G-TwYST Study C

a 90-day toxicity study in rats fed GM maize NK603

Statistical report

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For more information please visit the website www.biometris.nl or contact:

Biometris, Wageningen University & Research
P.O. Box 16
6700 AA Wageningen, The Netherlands

Visiting address:
Buildingnumber 107
Droevendaalsesteeg 1, 6708 PD Wageningen, The Netherlands

Phone: +31 317 480798 or +31 317 486001
E-mail: biometris@wur.nl

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Abstract

The purpose of oral toxicity study C in the EU project G-TwYST was to assess the effects of genetically modified (GM) maize NK 603, grown both with and without the use of RoundUp, when fed to rats for a period of 90 days at GM incorporation rates of 11%, 33% and 50% in the feed, if needed supplemented with near-isogenic non-GM maize to total maize inclusion rates of 33% or 50%. Differences between feeding groups were compared, using a recently developed method for equivalence testing, to differences between non-GM feeds obtained in previous studies performed in the EU project GRACE.

Given tentative settings for regulatory parameters, for a set of 648 comparisons involving body and organ weights, haematology and clinical chemistry, equivalence was established in 99% of cases, higher than the minimal nominal confidence level of the test which was 95%. Equivalence was found to be more likely than lack of equivalence in 100% of cases.

In addition to this primary analysis, the report also contains results for equivalence testing using external target effect sizes for a limited set of variables, results for classical statistical analysis of differences, graphs of standardised effect sizes such as performed in the GRACE study, results of factorial analysis, graphs showing correlations between variables associated with liver or kidney damage, and a post-hoc power analysis comparing trials with 33% and 50% maize inclusion rates.

1 Introduction

The purpose of oral toxicity study C in the EU project G-TwYST was to assess the effects of genetically modified (GM) maize NK 603, grown both with and without the use of RoundUp, when fed to rats for a period of 90 days at GM incorporation rates of 11%, 33% and 50% in the feed. The effects were assessed relative to the responses for rats fed the near-isogenic non-GM maize with total maize inclusion rates 33% or 50%, and these two maize inclusion rates were also compared.

This report describes the results of the statistical analysis of the data from Study C. In principle, the statistical analysis was performed according to section 8 of the study plan (Zeljenková and Steinberg 2015). Deviations from the study plan were as follows:

- The study plan specified a statistical analysis of data for males and females together, unless there were prior biological arguments or statistical indications to analyse males and females separately. However, toxicologists preferred separate statistical analysis of males and females for all variables, because it was thought that any specific non-target effect might be sex-specific.
- The study plan anticipated pre-specified limits for use in equivalence testing. However, such limits could not be established in an early phase of the project. Therefore, an alternative method for equivalence testing was developed (van der Voet *et al* 2017) and applied. This method makes use of historical non-GM data to obtain reference values for acceptable and normal variation in the observed variables. For the analysis of the data in G-TwYST, the data from non-GM varieties in the preceding GRACE project were available as historical data. Target effect sizes for a few variables were recently proposed by Hong *et al* (2017). Although these values have no formal status, equivalence tests were also performed using these effect sizes as originally planned.

Four schemes of statistical analysis were employed:

1. Equivalence tests, following the method developed in the G-TwYST project (van der Voet *et al*, 2017).
2. For a small number of variables: equivalence tests, based on target effect sizes suggested in Hong *et al* (2017).
3. Classical tests, in line with OECD Guidance document 116 (2012).
4. Standardised effect sizes, following the methods used in the GRACE project (Schmidt *et al*, 2016, 2017).

In addition, results from more integrated analyses were obtained:

5. Factorial analysis, employing the fact that the six GM feeding groups form a 2 × 3 factorial structure.
6. Correlation analysis, showing co-variation of effects for variables related to the same target organ.

This report is organised as follows. Section 2 describes the data, both the data from the current study and the non-GM data from the GRACE studies A-E as used in the equivalence tests. Section 3 describes data pre-processing procedures, such as summarising the growth and food intakes over time, outlier identification and assumptions checking. Section 4 presents the main results according to the six schemes of statistical analysis. Section 5 gives a summary and some evaluation of the methodology. Appendices to this report are provided as a separate document.

2   Data

2.1   Data in G-TwYST study C

Study C is a 90-day (sub-chronic) toxicity study in rats fed GM maize NK603. A full description of the data that have been measured is given in the study plan (document 632165 C/2017/GLP, Zeljenková and Steinberg 2015). There are eight feeding groups which are administrated to cages with 2 rats in each cage. Experimental units, i.e. cages, are organized in blocks of 8 cages, and the feeding groups are randomized within blocks. The design is thus a complete randomized block design with cage as the experimental unit. There are eight blocks with male rats and eight other blocks with female rats. Most of the measurements are on individual animals, only feed intake is measured on the cage level. All animals survived for three months.

The available files for G-TwYST study C are given in Table 1. The randomisation was provided in the two "*Random*" Excel files. The data in these files was converted to a single Excel file with blocking information "*00-Design-G-TwYST-Study-C.xlsx*". Organ weights were provided as absolute weights in Excel files "*Tab_9*" and "*Tab_11*" and as relative weights with respect to the final body weight in Excel files "*Tab_10*" and "*Tab_12*". It was checked that the data in these files were equivalent.

Table 1   Data files for G-TwYST study C.

Data files in G-TwYST study C	Date	Time	Size (b)
00-Design-G-TwYST-Study-C.xlsx	17-02-2018	11:38	12,199
Random__G-TwYST_C_Females.xlsx	15-02-2018	16:53	19,445
Random__G-TwYST_C_Males.xlsx	15-02-2018	16:53	18,948
Rev_1_Tab_1_Body weight_90_day_st_2C_G_TwYST_Mal.xlsx	20-02-2018	13:40	212,091
Rev_1_Tab_2_Body weight_90_day_st_2C_G_TwYST_Femal.xlsx	20-02-2018	13:36	213,393
Tab_3_Feed consumpt_90_day_st_2C_G_TwYST_Mal.xlsx	16-06-2017	06:56	164,951
Tab_4_Feed consumpt_90_day_st_2C_G_TwYST_Femal.xlsx	16-06-2017	08:06	166,046
Tab_5_Haematology_90_day_st_2C_G_TwYST_Mal.xlsx	29-06-2017	07:06	65,223
Tab_6_Haematology_90_day_st_2C_G_TwYST_Femal.xlsx	29-06-2017	07:20	66,634
Tab_7a_Clinical chem_bl_90_day_st_2C_G_TwYST_Mal.xlsx	20-09-2017	08:29	78,847
Tab_7b_Clinical chem_ur_90_day_st_2C_G_TwYST_Mal.xlsx	23-08-2017	13:51	62,428
Tab_8a_Clinical chem_bl_90_day_st_2C_G_TwYST_Femal.xlsx	23-08-2017	13:16	78,861
Tab_8b_Clinical chem_ur_90_day_st_2C_G_TwYST_Femal.xlsx	23-08-2017	13:46	62,155
Tab_9_Absolute weight organs_90_day_st_2C_G_TwYST_Mal.xlsx	06-07-2017	10:13	98,465
Tab_10_Relative weight organs_90_day_st_2C_G_TwYST_Mal.xlsx	06-07-2017	10:16	111,643
Tab_11_Absolute weight organs_90_day_st_2C_G_TwYST_Femal.xlsx	24-08-2017	13:22	96,590
Tab_12_Relative weight organs_90_day_st_2C_G_TwYST_Femal.xlsx	24-08-2017	13:38	109,590
Blood_urine_3 month_MC_G_TwYST_90day st2_C_Fem_time image_necr.xlsx	03-08-2017	08:36	94,801
Blood_urine_3 month_MC_G_TwYST_90day st2_C_Mal_time image_necr.xlsx	03-08-2017	08:34	101,501
CD markers G-TwYST C - database 9.2.2018.xlsx	12-02-2018	17:21	43,699
LTT- GTwYST C database 18.2.18.xlsx	18-02-2018	18:32	81,724
Phagocyt G-TwYST C 12.12.17 final.xlsx	13-12-2017	15:03	25,470

The definition of the eight feeding groups is given in Table 2. This includes the coding of the blinded treatments. In this report the Group labels (Con50 NK11-/50 NK50- NK11+/50 NK50+ Con33 NK33- NK33+), or shortened versions when necessary, will mostly be used.

Table 2   Feeding groups used in study C.

Factor	Levels/Labels							
Treat (blinded)	C	E	A	D	B	G	F	H
Group	Con50	NK11-/50	NK50-	NK11+/50	NK50+	Con33	NK33-	NK33+
FeedType	non-GM	GM	GM	GM	GM	non-GM	GM	GM
AmountMaize	50	50	50	50	50	33	33	33
AmountNK	0	11	50	11	50	0	33	33
RoundUp	No	No	No	Yes	Yes	No	No	Yes

Male and female rats were analysed separately. Since cage is the experimental unit an analysis of variance employs cage means with degrees of freedom as in Table 3. The main interest is in the difference between each of the six GM maize feeding groups and the two control non-GM feeding groups.

Table 3   Skeleton analysis of variance with degrees of freedom for cage means for a single sex.

Source of variation	8 cages/group	6 cages/group	5 cages/group
	d.f.	d.f.	d.f.
Block stratum	7	5	4
Block.Cage stratum			
Group	7	7	7
Residual	49	35	28
Total	63	47	39

The observed variables in Study C are given in Table 4; this table includes the grouping of variables. All variables are measured on the animal level; only feed intake is measured on the cage level. The Immunology and CellPhenotype measurement are only available for a subset of 9-12 animals per feeding group, i.e. 5 to 6 cages per feeding group. All other variables are available for 16 animals, i.e. for 8 cages, although there are occasionally missing values. Some ALP, ALT, CHOL and cHGB were given as bounds; the number of such values are given in Table 5. For most Urine variables only a limited number of values have been observed, see Table 6. It is evident that a statistical analysis of the Urine variables uColour, uBil, uNit, uProtein, uGlu, uHemogl and uUrobili is not very useful. Therefore, only the Urine variables uVol, uVolW, uLeu, uOsmoll, uKeton and upH were statistically analysed.

All variables were transformed to the natural logarithmic scale and then averaged to the cage level. This implies that, rather than looking at differences between feeding group means, ratios between the GM feeds and the corresponding Control feed are of interest. Only pH as measured in urine was not log transformed because the pH is already measured on the log scale. Since uLeu, uHemogl and uKeton have zero values, half of the smallest positive value was added to all observations before taking the logarithm.

Table 4 **List of grouped variables with abbreviated names, descriptions and measurement units. Grouping is indicated by the headers in the first column. The Grace column indicates whether the same variable was measured in the GRACE study. Urine variables given in red are not statistically analysed because only a limited number of values was observed (see Table 6 for details).**

Weights	Description	Unit	Grace
Weight 0-13	Body weight at weeks 0, 1 … 13	g/animal	X
Feed 1-13	Feed intake in week 1, 2 … 13	g/cage	X
BodyWeight	Body weight at the end of the trial, i.e. at week 13	g/animal	X
growthRate	Growth rate fitted to the weight over all weeks	1/week	X
FeedMean	Mean of feed intake over 13 weeks	g/animal/day	X
Haematology	**Description**	**Unit**	**Grace**
WBC	white blood cells	10^9/L	X
RBC	red blood cells	10^{12}/L	X
HGB	haemoglobin	g/dL	X
HCT	haematocrit	%	X
MCV	mean cell volume	fL	X
MCH	mean corpuscular haemoglobin	pg	X
MCHC	mean corpuscular haemoglobin concentration	g/dL	X
PLT	platelets	10^9/L	X
LYMR	relative lymphocytes count	%	-
LYMA	absolute count of lymphocytes	10^3/uL	X
ClinChem	**Description**	**Unit**	**Grace**
ALP	alkaline phosphatase	µkat/L	X
ALT	alanine aminotransferase	µkat/L	X
AST	aspartate aminotransferase	µkat/L	X
BIL	bilirubin	µmol/L	-
ALB	albumin	g/L	X
TP	total protein	g/L	X
Glu	glucose	mmol/L	X
CHOL	cholesterol	mmol/L	X
TAG	triglycerides	mmol/L	X
Crea	creatinine	mmol/L	X
Urea	urea	mmol/L	X
cHGB	haemoglobin	mg/dL	-
Ca	calcium	mmol/L	X
Cl	chloride	mmol/L	X
K	potassium	mmol/L	X
Na	sodium	mmol/L	X
P	phosphorus	mmol/L	X
Urine	**Description**	**Unit**	**Grace**
uVol	Urine Volume	ml	
uVolW	Urine Volume / bodyweight	ml /100g	
uColour	Urine Colour (1 – light yellow; 2 – yellow; 3 – dark yellow)	-	
uBil	bilirubin	µmol/L	
uLeu	leukocytes	leu/uL	
uNit	nitrites	neg/pos	
uOsmoll	osmolality	mOsm	
uProtein	total protein	g/L	
uGlu	glucose	mmol/L	
uHemogl	haemoglobin	ery/uL	
uKeton	ketone	mmol/L	

upH	pH	-	
uUrobili	urobilinogen	µmol/L	
Organs	**Description; all as percentage of BodyWeight**	**Unit**	**Grace**
Kidney	Percentage weight of kidney	%	X
Spleen	Percentage weight of spleen	%	X
Liver	Percentage weight of liver	%	X
AdrenGl	Percentage weight of adrenal gland	%	X
Heart	Percentage weight of heart	%	X
Thymus	Percentage weight of thymus	%	X
Testis	Percentage weight of testis (males)	%	X
Epididymis	Percentage weight of epididymis (males)	%	X
Uterus	Percentage weight of uterus (females)	%	X
Ovary	Percentage weight of ovary (females)	%	X
Brain	Percentage weight of brain	%	X
Immunology	**Description**	**Unit**	**Grace**
Monocytes	Phagocytic activity of monocytes	%	
Granulocytes	Phagocytic activity of granulocytes	%	
RespirBurst	Respiratory burst of phagocytes	%	
Con	Proliferative activity of lymphocytes stimulated with mitogen Concanavalin A	cpm (counts per minute)	
PHA	Proliferative activity of lymphocytes stimulated with mitogen phytohaemmagglutinin	cpm	
PWM	Proliferative activity of lymphocytes stimulated with pokeweed mitogen	cpm	
Med3d	Proliferative activity of non-stimulated lymphocytes 3 day-cultivation	cpm	
IprConA	Ratio of proliferative activity of lymphocytes stimulated with concanavalin A / proliferative activity of non-stimulated lymphocytes	-	
IprPHA	Ratio of proliferative activity of lymphocytes stimulated with phytohaemmagglutinin / proliferative activity of non-stimulated lymphocytes	-	
IprPWM	Ratio of proliferative activity of lymphocytes stimulated with pokeweed mitogen / proliferative activity of non-stimulated lymphocytes	-	
G4c1	Proliferative activity of lymphocytes stimulated with maize G4 protein, concentration 1	cpm	
G4c2	Proliferative activity of lymphocytes stimulated with maize G4 protein, concentration 2	cpm	
G4c3	Proliferative activity of lymphocytes stimulated with maize G4 protein, concentration 3	cpm	
NG2c1	Proliferative activity of lymphocytes stimulated with maize NG2 protein, concentration 1	cpm	
NG2c2	Proliferative activity of lymphocytes stimulated with maize NG2 protein, concentration 2	cpm	
NG2c3	Proliferative activity of lymphocytes stimulated with maize NG2 protein, concentration 3	cpm	
A6c1	Proliferative activity of lymphocytes stimulated with maize A6 protein, concentration 1	cpm	
A6c2	Proliferative activity of lymphocytes stimulated with maize A6 protein, concentration 2	cpm	
A6c3	Proliferative activity of lymphocytes stimulated with maize A6 protein, concentration 3	cpm	
Med6d	Proliferative activity of non-stimulated lymphocytes 6 day-cultivation	cpm	

