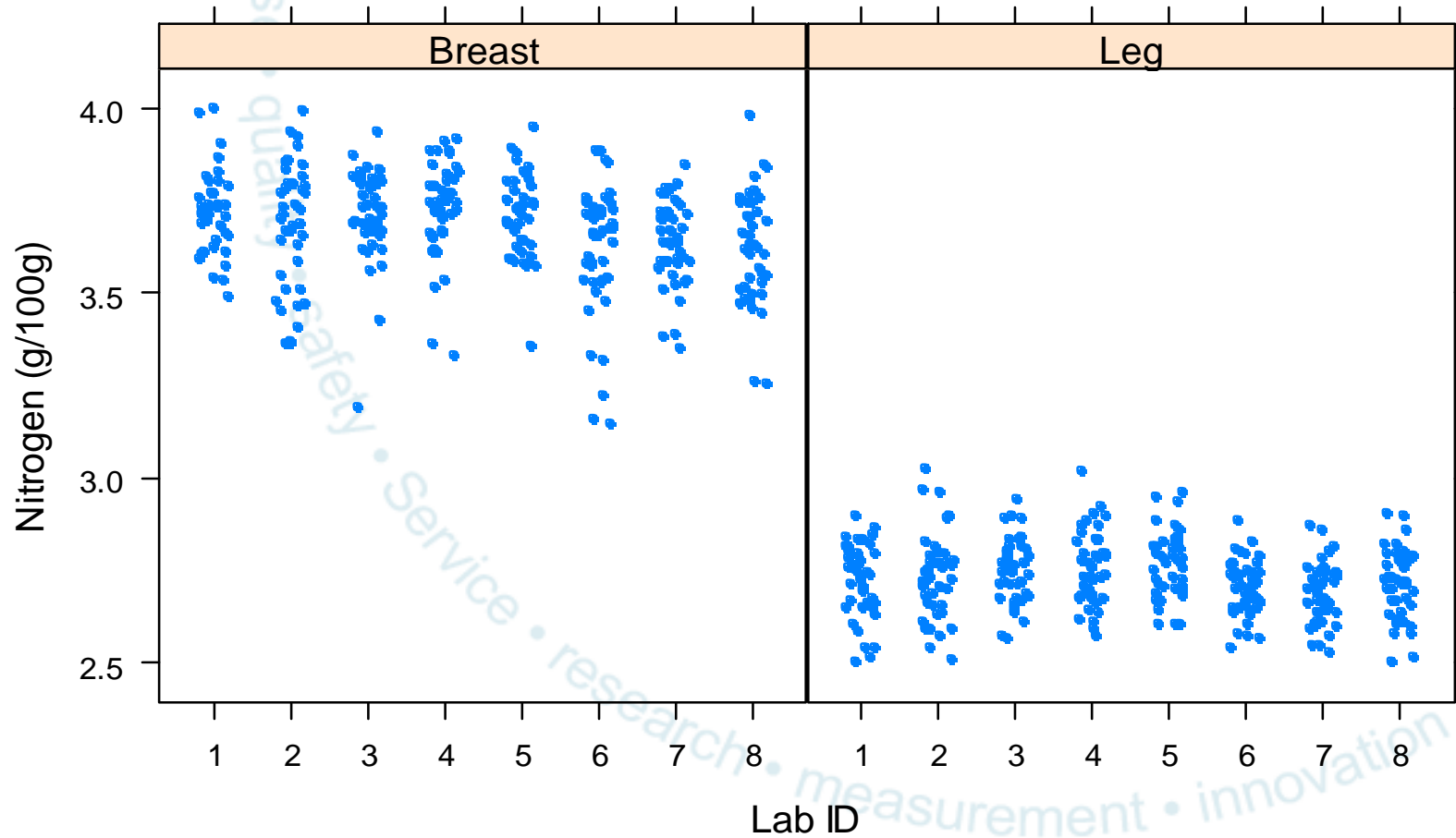
Results – Water (Moisture)

Moisture



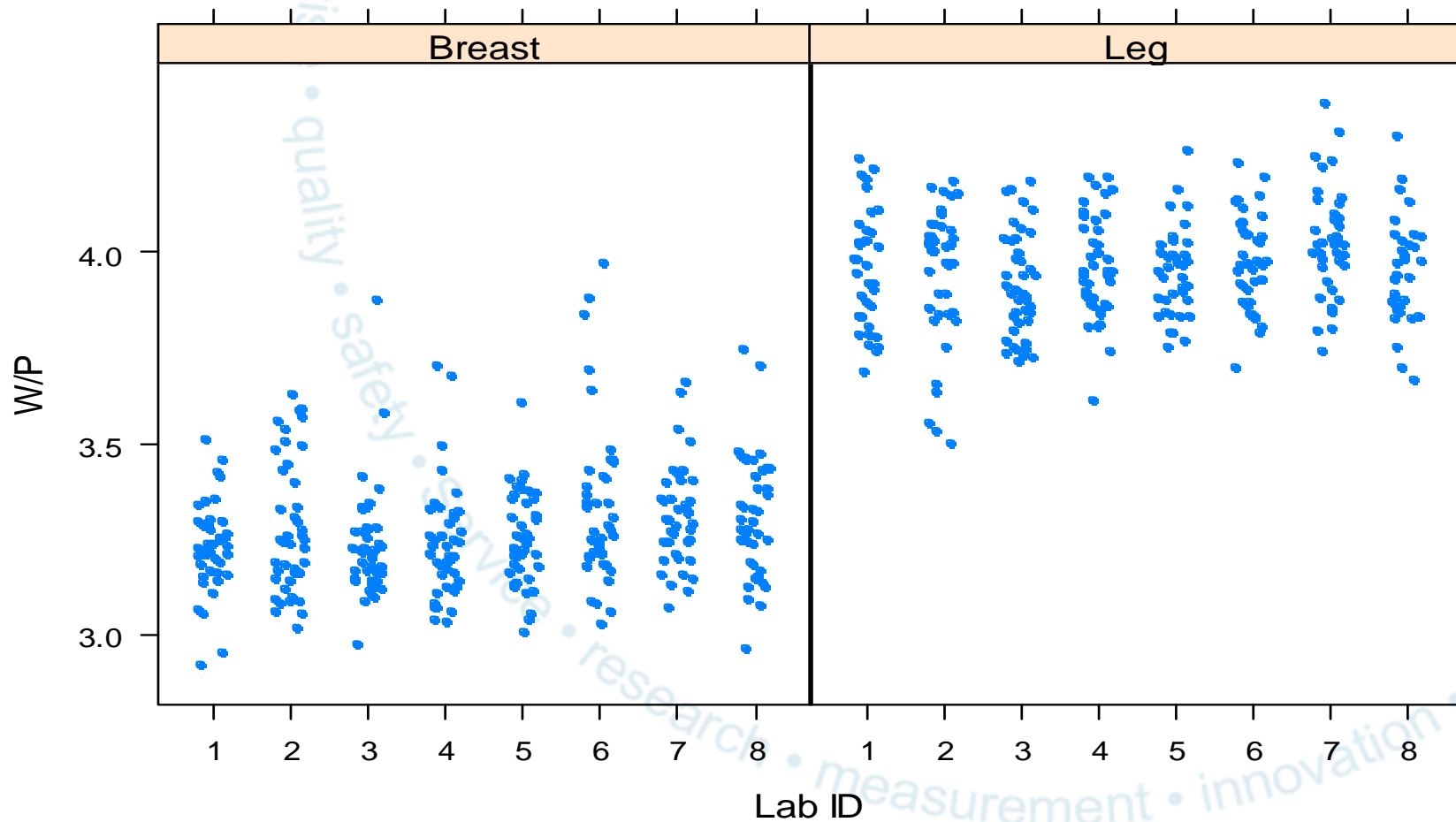
Results - Nitrogen

Nitrogen



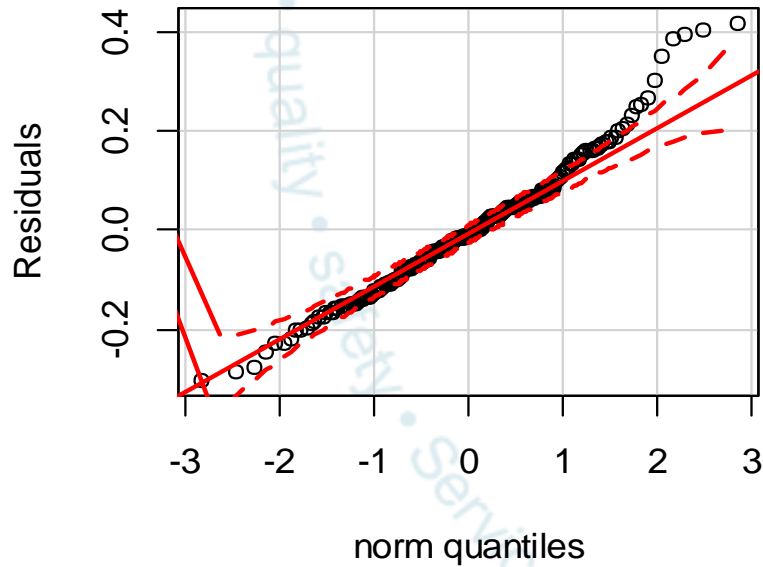
Results – W/P ratios

Water Protein Ratio

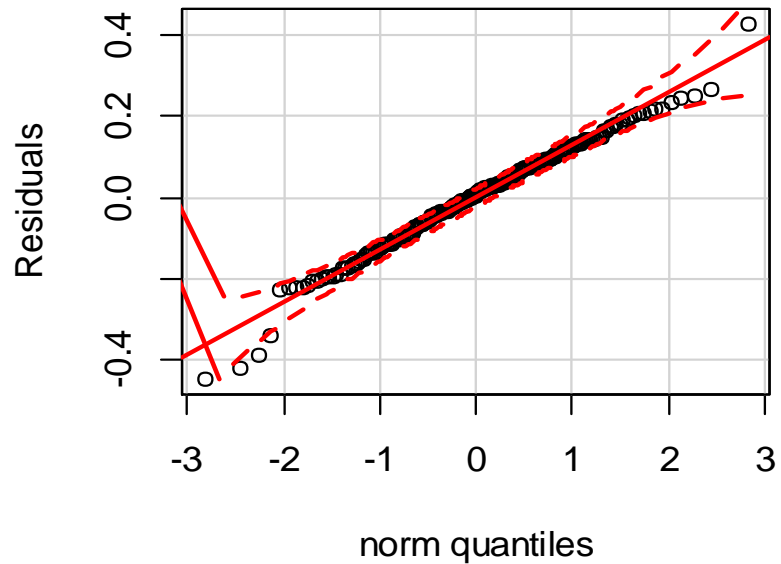


Results – distribution of data

Breast



Leg



Results – significance of factors



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Factor	Breast	Leg
Lab	✓	
Breed	×	×
Country	✓	✓
Gender	✓	×
Wcat	✓	×
Breed:Gender	×	
Breed:Country	✓	
Breed:Wcat	×	✓

✓ *p-values* < 0.05 × *p-values* > 0.05

Empty cells: factor was removed from the linear model.