IprG4c1	Ratio of proliferative activity of lymphocytes stimulated with maize G4 protein, concentration 1 / proliferative activity of non-stimulated lymphocytes	-	
IprG4c2	Ratio of proliferative activity of lymphocytes stimulated with maize G4 protein, concentration 2 / proliferative activity of non-stimulated lymphocytes	-	
IprG4c3	Ratio of proliferative activity of lymphocytes stimulated with maize G4 protein, concentration 3 / proliferative activity of non-stimulated lymphocytes	-	
IprNG2c1	Ratio of proliferative activity of lymphocytes stimulated with maize NG2 protein, concentration 1 / proliferative activity of non-stimulated lymphocytes	-	
IprNG2c2	Ratio of proliferative activity of lymphocytes stimulated with maize NG2 protein, concentration 2 / proliferative activity of non-stimulated lymphocytes	-	
IprNG2c3	Ratio of proliferative activity of lymphocytes stimulated with maize NG2 protein, concentration 3 / proliferative activity of non-stimulated lymphocytes	-	
IprA6c1	Ratio of proliferative activity of lymphocytes stimulated with maize A6 protein, concentration 1 / proliferative activity of non-stimulated lymphocytes	-	
IprA6c2	Ratio of proliferative activity of lymphocytes stimulated with maize A6 protein, concentration 2 / proliferative activity of non-stimulated lymphocytes	-	
IprA6c3	Ratio of proliferative activity of lymphocytes stimulated with maize A6 protein, concentration 3 / proliferative activity of non-stimulated lymphocytes	-	
CellPhenotype	Description	Unit	Grace
sp3	Spleen: percentage of cells, not labelled with monoclonal antibody anti-rat CD3	%	
sp3-4	Spleen: percentage of T-cytotoxic lymphocytes, cells double labelled with monoclonal antibodies anti-rat CD3 and anti-rat CD4	%	
sp3-8	Spleen: percentage of T-helper lymphocytes, cells double labelled with monoclonal antibodies anti-rat CD3 and anti-rat CD8	%	
sp3-45	Spleen: percentage of B-lymphocytes, cells anti-rat CD3 antigen negative and anti-rat CD45R antigen positive	%	
sp3-161	Spleen: percentage of NK-cells, cells anti-rat CD3 antigen negative and anti-rat CD161 antigen positive	%	
ln3	Lymph node: percentage of cells, not labelled with monoclonal antibody anti-rat CD3	%	
ln3-4	Lymph node: percentage of T-cytotoxic lymphocytes, cells double labelled with monoclonal antibodies anti-rat CD3 and anti-rat CD4	%	
ln3-8	Lymph node: percentage of T-helper lymphocytes, cells double labelled with monoclonal antibodies anti-rat CD3 and anti-rat CD8	%	
ln3-45	Lymph node: percentage of B-lymphocytes, cells anti-rat CD3 antigen negative and anti-rat CD45R antigen positive	%	
ty3	Thymus: percentage of cells, not labelled with monoclonal antibody anti-rat CD3	%	
ty3-4	Thymus: percentage of T-cytotoxic lymphocytes, cells double labelled with monoclonal antibodies anti-rat CD3 and anti-rat CD4	%	

ty3-8	Thymus: percentage of T-helper lymphocytes, cells double labelled with monoclonal antibodies anti-rat CD3 and anti-rat CD8	%
bm3	Bone marrow: percentage of cells, not labelled with monoclonal antibody anti-rat CD3	%
bm3-45	Bone marrow: percentage of T-cytotoxic lymphocytes, cells double labelled with monoclonal antibodies anti-rat CD3 and anti-rat CD4	%

Table 5   **Number of animals for which bounded values were provided. These bounds are given in the first column.**

Male rats								
Variable	Con50	NK11-/50	NK50-	NK11+/50	NK50+	Con33	NK33-	NK33+
ALT < 0.12	-	-	-	1	-	-	-	-
cHGB > 522.5	-	2	-	1	-	-	-	-
Female rats								
Variable	Con50	NK11-/50	NK50-	NK11+/50	NK50+	Con33	NK33-	NK33+
ALP < 0.17	-	-	-	-	-	-	-	1
ALT < 0.05	-	-	-	-	-	-	-	1
CHOL < 1.16	2	5	5	4	10	3	1	3
cHGB < 27.5	-	-	1	-	-	-	-	-
cHGB > 522.5	-	1	-	1	1	-	-	2

Table 6   **Urine variables with only a limited set of observed values which are given in the heading of each sub-table. The table entries give the number of times each value occurs for male and female rats. Only uLeu and uKeton were statistically analysed.**

uColour	**1**	**2**		
Male	-	128		
Female	1	127		
uBil	**0**	**50**		
Male	127	1		
Female	128	-		
uLeu	**0**	**25**	**100**	**500**
Male	81	45	2	-
Female	117	9	1	1
uNit	**0**			
Male	127			
Female	128			
uProtein	**0.00**	**0.25**		
Male	125	3		
Female	128	-		
uGlu	**1**			
Male	128			
Female	128			
uHemogl	**0**	**10**	**25**	**50**
Male	126	-	1	1
Female	127	1	-	-

uKeton	0.0	0.5	1.5	
Male	77	24	27	
Female	117	7	4	
uUrobili	**1**	**17**		
Male	127	1		
Female	128	-		

The variables in Table 4 exclude the following measurements in comparison to the study plan:

- Histopathological data. Reason: these were excluded from this statistical analysis in the study plan, and will be separately reported by the histopathological expert in the G-TwYST project.
- Periodic health status observations: morbidity, mortality, clinical signs. Rats were inspected twice daily for evidence of reaction to treatment or ill-health. The following clinical findings were observed at the end of the experiment
    1. Male animal 7 (NK50-) skin: alopecia- *r.coli ventralis, r.trachealis* (1-2cm)
    2. Male animal 58 (NK33+) skin: alopecia- *r.abdominis lateralis sinister* (3x2cm)
    3. Female animal 265 (NK11-/50) skin: alopecia- ventromedial area (3x1cm)
    4. Female animal 266 (NK11-/50) skin: alopecia- ventromedial area (3x1cm), *r.inguinalis sin.* 2x1cm)
    5. Female animal 287 (NK33-) skin: alopecia- ventromedial area (2x1cm)
    6. Female animal 228 (NK50+) skin: alopecia- *r. colli dorsalis*
    7. Female animal 328 (NK33+) skin: alopecia- *r. lumbalis*

    It was assumed that these findings were not related to the experiment.
- Organ weights: sternum with bone marrow, thyroid, parathyroid. These were not determined and this is in line with the OECD Test Guideline 408, which does not foresee such measurements. These measurement were erroneously included in the original study plan.
- In addition to the measurements in urine specified in the study plan, also bilirubine, leukocytes, nitrites, haemoglobin, ketone and urobilinogen were measured in urine.
- In addition to the measurements specified in the study plan, immunological measurements and measurements on the spleen, lymph node, thymus and bone marrow cell phenotypes were performed.

2.2   Reference data in GRACE studies

Data from the GRACE project are used as historic data to set equivalence limits. These data have been analysed before as part of the GRACE project (Schmidt and Schmidtke 2014, Schmidt *et al* 2015ab, 2016, 2017, Zeljenková *et al* 2014, 2016). Note that in the GRACE studies a completely randomized design, i.e. without blocking, was used. The GRACE data were retrieved from the Cadima website (https://www.cadima.info) at 29-11-2016. In GRACE five studies were conducted with several control (or reference) feeds as given in Table 7, see Schmidt et al (2017). In studies D and E only a single reference feed was used and, since the equivalence analysis corrects for differences between studies, these studies do not contribute to the between reference variation. In GRACE studies A, B and C, the reference feeds DKC6666 and SY-NEPAL were replicated. The degrees of freedom associated with the between reference feeds variance therefore equals 4. The degrees of freedom associated with the residual (between cages) variance varies between 50 and 78 since not all measurements were done on all rats in every study.

Table 7   Feeds which were used in the five GRACE studies with reference feeds in bold.

GRACE Study	Control	11% GMO	33% GMO	33% Conv-1	33% Conv-2
A	**DKC6666**	DKC6667-YG-11	DKC6667-YG-33	**PR33W82**	**SY-NEPAL**
B	**PR32T16**	PR33D48-11	PR33D48-33	**PR32T83**	**DKC6815**
C	**DKC6666**	DKC6667-YG-11	DKC6667-YG-33	-	**SY-NEPAL**
D	**DKC6666**	DKC6667-YG-11	DKC6667-YG-33	-	-
E	**PR32T16**	PR33D48-11	PR33D48-33	-	-

We re-analysed the GRACE data to enable a comparison with the G-TwYST data. This re-analysis is different from the analysis in the GRACE reports in the following ways:

- For the re-analysis all variables were transformed to the natural logarithmic scale and then averaged to the cage level; the thus obtained cage means were used in the statistical analysis;
- the exponential growth model (see section 3.1) was fitted to the weights observed in GRACE to obtain an estimate of the growth rate γ;
- The sum of the weights of organ pairs was analysed rather than the left and right organs;
- outliers were identified by applying Grubbs' outlier test at the 1% level on residuals of a one-way ANOVA which is conducted separately for each study. These outliers were set to missing.

Details of the re-analysis are given in the report on G-TwYST Study B (Goedhart & van der Voet, 2017). In Table 4 it is indicated which G-TwYST variables in Study C have also been measured in the GRACE studies.

3   Data pre-processing

GenStat programs "00-Males.gen" and "00-Females.gen" were used to combine all the data given in Table 1 into single Excel files, separately for males and females. All animals survived the experiment.

3.1   Growth curves and feed intake

For each individual rat an exponential growth curve $A + B\,R^{Week}$ was fitted to the observed weights. A re-parameterization of this curve is given by $A + B\exp(-\gamma\,Week)$ with the growth rate γ defined by $\gamma = -\log(R)$. Appendix 1 displays, for each rat separately, the observed weights along with the fitted curve and, in the bottom right corner, the resulting estimate of the growth rate γ. In general the exponential curve fits very well and it was therefore decided to only analyse the final weight observed after week 13, further called BodyWeight, and the estimated growth rate γ, further called growthRate. The mean weight for each feeding group is given in Figure 1, while the mean weight gain per day per animal in each week is given in Figure 2. Feed consumption for each cage in units g/animal/day is graphically depicted in Appendix 2. The mean feed consumption for each feeding group is given in Figure 3.
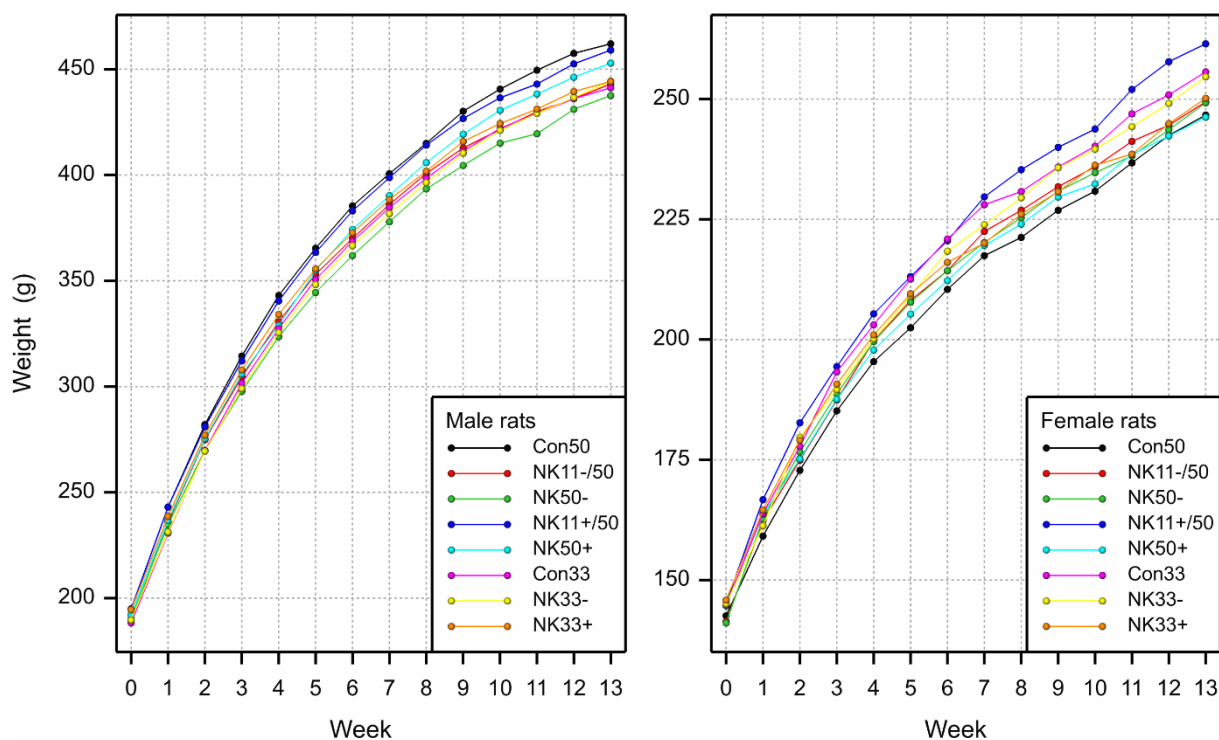
Figure 1   Mean body weights versus week for each feeding group for male rats (left) and for female rats (right).
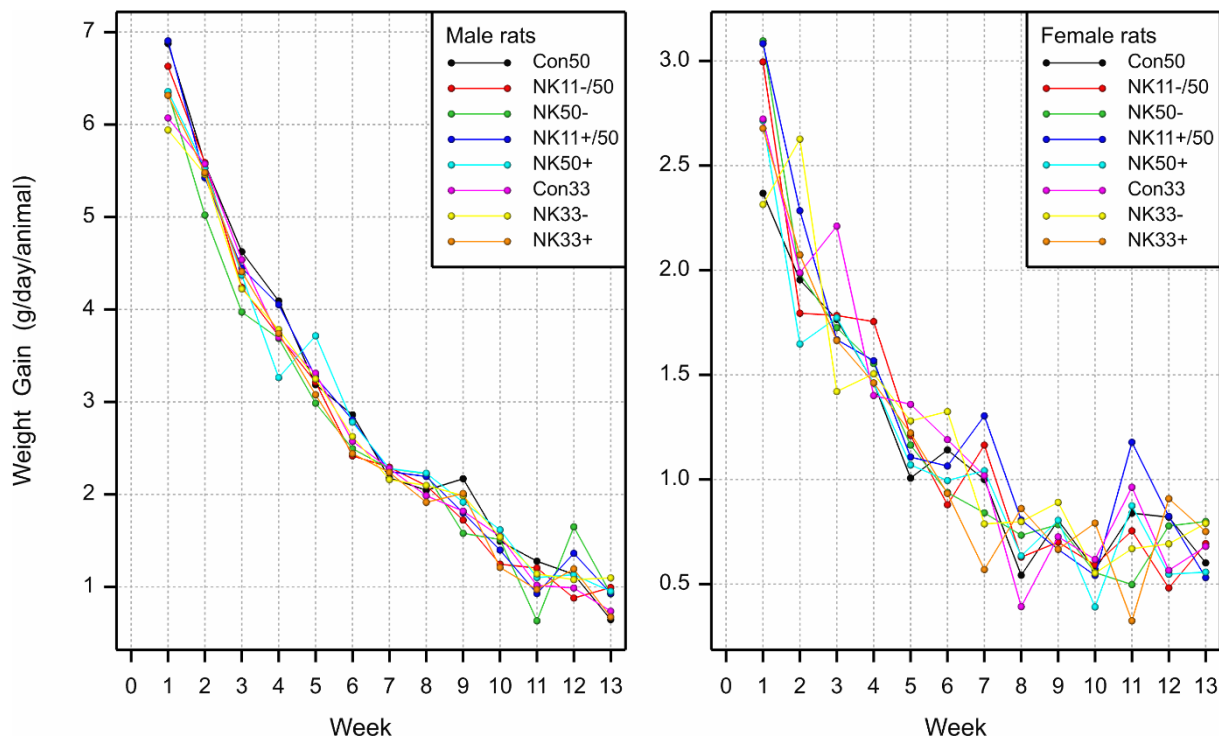