- Data set Italy and France excluded (unbalanced)
- Breed is not significant overall (Ross & Cobb)
- Breast data influenced by all factors except breed
- Leg data mostly influenced by country of origin

Results – pair-wise comparison



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Pairs	Breast	Leg
Po - Ge		✓
Sp - Ge	✓	
Sp - Ne	✓	
Sp - Po	✓	
Sp - UK	✓	
Male - Female	✓	

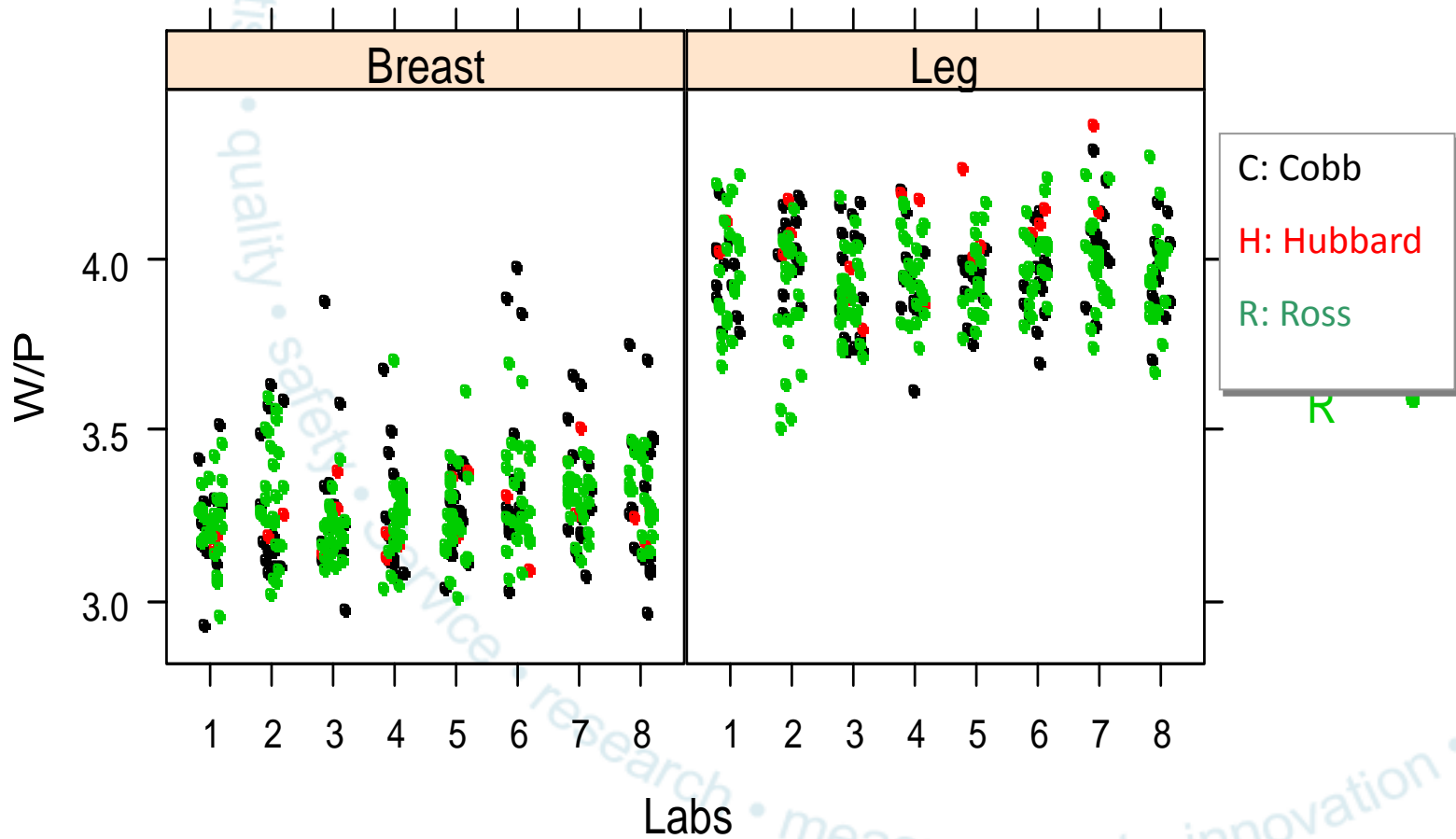
✓ *p-values* < 0.05

Spain shows differences from other countries for breast meat, while only Ge – Po differ for leg meat. The gender is only significant for breast meat.

- Spain shows a difference from other countries for the breast cut – higher values
- Poland differs from Germany for the leg cut - higher values