Figure 2   Mean body weights gain (g/day/animal) versus week for each feeding group for male rats (left) and for female rats (right).
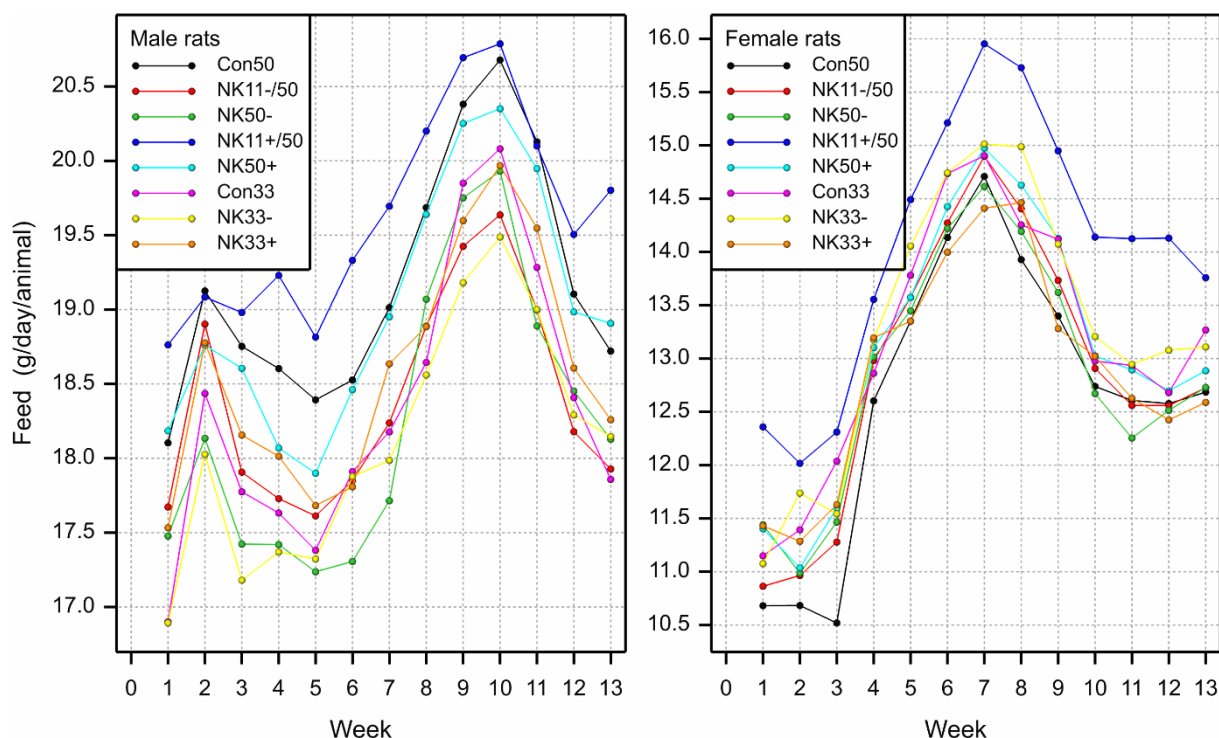
Figure 3 **Mean feed consumption (g/day/animal) versus week for each feeding group for male rats (left) and for female rats (right).**

3.2 Outliers and checking of ANOVA assumptions

The cage means, after log transformation, for each observed variable are statistically analysed by means of an analysis of variance using the model "Block + Group" according to the randomized block design. Grubbs' outlier test at the 1% level was applied to the residuals to detect outliers. This resulted in a number of outliers, given in Table 8, which were presented to the G-TwYST coordinator along with the blinded feeding group. Outliers were classified as either (1) typos or physiologically improbable values, or (2) values that might be realistic, or (3) values that should be kept. For the first category the values were set to missing, effectively removing the outlier completely. For the second category a statistical analysis without and with these outlier was performed. Values in the third category were kept in the dataset. The analyses presented in this report are without the outliers. Analyses including outliers are presented in Appendix 14.

Table 8 **Values that have been modified, deleted, or were considered as possible outliers, before unblinding of the feeding groups. The values were presented to the G-TwYST coordinator. He decided which values should be deleted (red), which values should be considered as possible outliers (yellow), and which values should be kept (white). Comments by the authors of this report are given in the last column.**

Sex	Variable	Animal	Cage	oldValue	newValue	Comment
Male	LYMR	109	55	45.6	*	The two smallest values occur in the same Cage; five smallest are 45.6, 55.8, 58.4, 61.2, 61.6
Male	LYMR	110	55	55.8	*	The two smallest values occur in the same Cage; five smallest are 45.6, 55.8, 58.4, 61.2, 61.7

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Sex	Variable	Animal	Cage	oldValue	newValue	Comment
Male	ALT	3	2	3.06	*	Large value; five largest are 0.86, 0.92, 1.80, 2.22, 3.06. In StudyA24 values 5.92 and 6.18 were considered outliers
Male	ALT	58	29	0.12	*	Small value (ALT,BIL,ALB,TP,K); five smallest are 0.12, 0.28, 0.37, 0.37, 0.37. In StudyA24 the value 0.08 was deleted
Male	ALT	61	31	2.22	2.22	Large value; five largest are 0.86, 0.92, 1.80, 2.22, 3.06
Male	BIL	58	29	84.8	*	Large value (ALT,BIL,ALB,TP,K); five largest are 13.3, 13.6, 25.6, 56.9, 84.8. In StudyA24 values 76 and 86 were deleted
Male	BIL	76	38	56.9	*	Large value (BIL,ALP,TP); five largest are 13.3, 13.6, 25.6, 56.9, 84.8. In StudyA24 values 76 and 85.9 were deleted
Male	ALB	58	29	58.7	58.7	Large value (ALT,BIL,ALB,TP,K); five largest are 41.9, 41.9, 42.7, 52.9, 58.7
Male	ALB	76	38	52.9	52.9	Large value (BIL,ALP,TP); five largest are 41.9, 41.9, 42.7, 52.9, 58.7
Male	TP	58	29	91.4	*	Large value (ALT,BIL,ALB,TP,K); five largest are 70.8, 70.9, 73.2, 83.7, 91.4. In StudyA24 values 96 and 100 are deleted
Male	TP	76	38	83.7	*	Large value (BIL,ALP,TP); five largest are 70.8, 70.9, 73.2, 83.7, 91.4. In StudyA24 values 96 and 100 are deleted
Male	K	58	29	10.1	*	Large value (ALT,BIL,ALB,TP,K); five largest are 5.8, 6.1, 6.9, 9.3 and 10.1
Male	AdrenGl_R	68	34	0.00181	*	Small value for Cage mean; five smallest are 0.0064, 0.0075, 0.0088, 0.0094, 0.0100
Male	AdrenGl_L	68	34	0.00454	*	Small value for Cage mean; five smallest are 0.0064, 0.0075, 0.0088, 0.0094, 0.0100
Male	Testis_R	39	20	0.1921	*	Small value for Cage mean; five smallest are 0.378, 0.698, 0.717, 0.720, 0.725
Male	Testis_L	39	20	0.1855	*	Small value for Cage mean; five smallest are 0.378, 0.698, 0.717, 0.720, 0.725
Sex	**Variable**	**Animal**	**Cage**	**oldValue**	**newValue**	**Comment**
Female	Ovary_R	286	110	1.416	*	Definitively Out of Range
Female	RBC	286	110	6.06	*	Small value (RBC,HCT); five smallest are 6.06, 6.79, 7.05, 7.10, 7.12
Female	HCT	286	110	35.2	*	Small value (RBC,HCT); five smallest are 35.2, 40.5, 40.7, 41.1, 41.4. in StudyA24 the value 27.7 was considered an outlier
Female	PLT	247	91	189.0	*	Small value; five smallest are 155, 189, 262, 420, 464. In StudyA24 the value 156 was considered an outlier
Female	PLT	277	106	420.0	*	Small value; five smallest are 155, 189, 262, 420, 464. In StudyA24 the value 156 was considered an outlier
Female	PLT	323	129	155.0	*	Small value; five smallest are 155, 189, 262, 420, 464. In StudyA24 the value 156 was considered an outlier
Female	PLT	324	129	262.0	*	Small value; five smallest are 155, 189, 262, 420, 464. In StudyA24 the value 156 was considered an outlier

Female	ALT	317	126	0.05	0.05	Small value; five smallest are 0.05, 0.19, 0.22, 0.22, 0.28
Female	ALB	289	112	4.1	*	Small value; five smallest are 4.1, 36.4, 37.6, 37.7, 38.5
Female	cHGB	323	129	522.5	*	Upper limit reached for 5 animals in total; only a single value in this cage
Female	Heart	250	92	0.175	*	Small value; five smallest are 0.175, 0.260, 0.261, 0.263, 0.264

Residual plots which include the outliers (i.e. including the yellow values in Table 8) are given in Figure 4. From this it is clear that these are indeed outliers. Residual plots for the variables for which Grubbs' outlier test is significant at the 1% level but which were not considered as outliers, i.e. the values with a white background in Table 8, are given in

Without these outliers, plots of cage means per feeding group on the original scale are given in Appendix 3, while plots of cage means after a log transformation of the individual data are given in Appendix 4. Normal probability plots of the ANOVA residuals, of an analysis on cage means after log transformation, are given in Appendix 5. To aid interpretation a 99% envelope is added to the probability plots, such that only values outside the envelop might be suspicious. Appendix 6 gives plots of residuals versus fitted values after the same analysis of variance. These residual plots are generally satisfactory implying that the ANOVA assumptions, homogeneity of variance and less importantly normality, are generally fulfilled.

3.3   Summary tables

Summary tables, on the original non-transformed scale, of means, standard deviations and coefficients of variation (%), classified by the feeding groups, are given in Table 9 for males and in Table 10 for females. These tables were obtained by first calculating cage means and then calculating the summary statistics. The number of cages per feeding group for which measurements are available is generally 8, except for the Immunology and CellPhenotype variables with 6 cages.

Figure 4 **Residuals along the y-axis versus fitted values along the x-axis resulting from an analysis of variance on log transformed cage means. Solid symbols denote outliers found by Grubbs' outlier test at the 1% level applied to the residuals; these values were considered to be outliers (the values with a yellow background in Table 8).**
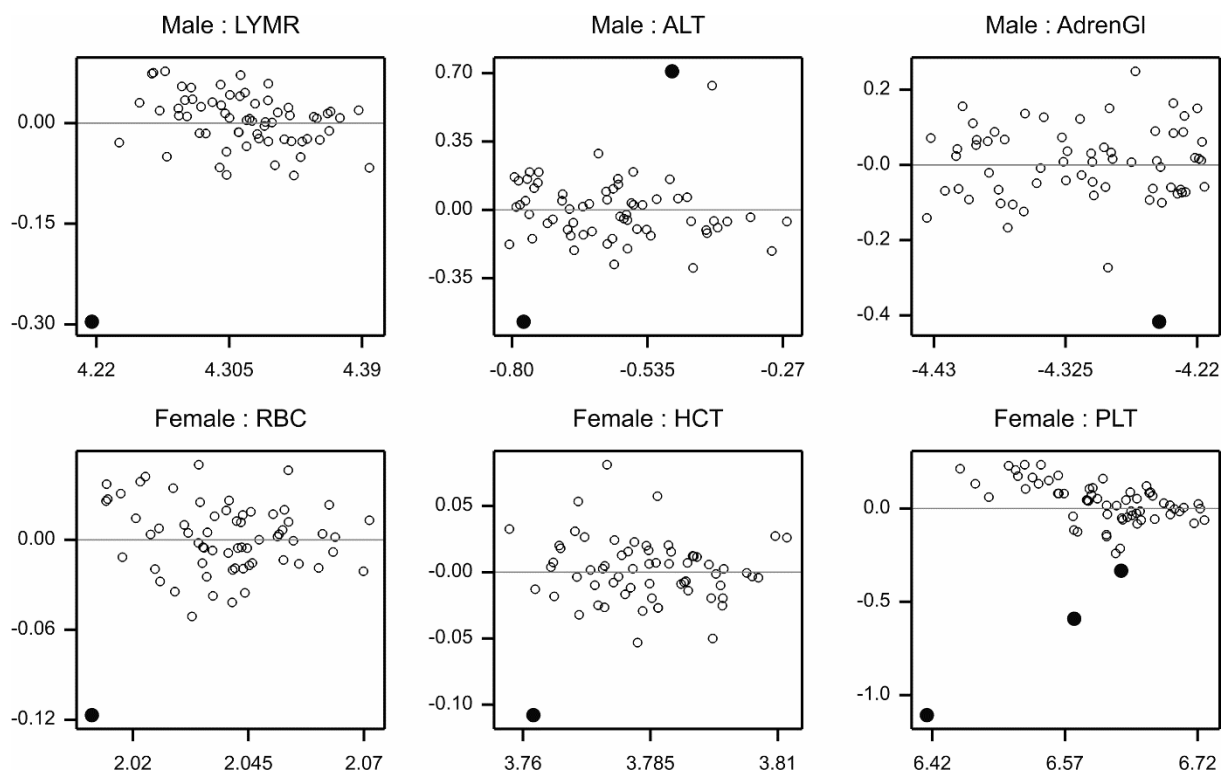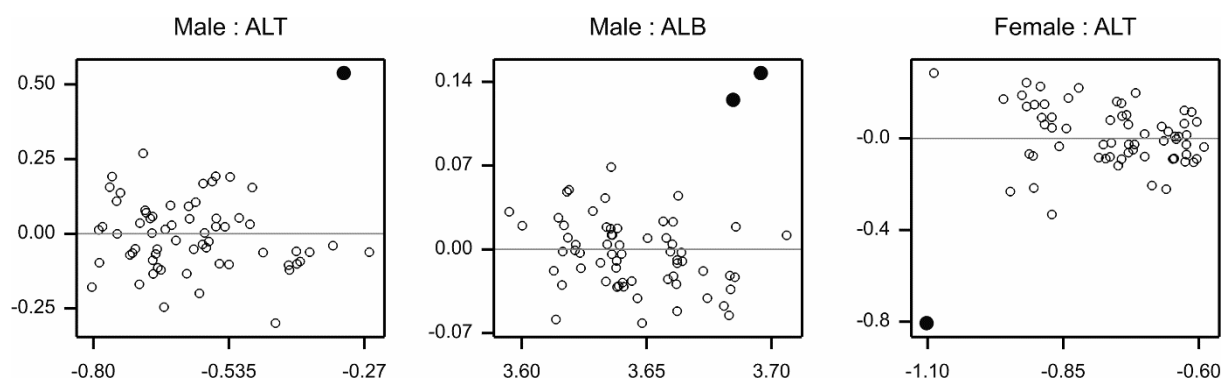

Figure 5 **Residuals along the y-axis versus fitted values along the x-axis resulting from an analysis of variance on log transformed cage means. Solid symbols denote outliers found by Grubbs' outlier test at the 1% level applied to the residuals; these values were not considered to be outliers (the values with a white background in Table 8).**

Table 9 **Summary statistics for male rats classified by the feeding groups: means (Mean), standard deviations (Sd) and coefficients of variation (CV). The summary statistics are obtained from cage means. Some variables are scaled.**

The number of cages (N) is given in subheadings. Exceptions from these numbers are for males for the following feeding groups: Control33 (LYMR, 7 cages), NK33- (Immunology, CellPhenotype, 5 cages), NK11+ (ln3-45, 5 cages) and NK50+ (Immunology, CellPhenotype, 5 cages).

Weights	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
Male (N=8)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
Bodyweight	462	28.0	6.0	443	29.5	6.7	438	17.9	4.1	459	26.8	5.8	453	32.8	7.2	441	26.7	6.1	444	30.1	6.8	444	25.7	5.8
growthRate	0.17	0.012	7.5	0.17	0.025	14.5	0.16	0.020	12.7	0.17	0.023	13.9	0.16	0.012	7.5	0.17	0.019	11.3	0.15	0.013	8.3	0.17	0.014	8.4
FeedMean	2.77	0.16	5.8	2.66	0.15	5.6	2.63	0.12	4.7	2.83	0.17	5.9	2.74	0.18	6.6	2.65	0.15	5.8	2.61	0.16	6.0	2.68	0.15	5.6
Haematology	**Con50**			**NK11-/50**			**NK50-**			**NK11+/50**			**NK50+**			**Con33**			**NK33-**			**NK33+**		
Male (N=8)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
WBC	7.98	1.53	19.2	8.01	1.31	16.4	8.56	1.56	18.2	7.41	1.01	13.6	8.46	1.45	17.2	8.36	2.06	24.7	8.24	1.15	14.0	8.22	1.53	18.6
RBC	8.63	0.14	1.6	8.67	0.21	2.4	8.54	0.17	2.0	8.71	0.21	2.4	8.54	0.20	2.3	8.63	0.21	2.4	8.64	0.18	2.0	8.79	0.20	2.2
HGB	15.7	0.47	3.0	16.1	0.41	2.6	15.7	0.22	1.4	16.2	0.36	2.2	15.9	0.48	3.0	15.9	0.40	2.5	16.0	0.28	1.8	15.8	0.32	2.1
HCT	46.8	1.27	2.7	47.3	1.17	2.5	46.5	0.72	1.5	47.8	0.88	1.8	46.9	1.09	2.3	46.9	0.84	1.8	47.2	0.84	1.8	47.0	1.00	2.1
MCV	54.3	0.94	1.7	54.6	0.79	1.5	54.4	0.54	1.0	54.9	1.14	2.1	55.0	0.94	1.7	54.4	0.85	1.6	54.6	0.75	1.4	53.5	0.68	1.3
MCH	18.2	0.38	2.1	18.6	0.33	1.8	18.4	0.26	1.4	18.6	0.54	2.9	18.6	0.26	1.4	18.5	0.53	2.9	18.5	0.40	2.2	18.0	0.43	2.4
MCHC	33.6	0.46	1.4	34.1	0.33	1.0	33.8	0.28	0.8	33.9	0.47	1.4	33.9	0.39	1.2	34.0	0.56	1.6	33.9	0.45	1.3	33.6	0.59	1.8
PLT	737	66	8.9	792	80	10.1	859	166	19.3	776	68	8.7	829	76	9.2	770	94	12.2	763	78	10.2	843	83	9.9
LYMR	74.5	3.13	4.2	74.5	2.88	3.9	76.3	2.27	3.0	75.7	3.00	4.0	75.8	2.62	3.5	75.2	2.99	4.0	77.9	3.13	4.0	73.0	3.74	5.1
LYMA	5.92	1.13	19.1	5.97	1.08	18.0	6.52	1.08	16.6	5.61	0.93	16.6	6.40	1.00	15.7	5.94	1.27	21.4	6.41	0.84	13.1	5.99	1.06	17.7
ClinChem	**Con50**			**NK11-/50**			**NK50-**			**NK11+/50**			**NK50+**			**Con33**			**NK33-**			**NK33+**		
Male (N=8)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
ALP	1.28	0.19	15.1	1.47	0.32	22.0	1.19	0.15	12.7	1.20	0.21	17.6	1.33	0.30	22.5	1.39	0.29	20.6	1.29	0.18	13.8	1.35	0.22	16.5
ALT	0.54	0.10	18.2	0.66	0.17	26.2	0.53	0.08	15.9	0.64	0.34	53.4	0.57	0.10	17.7	0.53	0.07	13.1	0.52	0.08	14.3	0.55	0.07	12.2
AST	2.37	0.37	15.8	2.92	1.01	34.6	2.46	0.54	22.0	2.67	0.68	25.6	2.57	0.71	27.5	2.42	0.45	18.7	2.28	0.30	13.1	2.39	0.24	9.9
BIL	7.47	0.72	9.6	8.92	3.22	36.1	7.86	1.57	20.0	8.04	1.39	17.3	8.21	0.94	11.4	7.50	0.91	12.1	7.59	0.81	10.7	7.72	0.78	10.1
ALB	37.5	0.64	1.7	39.3	2.95	7.5	38.3	0.82	2.1	39.0	3.75	9.6	38.4	0.67	1.8	38.4	1.58	4.1	38.2	1.48	3.9	38.3	1.36	3.5
TP	64.2	0.76	1.2	65.5	2.10	3.2	65.4	0.67	1.0	64.6	1.71	2.6	65.6	1.01	1.5	66.2	2.26	3.4	65.4	1.26	1.9	65.7	1.69	2.6
Glu	5.58	0.90	16.2	5.45	0.84	15.5	5.22	0.76	14.6	5.70	0.48	8.4	5.03	0.69	13.7	5.74	0.61	10.7	5.40	0.33	6.2	5.29	0.33	6.2
CHOL	2.13	0.26	12.1	2.11	0.17	8.2	2.23	0.14	6.2	2.25	0.09	3.9	2.26	0.26	11.6	2.15	0.24	11.3	2.08	0.17	8.4	2.11	0.22	10.6
TAG	1.13	0.30	26.1	1.12	0.17	15.5	1.18	0.17	14.6	1.18	0.23	19.4	1.20	0.24	19.7	1.07	0.24	22.5	1.27	0.24	18.5	1.12	0.22	19.2

	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
Crea	33.4	5.31	15.9	32.3	3.34	10.3	34.9	6.61	18.9	32.2	7.05	21.9	33.3	5.95	17.9	36.2	5.35	14.8	34.4	4.11	12.0	33.3	7.31	22.0
Urea	4.96	0.56	11.3	4.91	0.90	18.2	4.69	0.39	8.4	5.50	0.79	14.4	4.86	0.70	14.3	5.46	0.97	17.8	4.84	0.87	18.0	5.31	0.57	10.8
cHGB	66	15.2	23.0	138	98.2	71.1	89	42.0	47.2	106	78.7	74.2	107	37.7	35.2	87	18.1	20.8	74	21.5	29.1	89	25.8	29.1
Ca	2.39	0.041	1.7	2.37	0.043	1.8	2.40	0.038	1.6	2.39	0.041	1.7	2.39	0.034	1.4	2.39	0.035	1.4	2.40	0.047	2.0	2.39	0.036	1.5
Cl	100	1.03	1.0	100	1.90	1.9	101	2.19	2.2	100	2.63	2.6	99	1.36	1.4	99	1.95	2.0	100	2.42	2.4	100	2.31	2.3
K	4.44	0.21	4.8	4.89	0.88	18.1	4.66	0.37	7.9	4.50	0.24	5.2	4.56	0.21	4.7	4.56	0.27	5.9	4.51	0.37	8.2	4.43	0.19	4.4
Na	144	1.75	1.2	143	1.67	1.2	145	1.70	1.2	143	2.10	1.5	143	2.41	1.7	144	2.43	1.7	143	1.77	1.2	145	2.40	1.7
P	2.00	0.14	7.0	2.11	0.26	12.3	1.99	0.23	11.4	2.02	0.42	20.7	2.04	0.19	9.2	2.19	0.41	18.6	2.22	0.19	8.4	2.22	0.21	9.4

Urine	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
Male (N=8)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
uVol	23.8	7.96	33.5	23.0	7.24	31.5	25.2	5.56	22.1	25.3	7.23	28.5	25.5	9.05	35.5	18.9	3.65	19.3	21.1	5.02	23.8	20.7	8.10	39.0
uVolW	5.31	1.53	28.8	5.48	1.85	33.8	6.14	1.31	21.4	5.75	1.52	26.5	5.80	1.79	30.8	4.53	1.06	23.4	4.94	1.00	20.2	4.84	1.83	37.9
uLeu	12.5	11.6	92.6	9.4	8.8	94.3	4.7	6.5	138.0	7.8	9.3	119.0	6.2	9.4	151.2	12.5	6.7	53.5	9.4	8.8	94.3	20.3	24.0	118.3
uOsmoll	458	140	30.5	417	133	32.0	395	156	39.4	405	113	27.8	433	141	32.5	432	55	12.7	422	83	19.8	516	196	38.1
uKeton	0.50	0.60	119.5	0.34	0.38	109.5	0.37	0.33	87.3	0.22	0.53	240.0	0.47	0.65	138.0	0.31	0.44	140.2	0.62	0.46	74.1	0.44	0.42	95.4
upH	6.94	0.32	4.6	6.91	0.13	1.9	6.94	0.46	6.6	7.12	0.23	3.2	6.72	0.34	5.0	7.09	0.44	6.2	7.03	0.21	3.0	7.25	0.40	5.5

Organs	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
Male (N=8)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
Kidney * 10	5.35	0.32	6.0	5.55	0.32	5.7	5.47	0.38	7.0	5.61	0.19	3.4	5.54	0.21	3.8	5.43	0.27	4.9	5.33	0.44	8.3	5.79	0.44	7.6
Spleen * 10	1.63	0.14	8.3	1.62	0.13	8.1	1.70	0.16	9.3	1.68	0.19	11.1	1.70	0.17	10.1	1.67	0.16	9.7	1.67	0.16	9.8	1.68	0.17	9.9
Liver * 10	22.7	0.98	4.3	22.2	0.93	4.2	23.4	0.99	4.2	23.4	0.85	3.6	23.0	0.87	3.8	23.5	0.95	4.0	22.9	0.75	3.3	22.9	0.77	3.4
AdrenGl * 10	0.14	0.021	15.3	0.14	0.019	13.5	0.14	0.015	11.1	0.14	0.015	11.2	0.14	0.015	10.6	0.14	0.013	9.6	0.13	0.019	14.0	0.13	0.016	12.3
Heart * 10	2.50	0.10	4.1	2.48	0.18	7.1	2.58	0.07	2.6	2.56	0.24	9.3	2.55	0.11	4.3	2.56	0.08	3.3	2.42	0.13	5.5	2.61	0.17	6.4
Thymus * 10	0.95	0.11	11.3	0.90	0.12	12.9	0.97	0.13	13.3	1.01	0.16	15.4	0.92	0.11	12.0	0.96	0.14	14.4	0.87	0.14	15.9	0.96	0.11	11.5
Testis * 10	8.19	0.61	7.4	8.30	0.52	6.2	8.83	0.50	5.7	8.63	0.37	4.3	8.55	0.51	5.9	8.64	0.47	5.4	8.46	0.79	9.3	8.33	0.68	8.1
Epididymis * 10	2.80	0.28	9.9	2.80	0.29	10.3	2.98	0.22	7.5	2.96	0.13	4.3	2.91	0.18	6.1	2.93	0.14	4.9	2.80	0.30	10.8	2.90	0.14	4.7
Brain * 10	4.93	0.19	3.8	5.06	0.34	6.6	5.22	0.27	5.2	5.02	0.24	4.7	5.00	0.42	8.3	5.11	0.22	4.4	5.09	0.24	4.7	5.10	0.24	4.7

Immunology	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
Male (N=6)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
Monocytes	62.5	4.9	7.8	65.4	6.0	9.2	66.2	6.7	10.1	61.4	8.2	13.4	67.6	3.7	5.4	63.4	7.3	11.6	66.7	10.0	15.1	68.8	5.4	7.8
Granulocytes	82.4	2.58	3.1	80.3	4.46	5.6	82.8	4.44	5.4	81.9	6.73	8.2	81.5	4.63	5.7	82.7	2.98	3.6	83.9	4.28	5.1	84.0	2.45	2.9
RespirBurst	76.0	8.2	10.7	79.8	8.4	10.5	72.3	8.4	11.6	76.7	5.1	6.6	76.7	10.8	14.1	74.2	7.5	10.1	73.0	11.1	15.2	75.0	8.3	11.1
Con / 1000	88	64	72.6	105	78	74.2	136	112	82.8	129	64	49.7	98	48	48.5	155	56	36.3	176	49	27.7	135	74	54.7
PHA / 1000	45.6	33.1	72.6	50.3	34.6	68.8	51.2	48.4	94.7	68.9	35.2	51.1	38.4	30.3	78.7	59.3	41.2	69.4	77.7	27.6	35.5	52.0	36.3	69.8

	Con50 Mean	Sd	CV	NK11-/50 Mean	Sd	CV	NK50- Mean	Sd	CV	NK11+/50 Mean	Sd	CV	NK50+ Mean	Sd	CV	Con33 Mean	Sd	CV	NK33- Mean	Sd	CV	NK33+ Mean	Sd	CV
PWM / 1000	31.8	19.1	60.0	40.6	26.9	66.3	54.9	50.8	92.6	41.6	17.5	42.0	31.2	11.9	38.2	38.6	10.7	27.6	52.5	22.9	43.7	51.5	36.7	71.2
Med3d / 1000	1.83	1.46	79.6	2.28	2.21	97.1	3.15	3.21	101.9	2.16	1.30	60.1	1.47	0.69	46.7	2.63	1.23	46.8	3.65	1.08	29.7	3.18	3.12	98.3
IprConA	49.2	21.7	44.1	52.2	17.3	33.1	47.3	20.5	43.3	63.5	13.8	21.7	62.1	25.1	40.5	62.1	13.2	21.3	50.3	6.8	13.6	63.2	24.6	38.9
IprPHA	29.9	13.4	44.7	30.3	19.2	63.5	21.6	10.9	50.7	34.6	15.5	44.7	27.4	21.8	79.6	26.4	16.2	61.5	24.7	12.0	48.6	31.4	25.6	81.6
IprPWM	23.9	7.5	31.6	22.3	9.8	44.2	24.2	9.8	40.3	24.7	8.7	35.4	23.9	9.5	40.0	17.3	6.0	34.6	16.9	3.8	22.6	24.3	11.4	46.8
G4c1 / 1000	1.99	2.32	116.4	1.85	2.36	127.1	2.23	1.74	78.2	2.23	1.51	67.5	1.49	0.70	47.1	2.21	1.16	52.5	3.18	1.19	37.4	1.69	1.09	64.2
G4c2 / 1000	2.34	2.78	118.7	2.22	2.91	131.2	2.23	1.87	83.6	2.44	1.53	62.7	2.21	1.74	78.6	2.67	1.79	67.1	3.95	1.32	33.5	1.73	1.15	66.7
G4c3 / 1000	2.63	2.78	105.5	2.17	2.12	97.8	2.63	2.36	89.9	3.26	1.72	52.7	3.53	3.32	93.9	3.27	1.32	40.4	4.05	0.54	13.4	2.12	1.08	50.8
NG2c1 / 1000	1.98	2.25	113.7	2.05	2.15	105.2	2.45	1.92	78.5	2.26	1.53	67.7	2.12	1.18	55.9	2.20	0.98	44.7	3.65	1.18	32.4	1.97	1.26	64.1
NG2c2 / 1000	2.44	2.60	106.6	2.13	2.32	108.8	2.38	1.88	79.0	2.55	1.75	68.8	2.44	1.61	66.0	3.04	1.49	49.0	4.12	1.31	31.8	1.96	1.20	61.3
NG2c3 / 1000	2.42	2.37	98.0	2.26	2.43	107.7	2.45	2.05	83.7	2.97	1.49	50.1	3.95	4.47	113.1	3.45	1.31	38.1	4.62	0.67	14.5	2.19	0.97	44.4
A6c1 / 1000	2.33	2.71	116.2	2.47	2.91	118.1	2.30	1.88	81.9	2.62	1.74	66.4	2.16	1.00	46.3	3.12	1.49	47.7	4.26	1.28	30.1	2.14	1.53	71.7
A6c2 / 1000	2.45	2.71	110.9	2.73	3.29	120.5	2.20	1.77	80.4	2.60	1.78	68.4	3.05	1.76	57.7	3.20	1.59	49.8	4.16	1.75	42.1	2.24	1.59	70.8
A6c3 / 1000	2.32	2.48	107.0	2.12	1.96	92.7	2.20	1.86	84.6	2.60	1.30	50.0	3.88	4.43	114.1	3.19	1.12	35.1	3.52	0.46	13.1	2.06	1.02	49.5
Med6d / 1000	2.29	2.92	127.5	1.82	2.14	117.7	1.95	1.64	84.3	1.99	1.30	65.3	1.94	1.10	56.5	2.50	1.75	70.1	3.49	1.18	33.8	1.74	1.23	70.5
IprG4c1	1.34	0.74	55.7	1.40	0.79	56.6	2.00	1.26	63.1	1.21	0.24	20.0	1.13	0.44	38.7	1.12	0.63	56.2	0.92	0.12	13.5	1.08	0.23	21.3
IprG4c2	1.33	0.44	33.3	1.37	0.56	41.1	1.70	0.84	49.3	1.19	0.20	16.5	1.15	0.27	23.3	1.13	0.25	22.0	1.35	0.45	33.2	1.06	0.15	14.2
IprG4c3	1.73	0.67	38.9	2.05	1.46	71.2	1.70	0.88	51.8	1.91	0.37	19.3	1.73	0.94	54.4	1.69	0.92	54.2	1.45	0.44	30.2	1.58	0.67	42.2
IprNG2c1	1.29	0.75	58.0	1.42	0.78	54.9	1.90	1.08	56.9	1.47	0.58	39.5	1.33	0.29	22.1	1.10	0.42	38.3	1.08	0.08	7.4	1.24	0.26	21.0
IprNG2c2	1.66	0.62	37.1	1.42	0.28	19.9	1.73	0.71	41.3	1.21	0.22	18.6	1.26	0.16	12.5	1.28	0.48	37.3	1.26	0.20	15.9	1.28	0.22	17.3
IprNG2c3	1.77	0.82	46.3	1.82	0.94	51.9	1.64	0.75	46.0	1.79	0.40	22.5	1.81	0.98	54.3	1.68	0.90	53.2	1.58	0.50	31.8	1.69	0.75	44.1
IprA6c1	1.36	0.59	43.7	1.70	0.72	42.3	1.60	0.81	50.7	1.57	0.48	30.6	1.36	0.44	32.3	1.46	0.41	28.1	1.29	0.17	13.4	1.23	0.20	16.0
IprA6c2	1.56	0.66	42.5	1.49	0.42	28.3	1.57	0.63	40.2	1.29	0.28	21.3	1.90	1.32	69.4	1.40	0.25	18.1	1.25	0.27	21.9	1.40	0.38	27.2
IprA6c3	1.53	0.65	42.7	1.84	0.92	50.1	1.38	0.49	35.3	1.57	0.50	31.9	1.77	0.99	56.0	1.65	0.74	44.6	1.30	0.43	32.9	1.54	0.62	40.4
CellPhenotype	**Con50**			**NK11-/50**			**NK50-**			**NK11+/50**			**NK50+**			**Con33**			**NK33-**			**NK33+**		
Male (N=6)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
sp3	45.9	12.0	26.2	42.1	9.2	21.9	46.6	11.4	24.4	44.4	5.4	12.2	46.2	6.3	13.7	44.7	9.1	20.4	45.0	2.5	5.6	41.8	5.0	12.1
sp3-4	31.5	11.2	35.5	28.6	9.1	31.8	32.5	10.7	32.7	29.9	6.8	22.8	31.8	3.6	11.2	31.8	4.4	13.8	31.2	2.5	8.0	28.9	3.5	12.2
sp3-8	12.8	5.12	39.9	12.9	4.37	33.8	13.4	5.04	37.5	13.4	3.70	27.7	14.4	2.34	16.3	14.2	4.32	30.5	15.0	1.77	11.8	14.2	2.49	17.5
sp3-45	24.9	3.67	14.7	28.5	5.31	18.6	26.2	5.85	22.3	23.9	5.16	21.6	24.9	7.50	30.1	23.4	3.84	16.4	25.3	5.67	22.5	25.0	6.10	24.4
sp3-161	7.87	1.79	22.8	8.20	2.16	26.4	7.38	1.38	18.6	7.98	1.40	17.6	7.92	1.09	13.7	7.90	1.83	23.2	7.13	1.23	17.3	8.09	1.44	17.8
ln3	47.4	9.08	19.2	46.0	3.63	7.9	47.7	7.31	15.3	46.8	9.87	21.1	48.4	9.64	19.9	49.0	5.96	12.2	45.2	5.92	13.1	45.2	7.37	16.3
ln3-4	34.5	6.53	18.9	35.1	3.64	10.4	36.2	5.22	14.4	34.7	7.31	21.1	36.3	7.24	19.9	36.9	3.98	10.8	32.2	2.54	7.9	33.6	6.13	18.2
ln3-8	11.0	1.10	10.0	10.8	1.01	9.3	11.0	2.33	21.1	11.7	2.29	19.6	11.8	2.09	17.7	12.1	2.38	19.6	13.2	3.87	29.3	11.2	1.68	15.0