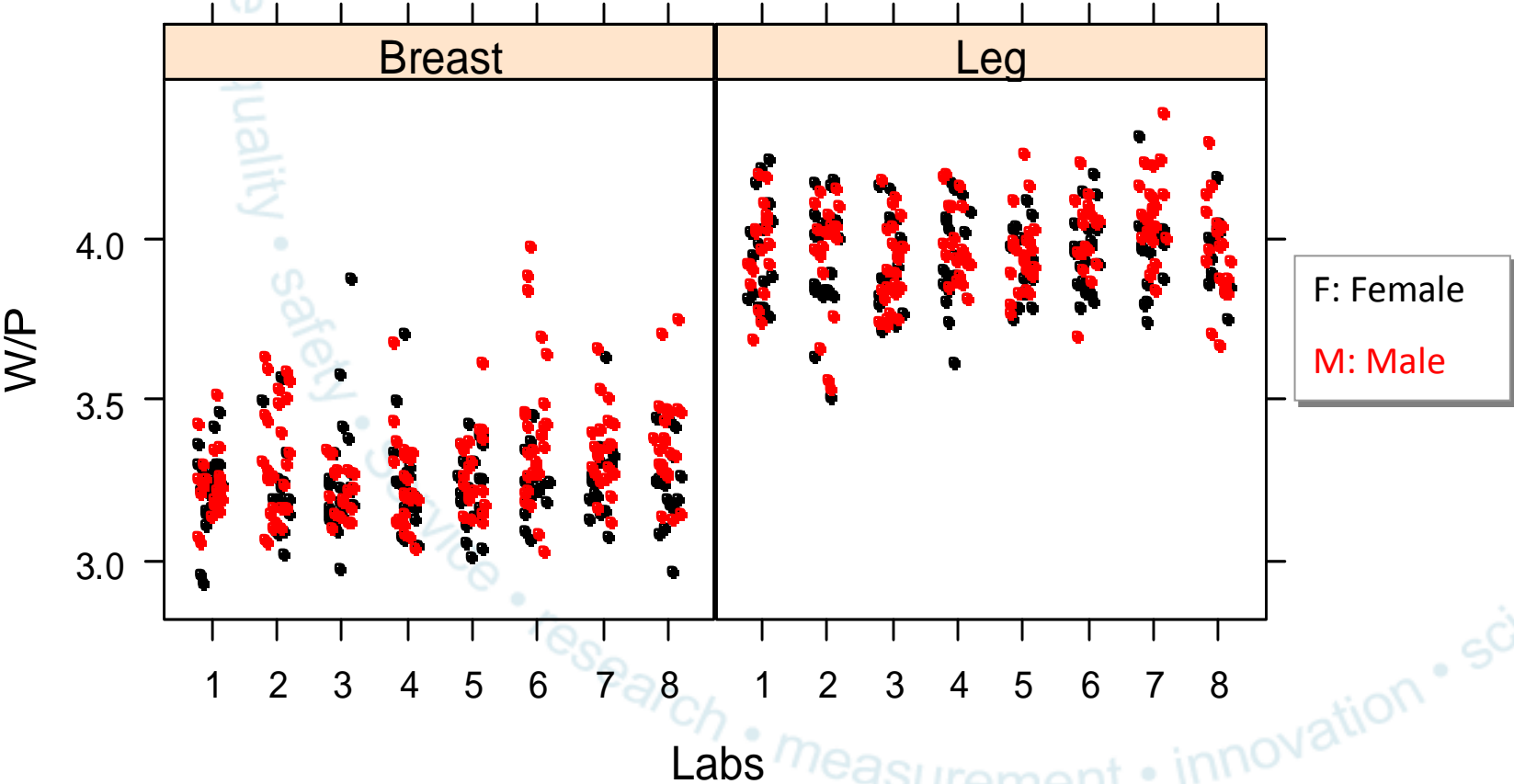
Results – Breed

W/P - Breed



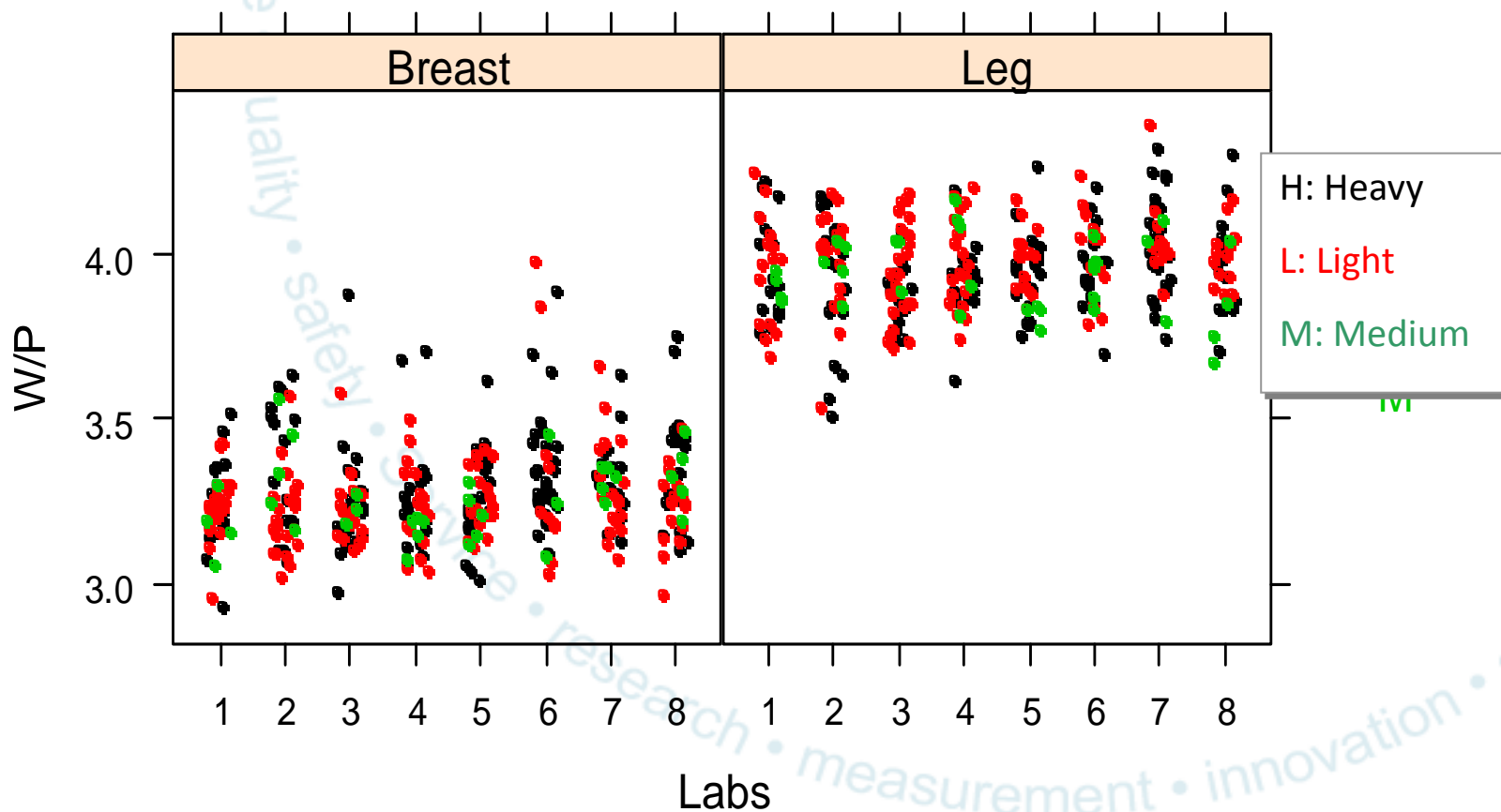
Results - Gender

W/P - Gender

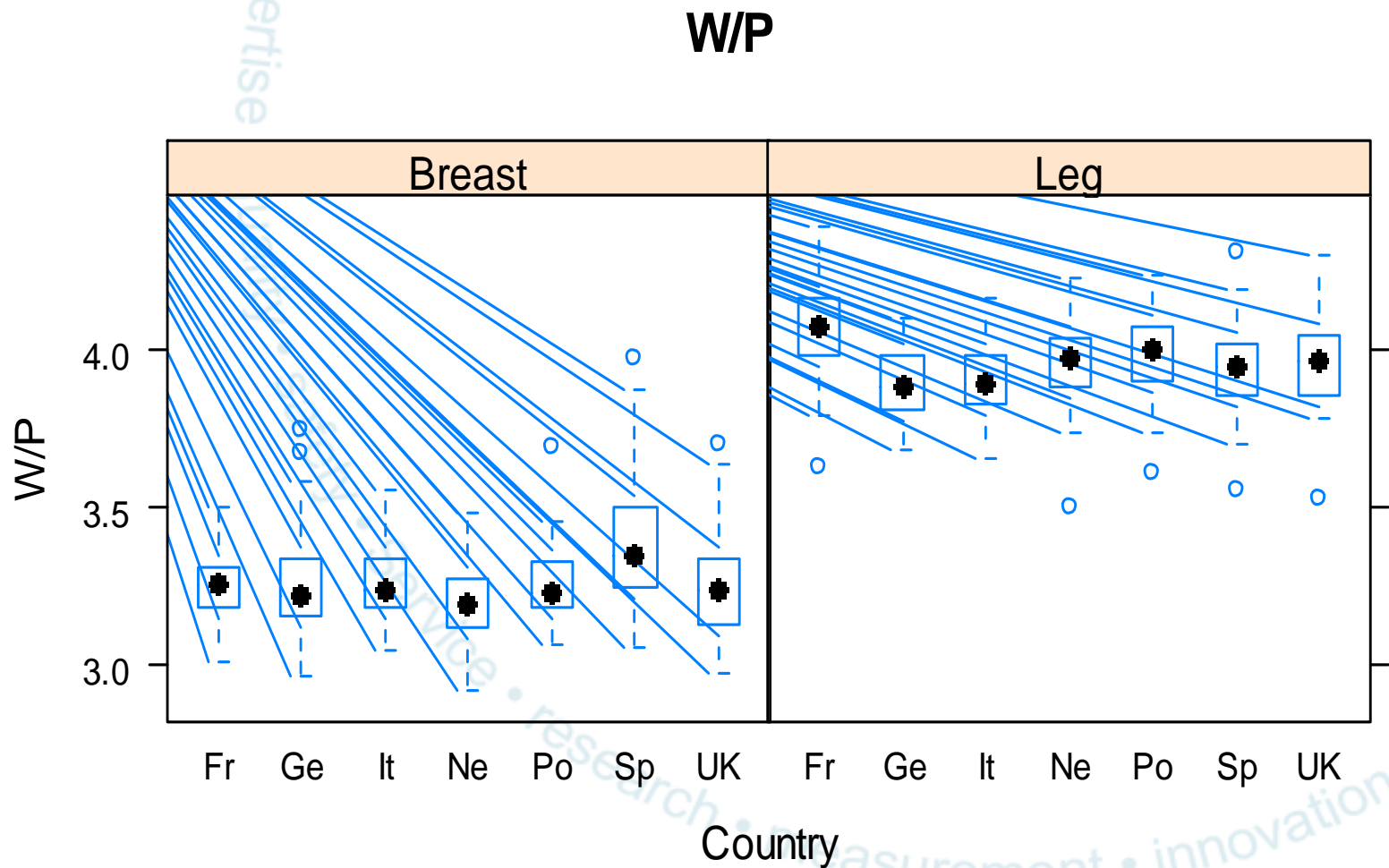


Results – Weight Category

W/P - Wcat



Results – Country of Origin



Significance of Differences – 2012 study



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- Statistically significant differences for:
 - Country of origin
 - Gender
- But....
 - Effects were small compared to dispersion for individual samples so not considered to be of practical significance