	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
ln3-45	29.7	3.2	10.9	33.5	14.2	42.5	27.8	11.9	42.9	31.5	8.2	26.2	32.6	13.8	42.4	35.3	11.9	33.8	31.3	8.5	27.3	31.6	15.7	49.6
ty3	21.1	3.72	17.6	18.4	2.44	13.2	19.8	0.61	3.1	19.8	3.50	17.6	19.7	2.37	12.0	18.2	3.84	21.1	20.1	2.80	14.0	19.6	1.73	8.8
ty3-4	17.5	2.76	15.7	15.5	1.95	12.6	17.1	0.38	2.2	16.8	2.50	14.9	16.4	2.35	14.3	15.4	2.49	16.1	17.7	2.85	16.1	17.0	1.81	10.7
ty3-8	11.3	1.41	12.5	9.5	2.81	29.7	9.7	2.21	22.8	11.3	3.49	30.9	9.5	1.93	20.4	9.4	3.09	32.8	9.1	1.73	19.0	10.3	2.40	23.4
bm3	7.7	5.8	75.1	9.2	9.2	99.5	7.2	5.8	79.8	5.9	3.7	62.6	6.3	4.5	71.3	12.5	11.7	93.9	5.7	3.6	62.4	13.7	11.1	81.1
bm3-45	53.9	20.4	37.8	57.2	20.6	36.0	60.2	24.2	40.3	57.9	19.0	32.8	59.3	22.4	37.8	57.8	20.5	35.5	66.5	6.9	10.3	60.8	9.4	15.5

Table 10 Summary statistics for female rats classified by the feeding groups: means (Mean), standard deviations (Sd) and coefficients of variation (CV). The summary statistics are obtained from cage means. Some variables are scaled.

The number of cages (N) is given in subheadings. For females there are only 5 observations available for the Immunology and CellPhenotype variables for feeding groups NK11-, NK33-, NK11+ and NK50+. In addition 7 cages, instead of 8, are available for PLT and cHGB in feeding group NK33+.

Weights	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
Female (N=8)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
Bodyweight	247	20.0	8.1	249	18.9	7.6	249	19.3	7.7	261	20.8	8.0	246	13.4	5.5	256	10.3	4.0	255	12.0	4.7	250	18.2	7.3
growthRate	0.13	0.021	16.3	0.16	0.034	21.8	0.16	0.035	22.2	0.13	0.020	15.1	0.14	0.026	18.4	0.15	0.038	24.9	0.14	0.028	20.7	0.15	0.034	22.7
FeedMean	1.83	0.15	8.2	1.86	0.15	7.8	1.86	0.11	5.7	2.03	0.16	8.0	1.89	0.14	7.6	1.90	0.09	4.9	1.92	0.10	5.3	1.86	0.10	5.2
Haematology	**Con50**			**NK11-/50**			**NK50-**			**NK11+/50**			**NK50+**			**Con33**			**NK33-**			**NK33+**		
Female (N=8)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
WBC	5.01	0.69	13.8	5.34	0.80	15.0	4.95	1.00	20.2	5.25	0.98	18.8	4.91	1.09	22.3	4.99	0.91	18.3	5.17	1.21	23.4	4.81	1.02	21.2
RBC	7.64	0.14	1.8	7.68	0.17	2.2	7.78	0.31	3.9	7.69	0.14	1.8	7.70	0.17	2.3	7.64	0.20	2.7	7.70	0.23	2.9	7.84	0.10	1.3
HGB	15.1	0.42	2.8	15.1	0.25	1.7	15.2	0.49	3.2	15.4	0.28	1.8	15.1	0.24	1.6	15.0	0.24	1.6	15.0	0.74	4.9	15.2	0.26	1.7
HCT	44.0	0.86	2.0	43.7	1.08	2.5	44.4	1.65	3.7	44.5	0.98	2.2	43.9	1.16	2.7	43.9	0.90	2.0	44.0	1.57	3.6	44.2	0.73	1.7
MCV	57.6	0.95	1.7	56.9	0.68	1.2	57.1	0.56	1.0	57.9	1.04	1.8	57.0	0.92	1.6	57.5	1.09	1.9	57.2	0.85	1.5	56.5	0.90	1.6
MCH	19.8	0.46	2.3	19.7	0.22	1.1	19.6	0.35	1.8	20.0	0.38	1.9	19.6	0.45	2.3	19.7	0.48	2.5	19.7	0.50	2.5	19.4	0.42	2.1
MCHC	34.4	0.37	1.1	34.5	0.35	1.0	34.3	0.52	1.5	34.6	0.50	1.5	34.4	0.69	2.0	34.3	0.40	1.2	34.4	0.56	1.6	34.3	0.58	1.7
PLT	787	36.2	4.6	807	57.3	7.1	745	92.7	12.4	734	68.6	9.3	760	58.3	7.7	785	45.7	5.8	773	74.8	9.7	824	46.2	5.6
LYMR	79.4	3.72	4.7	78.1	3.71	4.7	79.8	7.18	9.0	77.5	2.48	3.2	79.6	4.97	6.2	77.9	4.08	5.2	79.2	3.44	4.3	78.0	4.08	5.2
LYMA	3.97	0.59	15.0	4.16	0.61	14.7	3.96	0.89	22.4	4.05	0.66	16.2	3.92	1.09	27.7	3.91	0.89	22.8	4.09	0.98	23.9	3.79	0.92	24.3
ClinChem	**Con50**			**NK11-/50**			**NK50-**			**NK11+/50**			**NK50+**			**Con33**			**NK33-**			**NK33+**		
Female (N=8)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
ALP	0.66	0.14	22.0	0.59	0.11	18.5	0.64	0.13	20.2	0.63	0.13	20.0	0.68	0.10	14.7	0.64	0.08	13.1	0.72	0.14	19.5	0.62	0.16	25.5

	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
ALT	0.48	0.058	12.1	0.48	0.062	12.7	0.49	0.070	14.2	0.48	0.043	9.0	0.50	0.079	16.0	0.50	0.077	15.4	0.47	0.055	11.5	0.43	0.099	23.1
AST	2.22	0.45	20.1	2.36	0.57	24.2	2.44	0.60	24.7	2.29	0.58	25.3	2.29	0.43	18.8	2.40	0.30	12.4	2.21	0.38	17.4	2.48	0.67	26.8
BIL	6.2	1.61	25.9	8.7	5.71	66.0	6.6	0.90	13.6	9.0	4.94	55.0	8.1	5.16	64.1	6.5	1.06	16.4	8.0	4.20	52.5	10.4	8.46	81.2
ALB	44.0	2.00	4.5	42.7	1.48	3.5	44.7	1.87	4.2	43.1	1.59	3.7	42.7	2.54	5.9	44.4	2.39	5.4	46.1	3.07	6.7	44.7	2.08	4.7
TP	69.2	2.44	3.5	68.6	1.98	2.9	70.4	2.27	3.2	68.4	2.05	3.0	68.6	4.49	6.5	70.4	3.06	4.3	72.1	4.36	6.0	70.1	3.21	4.6
Glu	5.24	0.90	17.2	4.95	0.76	15.3	5.01	0.77	15.3	5.25	1.03	19.7	5.36	0.88	16.4	4.87	0.56	11.5	5.16	0.50	9.7	5.05	0.72	14.2
CHOL	1.63	0.23	14.4	1.57	0.41	26.1	1.65	0.44	26.9	1.54	0.30	19.2	1.34	0.19	14.2	1.83	0.34	18.7	1.95	0.35	17.7	1.68	0.29	17.5
TAG	0.52	0.09	16.9	0.51	0.13	25.4	0.48	0.06	11.5	0.48	0.09	18.9	0.43	0.11	26.2	0.56	0.12	21.0	0.56	0.07	12.8	0.54	0.15	28.7
Crea	36.5	3.11	8.5	38.5	6.08	15.8	37.7	4.34	11.5	37.6	3.50	9.3	34.0	4.00	11.8	35.4	3.91	11.1	35.3	3.06	8.7	37.6	3.58	9.5
Urea	5.93	0.59	10.0	6.47	0.80	12.4	5.87	0.64	10.8	6.36	0.54	8.6	5.85	0.81	13.8	5.48	0.33	6.1	5.96	0.76	12.8	5.90	0.55	9.3
cHGB	73	32.1	43.8	98	88.1	90.0	78	20.1	25.9	110	90.0	82.2	101	77.0	76.5	67	18.7	27.7	65	22.7	34.7	100	97.6	97.1
Ca	2.46	0.031	1.2	2.43	0.048	2.0	2.46	0.042	1.7	2.43	0.039	1.6	2.42	0.031	1.3	2.46	0.049	2.0	2.49	0.059	2.4	2.41	0.034	1.4
Cl	103	1.87	1.8	102	1.83	1.8	102	0.99	1.0	102	1.45	1.4	102	1.35	1.3	101	1.41	1.4	102	2.20	2.2	101	1.33	1.3
K	4.19	0.23	5.6	4.31	0.46	10.6	4.09	0.34	8.3	4.17	0.46	11.1	4.15	0.46	11.0	4.09	0.20	4.9	4.24	0.48	11.3	4.32	0.67	15.5
Na	144	1.28	0.9	143	1.69	1.2	144	1.39	1.0	143	0.90	0.6	143	1.51	1.1	143	2.11	1.5	144	1.49	1.0	144	1.62	1.1
P	1.65	0.40	24.0	1.63	0.32	19.8	1.64	0.17	10.6	1.66	0.30	18.3	1.71	0.36	21.1	1.62	0.36	22.4	1.84	0.30	16.1	1.87	0.38	20.5

Urine	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
Female (N=8)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
uVol	19.9	4.27	21.4	21.1	7.05	33.5	18.1	5.30	29.3	16.5	4.53	27.4	19.9	7.50	37.6	15.3	3.23	21.1	22.6	5.17	22.9	20.1	5.19	25.9
uVolW	8.49	1.69	19.8	9.11	3.88	42.6	7.73	2.21	28.6	6.73	2.24	33.2	8.59	3.02	35.1	6.38	1.38	21.7	9.38	2.33	24.9	8.49	2.43	28.6
uLeu	0.0	0.0		3.1	8.8	282.8	9.4	21.9	233.7	1.6	4.4	282.8	32.8	87.9	267.8	3.1	8.8	282.8	1.6	4.4	282.8	0.0	0.0	
uOsmoll	340	85	25.1	384	156	40.8	435	182	41.8	429	89	20.7	340	106	31.2	423	69	16.4	322	74	23.0	347	73	20.9
uKeton	0.12	0.27	213.8	0.03	0.09	282.8	0.19	0.35	185.2	0.09	0.27	282.8	0.06	0.12	185.2	0.03	0.09	282.8	0.06	0.12	185.2	0.00	0.00	
upH	6.44	0.26	4.0	6.44	0.32	5.0	6.44	0.18	2.7	6.53	0.25	3.8	6.56	0.18	2.7	6.53	0.25	3.8	6.78	0.34	5.0	6.75	0.35	5.2

Organs	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
Female (N=8)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
Kidney * 10	5.81	0.36	6.2	5.89	0.48	8.2	6.00	0.29	4.8	6.01	0.49	8.1	6.28	0.41	6.5	5.77	0.23	4.0	5.79	0.50	8.6	5.88	0.42	7.2
Spleen * 10	2.14	0.07	3.5	2.20	0.31	14.1	2.33	0.32	13.8	2.22	0.20	9.2	2.26	0.25	10.9	2.13	0.20	9.6	2.23	0.21	9.6	2.09	0.12	5.7
Liver * 10	24.0	0.65	2.7	24.1	2.30	9.5	24.6	1.37	5.6	23.5	1.28	5.4	23.8	1.12	4.7	24.4	1.01	4.2	25.1	1.90	7.6	23.5	0.71	3.0
AdrenGl * 10	0.30	0.010	3.5	0.29	0.022	7.6	0.32	0.034	10.8	0.31	0.024	7.7	0.31	0.026	8.5	0.31	0.031	10.0	0.30	0.035	11.8	0.30	0.021	7.0
Heart * 10	3.02	0.13	4.3	2.97	0.13	4.5	3.13	0.15	4.8	3.12	0.21	6.8	3.20	0.26	8.1	3.13	0.25	8.1	3.01	0.18	6.0	2.98	0.09	3.1
Thymus * 10	1.34	0.16	12.0	1.34	0.20	14.8	1.31	0.18	13.8	1.16	0.12	9.9	1.24	0.16	12.5	1.31	0.22	16.5	1.26	0.16	12.8	1.27	0.11	8.9
Uterus * 10	2.59	0.70	26.8	2.98	0.83	28.0	2.73	0.70	25.7	2.49	0.55	22.2	2.57	0.59	23.1	2.53	0.40	15.7	2.70	0.66	24.5	2.62	0.62	23.7
Ovary * 10	0.40	0.053	13.4	0.39	0.052	13.4	0.40	0.063	16.0	0.40	0.034	8.5	0.46	0.087	19.0	0.40	0.028	6.9	0.37	0.058	15.7	0.39	0.065	16.7

Brain * 10	8.55	0.49	5.7	8.53	0.69	8.1	8.58	0.72	8.4	8.23	0.56	6.8	8.68	0.77	8.9	8.07	0.20	2.4	8.32	0.43	5.2	8.33	0.56	6.7
Immunology	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
Female (N=6)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
Monocytes	63.8	8.8	13.8	65.8	6.4	9.7	59.8	11.0	18.3	62.5	9.1	14.5	56.9	8.4	14.8	64.3	6.5	10.0	63.0	6.5	10.4	61.3	7.0	11.5
Granulocytes	80.7	4.68	5.8	78.9	4.87	6.2	73.8	4.80	6.5	76.4	7.57	9.9	74.4	4.99	6.7	78.9	3.63	4.6	77.0	4.18	5.4	76.9	3.43	4.5
RespirBurst	81.0	7.6	9.4	79.6	4.6	5.8	73.1	6.5	8.9	77.1	9.4	12.2	74.9	6.2	8.3	76.8	5.5	7.2	74.4	8.0	10.8	73.4	10.0	13.7
Con / 1000	76	19.8	26.1	69	61.2	89.1	81	40.9	50.5	117	76.4	65.2	55	32.7	59.8	79	41.6	53.0	63	57.2	91.5	60	31.4	52.0
PHA / 1000	26.9	14.6	54.3	31.0	21.5	69.5	34.0	23.3	68.5	53.8	44.9	83.4	15.7	2.8	17.8	32.7	21.4	65.4	26.0	28.3	108.6	22.3	18.0	80.8
PWM / 1000	20.9	4.5	21.7	26.5	21.7	82.0	25.1	14.6	58.3	36.7	27.3	74.3	15.0	3.9	26.0	24.9	11.7	46.8	18.4	11.6	62.9	20.0	12.6	62.8
Med3d / 1000	1.98	0.47	23.7	1.92	1.11	57.7	3.12	1.66	53.2	3.24	1.36	41.8	1.65	1.21	73.2	1.77	1.08	61.3	1.56	0.91	58.6	1.65	0.88	53.5
IprConA	34.2	7.1	20.9	31.9	17.7	55.4	28.3	15.8	56.0	32.9	12.5	37.9	36.6	17.5	47.8	42.5	11.8	27.7	35.0	17.3	49.6	34.8	12.3	35.3
IprPHA	12.7	6.7	52.4	17.6	11.1	63.2	15.5	10.3	66.7	15.9	9.0	56.3	15.2	7.0	45.8	23.6	18.3	77.3	14.4	10.1	69.8	14.4	7.4	51.4
IprPWM	11.1	1.61	14.6	15.1	4.59	30.4	9.5	3.81	40.3	10.6	4.53	42.7	13.0	4.78	36.9	15.9	5.97	37.5	12.5	2.84	22.7	12.8	2.98	23.3
G4c1 / 1000	2.32	0.31	13.4	2.17	1.32	60.5	2.54	0.94	37.2	3.48	1.57	45.1	1.99	0.81	40.9	1.89	0.44	23.2	1.89	1.01	53.3	2.24	0.98	43.7
G4c2 / 1000	2.53	0.52	20.5	2.53	1.45	57.0	2.83	0.82	28.9	3.41	1.52	44.7	2.43	1.07	44.1	2.16	0.65	30.1	2.37	0.96	40.4	2.59	1.03	39.7
G4c3 / 1000	2.77	0.66	23.9	2.59	0.79	30.5	2.78	0.81	29.1	3.12	1.27	40.5	2.33	1.30	55.8	2.17	0.59	27.2	2.47	1.09	43.9	2.80	0.89	31.7
NG2c1 / 1000	2.30	0.31	13.7	2.16	0.90	41.7	2.47	1.00	40.4	3.43	1.99	58.0	1.93	0.80	41.3	1.96	0.47	23.8	2.22	0.89	40.2	2.39	0.64	26.7
NG2c2 / 1000	2.56	0.49	19.3	2.58	1.25	48.3	2.88	1.30	45.2	3.31	1.34	40.5	2.36	1.00	42.5	2.18	0.71	32.5	2.64	0.93	35.1	2.81	0.74	26.3
NG2c3 / 1000	2.67	0.73	27.1	2.59	0.55	21.1	2.94	1.36	46.3	3.27	1.23	37.6	2.50	1.26	50.2	2.15	0.59	27.4	2.69	0.99	36.8	2.79	0.82	29.2
A6c1 / 1000	2.44	0.91	37.1	2.54	0.62	24.5	2.86	0.92	32.1	3.29	0.99	30.0	1.86	0.84	45.0	2.18	0.71	32.6	2.66	0.91	34.3	2.91	0.95	32.6
A6c2 / 1000	1.98	0.77	38.8	2.73	0.56	20.7	2.70	0.93	34.5	3.20	1.43	44.6	2.60	1.36	52.6	1.97	0.56	28.4	2.72	0.90	33.1	2.81	0.75	26.9
A6c3 / 1000	2.42	0.59	24.3	2.34	0.36	15.3	2.72	1.21	44.3	3.16	1.40	44.4	2.16	0.90	41.7	1.95	0.50	25.8	2.44	0.73	29.7	2.49	1.02	41.0
Med6d / 1000	2.57	0.68	26.4	2.45	0.87	35.4	2.48	0.81	32.5	2.66	1.03	38.8	1.70	0.95	56.2	1.91	0.62	32.3	2.18	0.95	43.8	2.31	0.97	41.9
IprG4c1	0.95	0.31	32.4	0.93	0.34	36.3	1.08	0.27	24.7	1.43	0.32	22.0	1.32	0.44	33.1	1.00	0.21	21.1	0.99	0.45	45.7	1.02	0.31	30.1
IprG4c2	1.10	0.25	22.3	1.13	0.41	36.2	1.32	0.26	19.6	1.34	0.34	25.4	1.50	0.43	28.6	1.18	0.20	16.9	1.30	0.59	45.1	1.22	0.38	30.7
IprG4c3	1.17	0.39	33.3	1.26	0.25	19.4	1.38	0.24	17.2	1.21	0.26	21.5	1.42	0.43	30.6	1.27	0.28	22.1	1.24	0.38	30.5	1.29	0.21	16.0
IprNG2c1	1.00	0.35	34.7	0.98	0.27	28.0	1.11	0.36	32.3	1.37	0.56	41.0	1.31	0.30	22.9	1.10	0.25	22.4	1.07	0.28	25.8	1.11	0.40	36.5
IprNG2c2	1.19	0.21	17.8	1.21	0.34	28.2	1.22	0.39	32.3	1.35	0.24	17.5	1.63	0.52	31.6	1.27	0.31	24.5	1.31	0.36	27.8	1.48	0.64	43.2
IprNG2c3	1.35	0.72	52.9	1.26	0.33	26.5	1.33	0.39	29.1	1.28	0.27	21.0	1.73	0.82	47.3	1.39	0.36	25.9	1.42	0.57	40.1	1.48	0.41	27.5
IprA6c1	1.08	0.19	17.7	1.23	0.22	17.7	1.26	0.20	15.7	1.42	0.47	33.0	1.23	0.43	35.5	1.24	0.24	19.8	1.34	0.37	27.3	1.49	0.24	16.0
IprA6c2	1.00	0.27	27.1	1.38	0.23	16.6	1.42	0.41	28.6	1.27	0.45	35.2	1.67	0.50	29.7	1.30	0.32	24.5	1.48	0.60	40.3	1.56	0.55	34.9
IprA6c3	1.08	0.24	22.3	1.12	0.25	22.7	1.24	0.35	28.0	1.26	0.24	19.4	1.49	0.70	47.4	1.27	0.37	29.2	1.32	0.45	34.0	1.24	0.45	36.0

CellPhenotype	Con50			NK11-/50			NK50-			NK11+/50			NK50+			Con33			NK33-			NK33+		
Female (N=6)	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV	Mean	Sd	CV
sp3	50.7	17.7	34.9	52.0	15.7	30.3	50.9	14.9	29.3	52.8	18.3	34.6	58.6	13.9	23.7	52.7	13.9	26.4	44.5	19.3	43.3	46.9	18.3	39.0
sp3-4	25.6	2.66	10.4	26.7	5.12	19.2	26.2	3.92	15.0	24.8	2.63	10.6	26.5	1.19	4.5	27.2	3.27	12.0	22.6	4.00	17.7	24.8	1.91	7.7
sp3-8	10.9	1.69	15.5	12.3	2.28	18.5	12.8	2.73	21.3	12.2	1.61	13.2	12.1	2.50	20.7	12.8	2.69	21.0	11.2	2.96	26.3	11.1	3.37	30.4
sp3-45	32.1	3.95	12.3	28.3	3.20	11.3	27.9	3.11	11.1	31.0	1.85	6.0	29.8	5.41	18.2	28.2	3.49	12.4	28.6	5.78	20.2	28.0	3.10	11.1
sp3-161	7.30	0.91	12.5	6.68	0.79	11.8	6.30	0.74	11.7	7.62	1.23	16.2	6.42	0.60	9.4	6.92	0.96	13.9	7.29	0.78	10.7	7.16	0.71	9.9
ln3	53.2	7.72	14.5	50.5	5.03	10.0	55.1	5.59	10.1	49.4	8.75	17.7	56.4	4.16	7.4	53.1	7.18	13.5	51.7	6.24	12.1	52.0	8.26	15.9
ln3-4	38.1	5.94	15.6	36.8	2.58	7.0	39.8	5.63	14.1	35.6	5.00	14.1	43.2	3.57	8.3	38.5	5.49	14.2	39.0	4.65	11.9	36.7	5.35	14.6
ln3-8	14.1	2.17	15.4	13.1	2.78	21.2	15.2	2.04	13.4	14.1	3.25	23.0	12.7	1.26	9.9	14.0	3.02	21.6	13.0	1.87	14.4	14.2	3.48	24.5
ln3-45	27.8	5.7	20.6	24.0	6.5	27.3	24.7	5.2	21.1	33.5	13.0	38.8	20.2	3.2	15.9	21.0	4.9	23.2	28.3	8.2	28.9	31.4	6.8	21.6
ty3	17.2	1.71	9.9	18.0	2.77	15.3	18.4	3.74	20.4	18.8	2.93	15.6	18.5	2.02	10.9	18.8	1.76	9.4	19.4	3.34	17.2	19.4	2.96	15.2
ty3-4	15.5	1.23	7.9	16.9	3.07	18.2	16.5	3.17	19.3	17.3	2.53	14.6	16.8	1.77	10.6	17.1	2.15	12.6	16.9	3.09	18.2	17.7	2.75	15.5
ty3-8	8.60	1.34	15.6	7.97	1.15	14.5	7.90	0.85	10.8	9.95	3.11	31.3	8.66	1.04	12.0	8.33	0.60	7.2	8.90	1.32	14.8	8.78	2.41	27.4
bm3	11.2	4.6	41.3	13.9	5.4	38.6	16.3	10.1	61.9	15.8	8.8	55.9	16.7	4.8	28.6	18.0	9.6	53.5	7.9	2.8	35.8	16.1	3.1	19.5
bm3-45	62.3	7.88	12.7	62.6	8.90	14.2	60.7	5.54	9.1	59.7	5.12	8.6	53.9	4.96	9.2	55.6	7.18	12.9	62.8	4.57	7.3	55.8	4.01	7.2

4   Statistical analysis

A main interest in G-TwYST Study C was whether maize inclusion rate 33% can be replaced by a maize inclusion rate of 50% in order to establish a higher exposure. There was thus a primary interest in the comparison between feeds with 50% maize and the corresponding feed with 33% maize. In addition the GM feeds with 50% maize were compared to the non-GM control feed with 50% maize, and similarly for the feeds with 33% inclusion rate. Therefore the following nine comparisons were statistically tested:

1. NK11-/50   vs Con50
2. NK50-   vs Con50
3. NK11+/50   vs Con50
4. NK50+   vs Con50
5. Con50   vs Con33
6. NK50-   vs NK33-
7. NK50+   vs NK33+
8. NK33-   vs Con33
9. NK33+   vs Con33

The comparisons 1-4 are grouped in the Figures and Tables below, and so are the comparisons 5-9.

In section 4.7 a post-hoc power analysis is performed in which the power of a trial with only 50% maize feeds is compared to the power of a trial with only 33% maize feeds.

4.1   Equivalence testing using historical data

4.1.1   Method

Equivalence testing was introduced for GM safety assessment for compositional data in the EFSA guidance for risk assessment of food and feed from GM plants (EFSA 2011a). In the context of 90-day studies in rodents, EFSA (2014) recognized the potential advantages of equivalence testing and recommended further investigation. In response to this , an equivalence test was developed in the G-TwYST project. This test compares the difference between a test (T) and a control (C) feed, obtained simultaneously in a current study, to the typical differences between reference (R) varieties obtained in one or more historical studies (van der Voet *et al*, 2017). The equivalence test is corrected for between-study differences, and the within-study variation between references R is used to set equivalence limits for the difference between T and C in the current study. The so-called Distribution Wise Equivalence (DWE) criterion is used in this test. An equivalence limit for the current study is set using the concept of desired power in a simplified situation, where there is no between-reference variation, where the historical and current studies have the same residual variance, and where the current study is assumed to have a sample size as approved by a regulator. The method is fully described in van der Voet *et al* (2017) .

The equivalence test of van der Voet *et al* (2017) requires, employing the historic GRACE studies, calculation of the within-study between reference feeds sums of squares (SS_R), the residual sums of squares (SS_E) and their associated degrees of freedom df_R and df_E. It also involves the effective unit replication n_{eff} which is necessary to estimate the between reference variance employing the mean squares for feeds and for residuals. The required values based on the five GRACE studies A-E are given in Appendix 7. The test also requires, for the current G-TwYST study, estimates of differences

between the GMO feeds and the control feed, as well as the residual sums of squares and the associated degrees of freedom resulting from an analysis of variance. These are given in Appendix 8.

The equivalence limit θ_0 for the DWE criterion is only based on the design values of the historical studies and on three regulatory values: the minimal regulatory sample size n_0, a probability α which defines a $100(1 - \alpha)$% confidence interval for the difference in the current study, and a probability β which defines the desired power $1 - \beta$ for the equivalence test. We used values $n_0 = 8$, $\alpha = 0.05$ and $\beta = 0.05$ resulting in a power of 0.95. Note that the regulatory sample size $n_0 = 8$ equals the replication, i.e. the number of cages, for most variables in both the GRACE and the G-TwYST studies. Furthermore the equivalence limit θ_0 is calculated by simulating a large number of datasets in a simplified situation, where for each datasets an upper $100(1 - \alpha)$% percentile, θ_{upp}^0, for the DWE criterion is approximated by a large number of so-called GPQ samples. We simulated 40,000 datasets with 15,000 GPQ samples for each dataset. Note that θ_0 is calculated as the upper $100(1 - \beta)$% percentile of the thus obtained 40.000 values of θ_{upp}^0. The DWE criterion for the current dataset was approximated by means of 100,000 GPQ samples. Note that the equivalence limit θ_0 is calculated assuming a regulatory sample size $n_0 = 8$ which implies 14 degrees of freedom for error in the current study. The current G-TwYST study indeed has replication 8 but has 49 degrees of freedom for error.