Comparison with 1993 Study



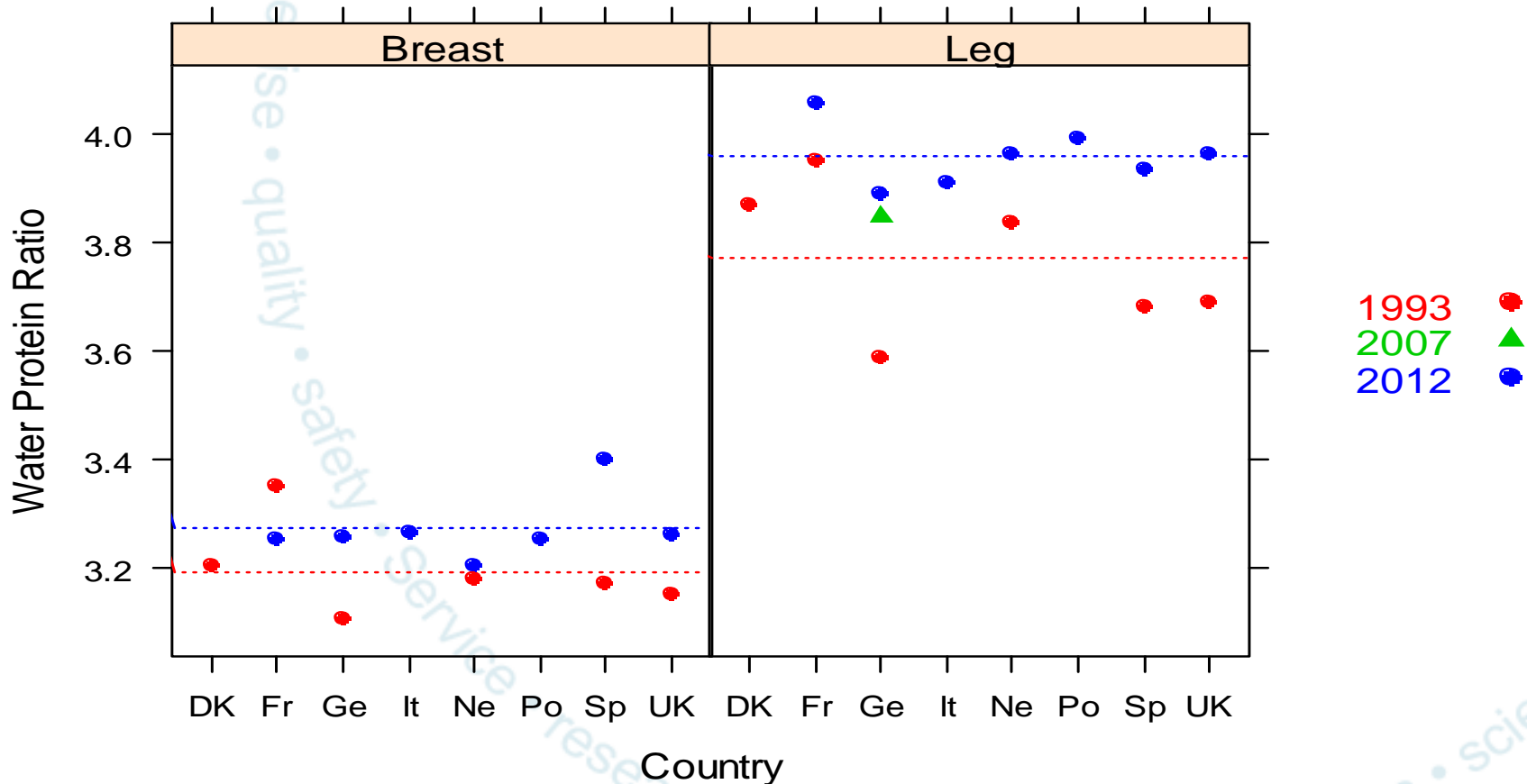
Differences in approach

Activity	1993 Study	2012 Study
Number of samples	120	336
Sample Type	Ideally same breed but not specified	Breed selected on information from Industry; Ross, Cobb & Hubbard
Sampling	By individual NRLs	By UK NRL + local NRL
Sample homogenisation	By individual NRLs	By one NRL
Analysis	By individual NRLs	Stratified sampling plan
Quality control	Pre-study check	Within study checks
Countries	DK, DE, ES, FR, NL & UK	DE, ES, FR, IT, NL, PL & UK
Laboratories	6	8

Results – comparison 1993 study



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Water protein ratios per country comparing 1993, 2007 and 2012 studies, dotted lines are the overall means.

Results – comparison 1993 study



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Breast fillets – precision of water/protein ratio

Country	Current 2012 Study			Previous 1993 Study		
	Mean	Std Dev	No. observations	Mean	Std Dev	No. observations
France	3.253	0.107	48	3.348	not stated	20
Germany	3.257	0.152	48	3.102	not stated	20
Italy	3.265	0.123	48	-----	-----	-----
The Netherlands	3.202	0.129	47	3.176		20
Poland	3.253	0.120	48	-----	-----	-----
Spain	3.398	0.212	48	3.171	not stated	20
UK	3.260	0.164	48	3.147	not stated	20
Denmark	-----	-----	-----	3.203	not stated	20
Overall	3.270	0.157	335	3.191	0.12	120

2012 mean and dispersion slightly higher than in 1993

Results – comparison 1993 study



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Leg cuts - precision of water/protein ratio

Country	Current 2012 Study			Previous 1993 Study		
	Mean	Std Dev of mean	No. observations	Mean	Std Dev of mean	No. observations
France	4.058	0.148	42	3.950	not stated	20
Germany	3.891	0.113	43	3.588	not stated	20
Italy	3.908	0.116	45	-----	-----	-----
The Netherlands	3.964	0.138	46	3.838	not stated	20
Poland	3.990	0.137	46	-----	-----	-----
Spain	3.934	0.139	45	3.682	not stated	20
UK	3.963	0.139	45	3.690	not stated	20
Denmark	-----	-----	-----	3.870	not stated	20
Overall	3.958	0.141	312	3.770	0.17	120