The DWE equivalence test results in a DWE interval as a so-called equivalence limit scaled difference (ELSD), which can be used both for difference and for equivalence testing. The hypothesis of no difference is rejected in case the interval does not contain zero, while the non-equivalence hypothesis is rejected when the interval fully lies inside the interval (-1,1). For further interpretation, the confidence intervals are also presented at the original ratio scale, with inclusion of the estimated equivalence limits (red bars) and their uncertainty (blue bars). Note that the latter graphs cannot be used directly for performing the equivalence test. However, they show the effects and equivalence limits at a more familiar scale.

4.1.2 Results
All equivalence tests assume that the historical GRACE precision results, which were obtained for maize inclusion rates of 33%, also apply to feeds in this study, including to feeds with a maize inclusion rate of 50%. The DWE intervals showing the main results of the equivalence tests for 36 variables are given in Figure 6 to Figure 9. For further interpretation the 95% confidence intervals for the ratios are given in Table 11 to Table 14. These intervals are based on an ANOVA with all eight feeding groups. Intervals and limits at the ratio scale are given in Figure 10 to Figure 18.

The DWE equivalence test depends, among other things, on the ratio of the residual variance of the current study and the residual variance of the historical studies. In case this ratio is small the corresponding DWE interval will generally be short. The ratio of the residual variances is given in Figure 19. Most ratios are around 100% or less, indicating that the current G-TwYST Study C is at least as precise as the historical GRACE studies. Ratios larger than 150% were observed for ALT and AST in males, and for ALP, CHOL, P, Kidney and Uterus in females. These endpoints are labelled by means of a golden background in Figure 6 to Figure 18.

Among 324 equivalence tests for males (36 variables × 9 comparisons), there were no failures to prove equivalence (i.e. the hypothesis of non-equivalence is always rejected). For females there were five (1.5%) of such failures: three failures for CHOL (NK50+ vs Con50, NK50- vs NK33-, and NK50+ vs

NK33+), one for P (NK33+ vs Con33) and one for Kidney (NK50+ vs Con50). In all these five female cases the median estimate was within the equivalence limits, therefore equivalence is still more likely than lack of equivalence according to the terminology of EFSA (2011a). From Figure 19 it can be seen that these are all cases where the G-TwYST study C was less precise than the historical studies on average (residual variance more than 1.5 higher than in the historical GRACE studies).

Although not the primary result of the equivalence analysis, it can also be observed from the graphs and tables that, for those variables for which the equivalence test is performed, the number of significant differences, employing t-tests, equals 46 (7.1% of 648 difference tests), which is close to the 5% level of the test. Only in four of these cases (3 × CHOL and 1 × Kidney) there was both a significant difference and a failure to show equivalence.

For all 1584 difference tests, i.e. including those for which the equivalence test was not performed, 90 t-tests were significant at the 5% significance level which is 5.7% of the tests (see Table 11 to Table 14).
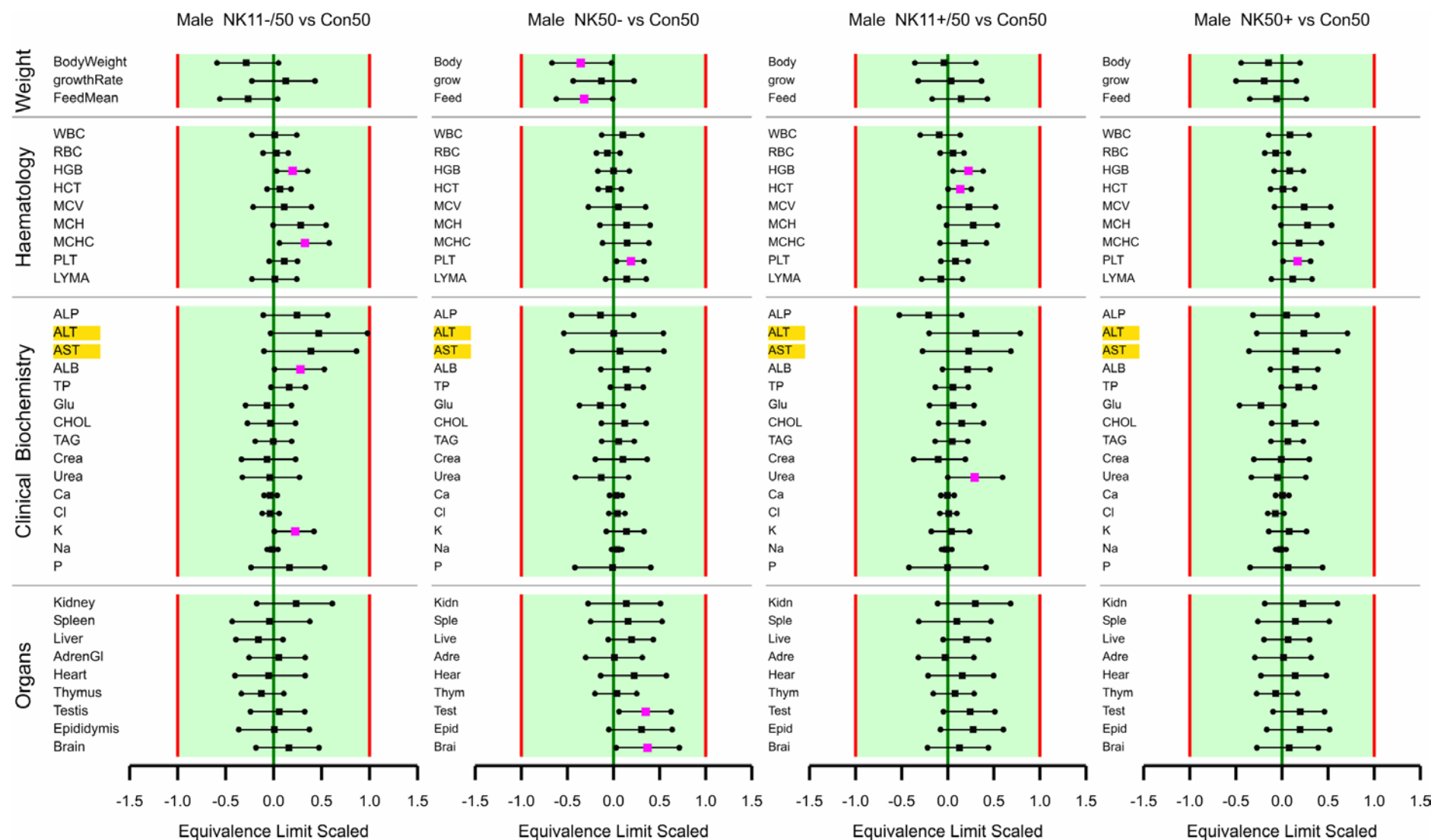
Figure 6 Equivalence testing of GM 50% maize feeds versus the corresponding non-GM control 50% maize feed for males. For estimates on the left of zero the 50% feed has a smaller mean than the corresponding control feed. Also see Table 11 / Figure 10 / Figure 11.
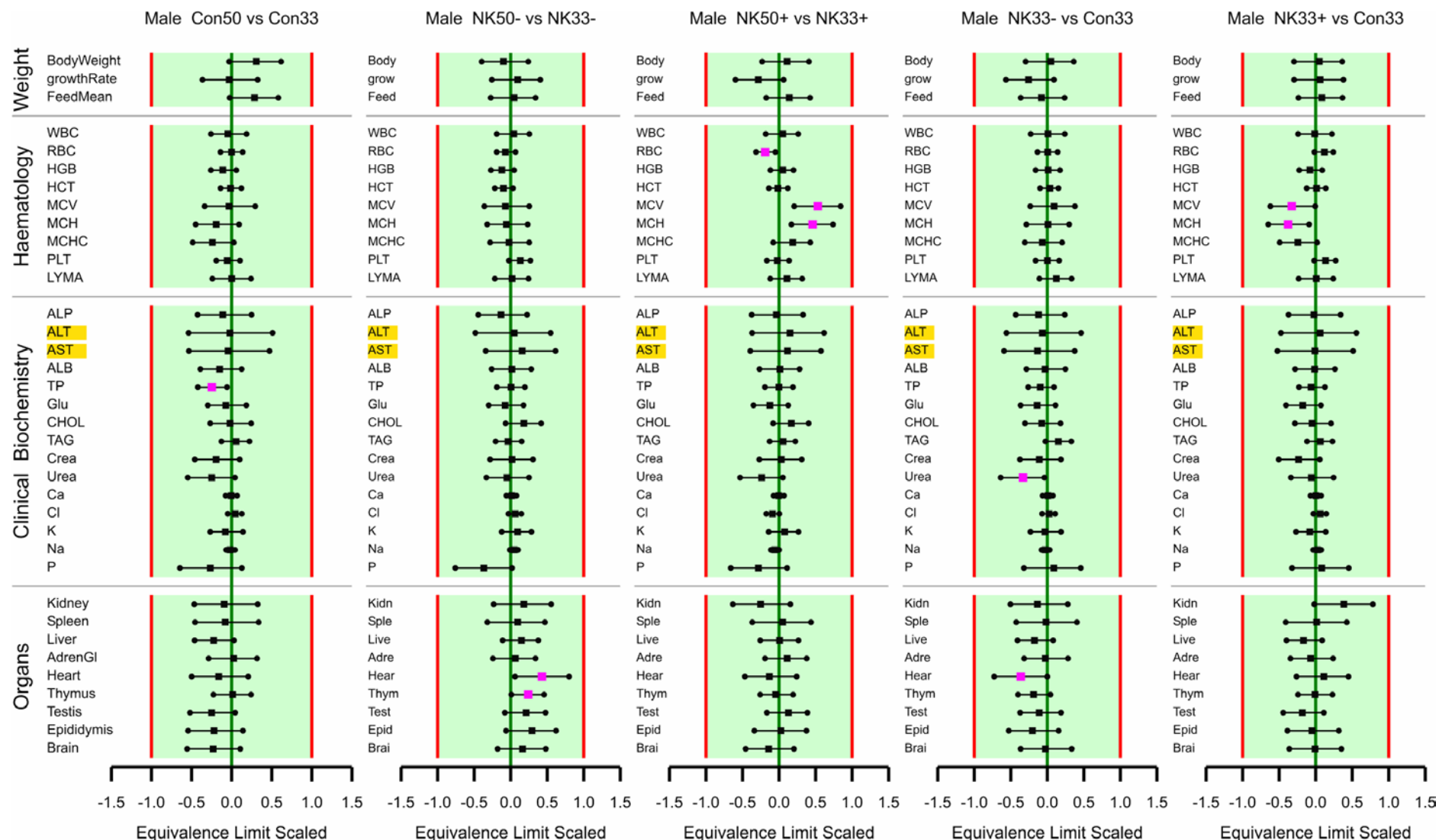
Figure 7   EQ testing of 50% maize feeds versus the corresponding 33% feeds for males, and for the GM 33% feeds versus the non-GM 33% feed. For estimates on the left of zero the 50% feed has a smaller mean than the 33% feed. Also see Table 12 / Figure 18 / Figure 12 / Figure 13.
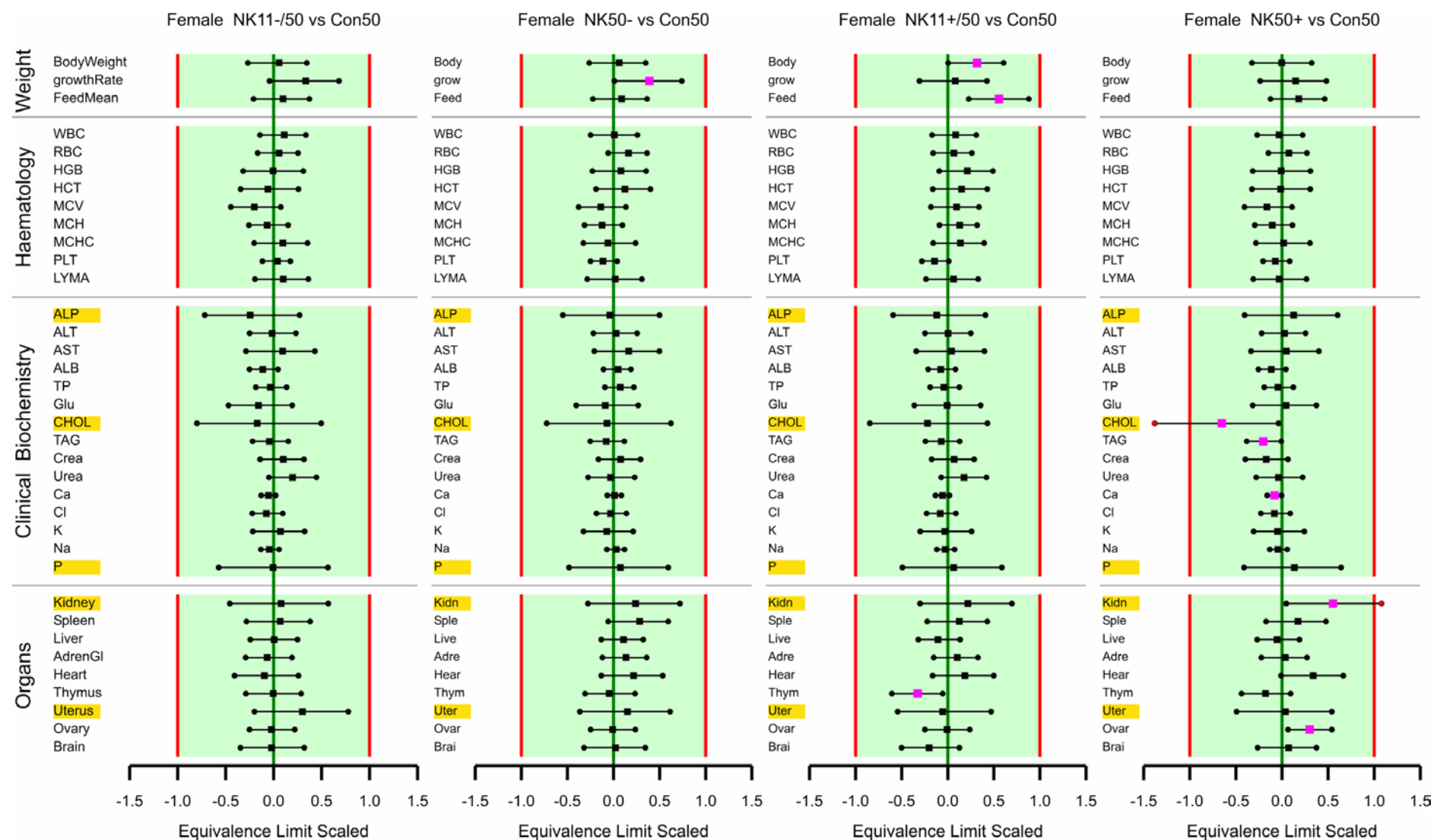
Figure 8   Equivalence testing of GM 50% maize feeds versus the corresponding non-GM control 50% maize feed for females. For estimates on the left of zero the 50% feed has a smaller mean than the corresponding control feed. Also see Table 13 / Figure 14 / Figure 15.
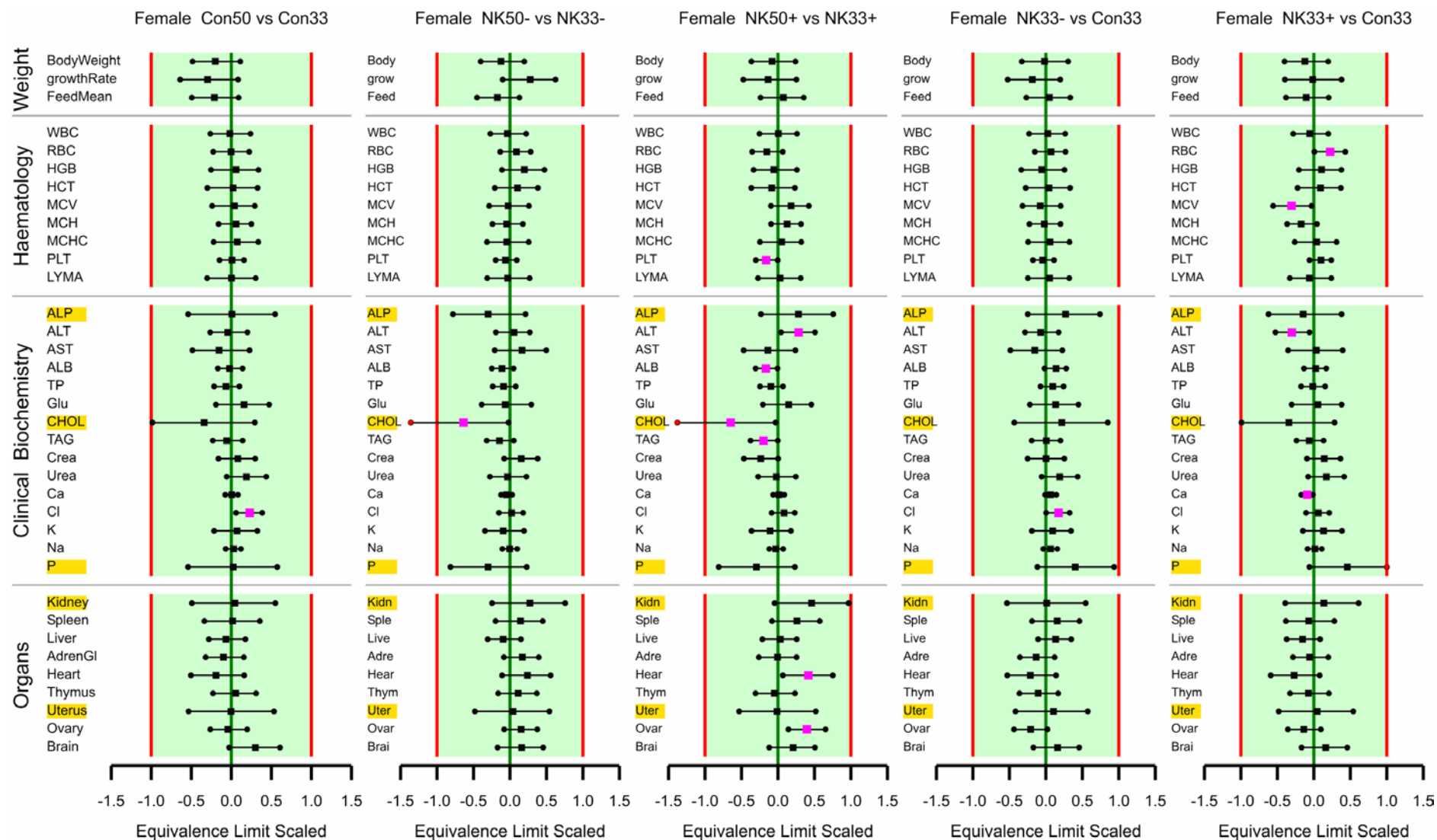
Figure 9 **EQ testing of 50% maize feeds versus the corresponding 33% feeds for females, and for the GM 33% feeds versus the non-GM 33% feed. For estimates on the left of zero the 50% feed has a smaller mean than the 33% feed. Also see Table 14 / Figure 18 / Figure 16 / Figure 17.**