2012 mean slightly higher but dispersion slightly lower than in 1993

Differences in Means

Cut:	Breast				Leg			
	Mean Water g/100g	Change	Mean Protein g/100g	Change	Mean Water g/100g	Change	Mean Protein g/100g	Change
1993	73.94	1.40%	23.19	-0.95%	66.19	1.80%	17.56	-2.85%
2012	74.99		22.97		67.40		17.06	



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Interpretation

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Upper Limit Calculation – 1993 Study



$$\bar{y} = \bar{x} + \Delta + t_v * \sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)} * \sqrt{\frac{(n_1 - 1) * s_1^2 + (n_2 - 1) * s_2^2}{(n_1 - 1) + (n_2 - 1)}}$$

Where:

n_1 & s_1 = number of observation and standard deviation in the study

n_2 & s_2 = future number of observations and standard deviation

t_v = students t value with $n_1 + n_2 - 2$ degrees of freedom

Δ = **tolerance** (set at 0.3*)

\bar{x} = overall mean in the current study

* "Means a water uptake of about 7%"

Upper Limit Calculation – 1993 Study

Tolerance (Δ) is calculated as below:

$$\Delta = \frac{100 * E_W}{E_W * P_{phys} - 100 * P_{phys}}$$

- Tolerance takes into account any Extraneous Water in the sample that may have been taken up for a given chilling process
- 1993 study calculated limits using a tolerance value $\Delta = 0.3$ which relates to:
 - 6.5% extraneous water in breast cuts
 - 5% extraneous water in leg cuts.
- Same calculation was performed using 2012 data and tolerance value $\Delta = 0.3$ relates to:
 - 6.5% extraneous water in breast cuts
 - 4.9% extraneous water in leg cuts

W/P ratio upper limit calculations 1993 study



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The limits set in Regulation (EC) 543/2008 were derived by:

- 1) calculating the tolerance values for 2%, 4% and 6% extraneous water
- 2) performing the upper limit calculations using these values for 5 cuts

W/P ratio upper limit calculations

1993 study



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Cut	Extraneous water (%)	Calculated Limits	Upper Limit in Legislation
Breast*	2	3.39	3.40
	4	3.48	3.40
	6	3.57	3.40
Leg	2	4.04	4.05
	4	4.16	4.15
	6	4.29	4.30

* The legislation used just one value for chicken breast without skin regardless of chilling method

W/P ratio upper limit calculations 2012 study



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The proposed new limits have been calculated using the following:

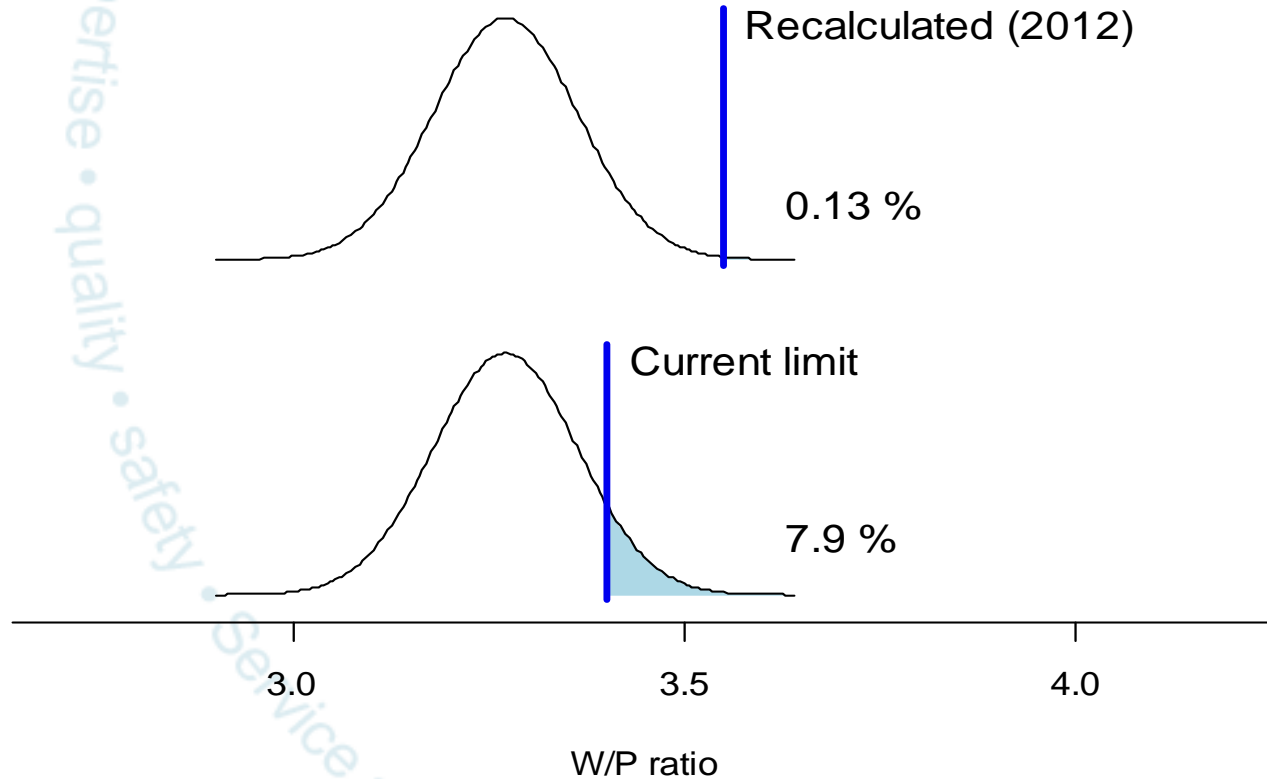
- Tolerance (Δ) calculated using the 2012 data
- Assumed an average/composite of 5 cuts (as stated in Regulation (EC) 543/2008)
- Assumed the five test portions will be from the same country (**different to 1993**)
- The sample(s) will be analysed in one laboratory (**different to 1993**)

Proposed Upper Limits for W/P Ratio - Breast



Cut	Extraneous water (%)	Proposed Limit (95%)	Upper Limit in Legislation
Breast	2	3.55	3.40
	4	3.64	
	6	3.74	

Failure Rate – Breast meat



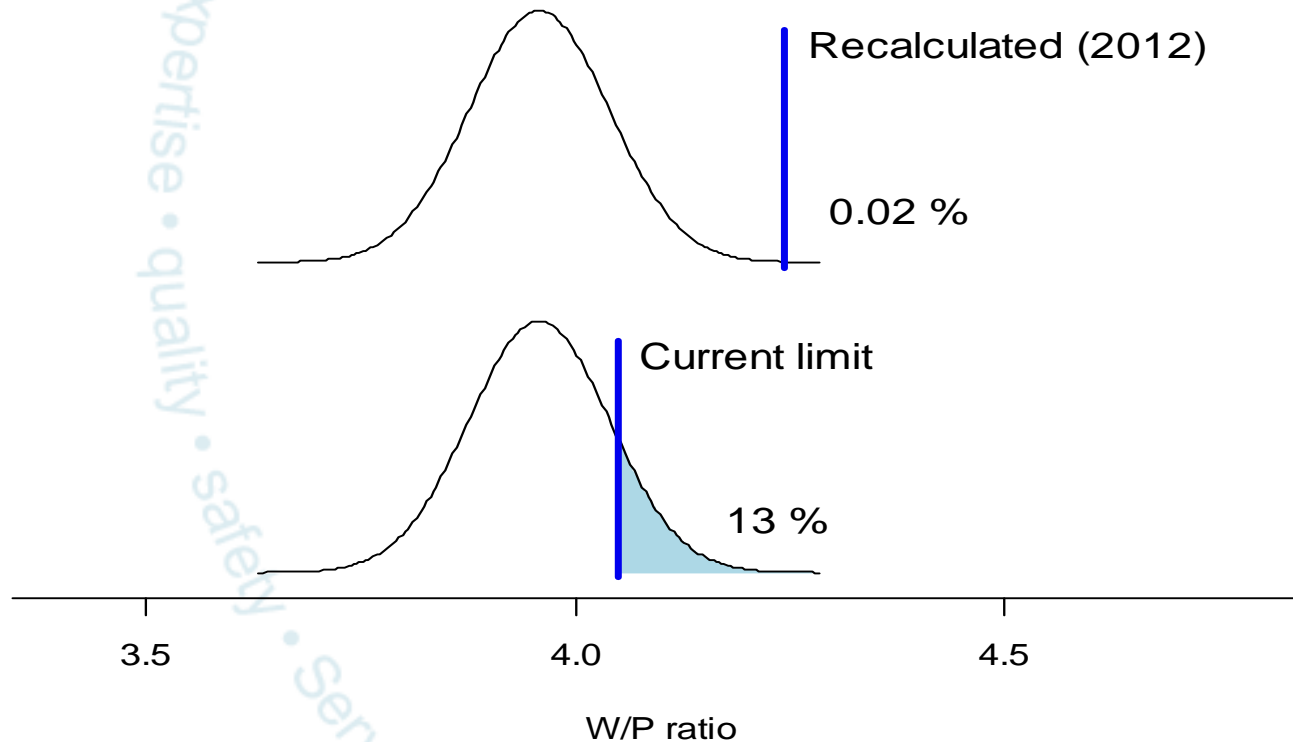
- Graphs show number of samples from the 2012 study that would be expected to fail the current legislative limit for air chilling (2% extraneous water) compared with the proposed new limit.
- Samples do not have any extraneous water.

W/P Ratio Leg

Proposed Upper Limits

Cut	Extraneous water (%)	Proposed Limit (95%)	Upper Limit in Legislation
Leg	2	4.25	4.05
	4	4.37	4.15
	6	4.50	4.30

Failure Rate – Leg cuts



- Graphs show number of samples from the 2012 study that would expected to fail at the current legislative limit for air chilling (2% extraneous water) compared with the proposed new limit.
- Samples do not have any extraneous water.



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Conclusions and Recommendations

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Conclusions

Compared to 1993 Data, 2012 Data shows:

- Mean water content has increased by 1-2%
- Mean protein content has decreased by 1-3%
- W/P ratio for chicken breast cuts:
 - mean and standard deviation are slightly higher
- W/P ratio for chicken leg cuts:
 - Mean is slightly higher
 - Standard deviation slightly lower
- Upper limit calculations are higher

Recommendations (1)

- The limits in the regulation should be revised to reflect the increase in physiological water content of chickens

It is recommended that:

- Chicken breasts (without skin) are to be set at:
 - 3.55 for air chilling (2% extraneous water)
 - 3.65 for air spray chilling (4% extraneous water)
 - 3.75 for immersion chilling (6% extraneous water)
- Chicken leg cuts (with skin & bone) are to be set at:
 - 4.25 for air chilling (2% extraneous water)
 - 4.40 for air spray chilling (4% extraneous water)
 - 4.50 for immersion chilling (6% extraneous water)

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Further Information



- The report will be published at:
 - http://ec.europa.eu/agriculture/external-studies/index_en.htm
 - http://circa.europa.eu/Public/irc/agri/pig/library?l=/poultry_public_domain&vm=detailed&sb=Title
- For further information contact:
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