Table 11   95% Confidence interval plus estimate for the ratio Δ of the GMO feeds with 50% maize inclusion rate versus the corresponding non-GM control feed for males. Ratios with corresponding 95/99% intervals that do not encompass the value 1 are coloured red/gold; this is equivalent to a significant difference according to a 5/1% t-test.

Weights	NK11-/50 vs Con50			NK50- vs Con50			NK11+/50 vs Con50			NK50+ vs Con50		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
BodyWeight	0.91	0.96	1.01	0.90	0.95	1.00	0.94	0.99	1.05	0.93	0.98	1.03
growthRate	0.99	1.01	1.03	0.98	0.99	1.01	0.98	1.00	1.02	0.97	0.99	1.01
FeedMean	0.91	0.96	1.01	0.91	0.95	1.00	0.97	1.02	1.07	0.94	0.99	1.04
Haematology	**NK11-/50 vs Con50**			**NK50- vs Con50**			**NK11+/50 vs Con50**			**NK50+ vs Con50**		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
WBC	0.85	1.01	1.19	0.91	1.08	1.27	0.79	0.93	1.10	0.90	1.07	1.26
RBC	0.98	1.00	1.03	0.97	0.99	1.01	0.99	1.01	1.03	0.97	0.99	1.01
HGB	1.00	1.03	1.05	0.98	1.00	1.02	1.01	1.03	1.05	0.99	1.01	1.03
HCT	0.99	1.01	1.03	0.97	0.99	1.01	1.00	1.02	1.04	0.98	1.00	1.02
MCV	0.99	1.01	1.02	0.99	1.00	1.02	1.00	1.01	1.03	1.00	1.01	1.03
MCH	1.00	1.02	1.05	0.99	1.01	1.03	1.00	1.02	1.04	1.00	1.02	1.04
MCHC	1.00	1.02	1.03	0.99	1.01	1.02	1.00	1.01	1.02	1.00	1.01	1.02
PLT	0.97	1.09	1.23	1.03	1.16	1.31	0.94	1.07	1.20	1.01	1.14	1.29
LYMR	0.96	1.00	1.04	0.99	1.03	1.06	0.98	1.02	1.06	0.98	1.02	1.06
LYMA	0.86	1.01	1.19	0.94	1.11	1.30	0.81	0.95	1.11	0.92	1.09	1.28
ClinChem	**NK11-/50 vs Con50**			**NK50- vs Con50**			**NK11+/50 vs Con50**			**NK50+ vs Con50**		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
ALP	0.95	1.13	1.34	0.78	0.93	1.11	0.76	0.90	1.07	0.86	1.03	1.22
ALT	0.99	1.15	1.34	0.86	1.00	1.16	0.94	1.10	1.28	0.92	1.07	1.25
AST	0.96	1.17	1.42	0.84	1.03	1.25	0.90	1.10	1.34	0.87	1.06	1.29
BIL	1.01	1.13	1.28	0.92	1.04	1.17	0.94	1.06	1.20	0.97	1.09	1.23
ALB	1.00	1.04	1.09	0.98	1.02	1.07	0.99	1.03	1.08	0.98	1.02	1.07
TP	1.00	1.02	1.04	1.00	1.02	1.04	0.98	1.01	1.03	1.00	1.02	1.05
Glu	0.86	0.97	1.09	0.83	0.94	1.05	0.92	1.03	1.15	0.80	0.90	1.01
CHOL	0.89	0.99	1.09	0.95	1.05	1.16	0.96	1.06	1.17	0.96	1.06	1.17
TAG	0.82	1.00	1.22	0.87	1.07	1.30	0.86	1.06	1.29	0.88	1.07	1.31
Crea	0.86	0.97	1.10	0.93	1.04	1.17	0.85	0.96	1.08	0.88	1.00	1.12
Urea	0.89	0.99	1.09	0.86	0.95	1.06	1.00	1.11	1.23	0.89	0.98	1.09
cHGB	1.09	1.52	2.12	0.84	1.17	1.63	0.92	1.28	1.78	1.09	1.51	2.11
Ca	0.98	0.99	1.01	0.99	1.01	1.02	0.98	1.00	1.01	0.99	1.00	1.02
Cl	0.98	0.99	1.01	0.99	1.01	1.02	0.99	1.00	1.02	0.97	0.99	1.00
K	1.00	1.08	1.16	0.98	1.05	1.12	0.94	1.01	1.09	0.96	1.03	1.10
Na	0.99	1.00	1.01	1.00	1.01	1.02	0.99	1.00	1.01	0.99	1.00	1.01
P	0.94	1.05	1.18	0.89	1.00	1.12	0.89	1.00	1.12	0.91	1.02	1.14
Urine	**NK11-/50 vs Con50**			**NK50- vs Con50**			**NK11+/50 vs Con50**			**NK50+ vs Con50**		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
uVol	0.75	1.00	1.33	0.84	1.13	1.51	0.85	1.14	1.52	0.79	1.06	1.42
uVolW	0.78	1.04	1.38	0.90	1.20	1.59	0.87	1.15	1.53	0.82	1.08	1.44
uLeu	0.58	0.87	1.32	0.47	0.71	1.07	0.54	0.81	1.23	0.50	0.76	1.15
uOsmoll	0.71	0.92	1.19	0.66	0.86	1.10	0.71	0.92	1.18	0.72	0.93	1.19
uKeton	0.44	0.77	1.35	0.51	0.90	1.57	0.37	0.64	1.12	0.53	0.93	1.63
upH	0.69	0.97	1.37	0.71	1.00	1.41	0.86	1.21	1.70	0.57	0.80	1.13
Organs	**NK11-/50 vs Con50**			**NK50- vs Con50**			**NK11+/50 vs Con50**			**NK50+ vs Con50**		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
Kidney	0.97	1.04	1.11	0.96	1.02	1.09	0.98	1.05	1.12	0.97	1.04	1.10

	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
Spleen	0.90	0.99	1.09	0.95	1.04	1.14	0.93	1.02	1.12	0.94	1.04	1.14
Liver	0.94	0.98	1.01	0.99	1.03	1.07	0.99	1.03	1.07	0.97	1.01	1.05
AdrenGl	0.92	1.02	1.13	0.90	1.00	1.11	0.89	0.99	1.10	0.91	1.01	1.12
Heart	0.94	0.99	1.05	0.98	1.03	1.09	0.97	1.02	1.08	0.97	1.02	1.08
Thymus	0.81	0.93	1.06	0.90	1.02	1.16	0.92	1.05	1.19	0.84	0.96	1.10
Testis	0.95	1.01	1.08	1.01	1.08	1.15	0.99	1.05	1.12	0.98	1.04	1.11
Epididymis	0.93	1.00	1.08	0.99	1.07	1.15	0.98	1.06	1.15	0.97	1.04	1.13
Brain	0.97	1.03	1.08	1.00	1.06	1.11	0.97	1.02	1.07	0.96	1.01	1.07
Immunology	**NK11-/50 vs Con50**			**NK50- vs Con50**			**NK11+/50 vs Con50**			**NK50+ vs Con50**		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
Monocytes	0.94	1.03	1.13	0.94	1.03	1.13	0.89	0.98	1.07	0.93	1.03	1.13
Granulocytes	0.92	0.97	1.02	0.94	0.99	1.04	0.95	0.99	1.04	0.92	0.97	1.03
RespirBurst	0.93	1.01	1.09	0.91	0.98	1.06	0.94	1.01	1.09	0.90	0.97	1.06
Con	0.57	1.32	3.05	0.54	1.23	2.81	0.82	1.84	4.15	0.41	1.00	2.45
PHA	0.54	1.15	2.43	0.52	1.09	2.29	0.70	1.45	2.99	0.42	0.93	2.06
PWM	0.64	1.14	2.04	0.67	1.19	2.10	0.74	1.29	2.26	0.46	0.84	1.56
Med3d	0.52	1.09	2.28	0.54	1.12	2.31	0.59	1.21	2.48	0.36	0.79	1.73
IprConA	0.85	1.21	1.72	0.78	1.10	1.56	1.08	1.51	2.13	0.88	1.27	1.85
IprPHA	0.69	1.06	1.63	0.64	0.98	1.50	0.79	1.19	1.81	0.75	1.18	1.87
IprPWM	0.72	1.05	1.54	0.74	1.07	1.55	0.74	1.06	1.53	0.72	1.07	1.60
G4c1	0.49	1.08	2.42	0.56	1.23	2.71	0.64	1.39	3.03	0.46	1.08	2.54
G4c2	0.41	1.05	2.73	0.42	1.07	2.73	0.52	1.32	3.31	0.40	1.10	3.02
G4c3	0.46	1.12	2.70	0.37	0.88	2.09	0.68	1.60	3.76	0.54	1.39	3.54
NG2c1	0.52	1.19	2.73	0.54	1.23	2.76	0.70	1.56	3.46	0.57	1.36	3.28
NG2c2	0.34	0.91	2.41	0.35	0.92	2.40	0.41	1.06	2.72	0.36	1.02	2.88
NG2c3	0.44	1.08	2.69	0.36	0.88	2.15	0.63	1.51	3.65	0.52	1.38	3.63
A6c1	0.52	1.28	3.15	0.42	1.01	2.45	0.66	1.58	3.78	0.47	1.23	3.21
A6c2	0.37	0.99	2.65	0.34	0.90	2.35	0.46	1.19	3.08	0.48	1.36	3.86
A6c3	0.48	1.20	2.96	0.35	0.85	2.06	0.61	1.45	3.49	0.57	1.49	3.91
Med6d	0.36	1.05	3.09	0.30	0.87	2.51	0.48	1.36	3.87	0.40	1.27	3.99
IprG4c1	0.67	1.03	1.60	0.92	1.41	2.17	0.67	1.02	1.56	0.53	0.85	1.36
IprG4c2	0.74	1.00	1.36	0.91	1.23	1.66	0.72	0.97	1.30	0.62	0.87	1.20
IprG4c3	0.69	1.06	1.64	0.66	1.01	1.54	0.78	1.18	1.78	0.69	1.09	1.73
IprNG2c1	0.78	1.14	1.65	0.97	1.40	2.02	0.80	1.14	1.63	0.72	1.07	1.59
IprNG2c2	0.62	0.86	1.20	0.76	1.05	1.46	0.56	0.78	1.07	0.56	0.80	1.14
IprNG2c3	0.73	1.03	1.46	0.71	1.01	1.42	0.79	1.11	1.56	0.75	1.08	1.57
IprA6c1	0.85	1.22	1.75	0.81	1.16	1.65	0.82	1.16	1.64	0.66	0.97	1.42
IprA6c2	0.65	0.95	1.38	0.71	1.03	1.49	0.61	0.87	1.26	0.72	1.07	1.60
IprA6c3	0.79	1.14	1.65	0.67	0.97	1.40	0.74	1.06	1.52	0.79	1.18	1.74
CellPhenotype	**NK11-/50 vs Con50**			**NK50- vs Con50**			**NK11+/50 vs Con50**			**NK50+ vs Con50**		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
sp3	0.77	0.96	1.21	0.83	1.03	1.29	0.79	0.99	1.23	0.84	1.07	1.37
sp3-4	0.71	0.95	1.27	0.79	1.05	1.40	0.75	1.00	1.32	0.82	1.11	1.52
sp3-8	0.76	1.10	1.58	0.75	1.08	1.54	0.76	1.08	1.54	0.93	1.37	2.02
sp3-45	0.87	1.04	1.24	0.86	1.03	1.22	0.80	0.95	1.13	0.80	0.96	1.16
sp3-161	0.74	0.90	1.10	0.78	0.94	1.14	0.84	1.02	1.24	0.74	0.91	1.12
ln3	0.78	0.92	1.08	0.88	1.03	1.21	0.83	0.97	1.13	0.84	1.00	1.18
ln3-4	0.81	0.96	1.12	0.91	1.07	1.25	0.84	0.98	1.15	0.85	1.01	1.20
ln3-8	0.77	0.94	1.14	0.85	1.03	1.24	0.87	1.05	1.26	0.87	1.07	1.31
ln3-45	0.73	0.96	1.28	0.75	0.98	1.30	0.76	1.01	1.35	0.73	0.99	1.34
ty3	0.75	0.90	1.08	0.81	0.96	1.15	0.79	0.94	1.13	0.76	0.92	1.11
ty3-4	0.76	0.90	1.05	0.83	0.97	1.14	0.82	0.96	1.13	0.76	0.91	1.08
ty3-8	0.73	0.90	1.11	0.75	0.92	1.14	0.81	0.99	1.22	0.70	0.88	1.10

bm3	0.75	1.21	1.95	0.67	1.07	1.71	0.56	0.89	1.42	0.56	0.93	1.54
bm3-45	0.95	1.04	1.13	0.96	1.05	1.14	1.01	1.11	1.21	0.99	1.09	1.20

Table 12   95% Confidence interval plus estimate for the ratio Δ of the feeds with 50% maize inclusion rate versus the corresponding 33% inclusion rate for males, and for the GM 33% feeds versus the non-GM 33% feed. Ratios with corresponding 95/99% intervals that do not encompass the value 1 are coloured red/gold; this is equivalent to a significant difference according to a 5/1% t-test.

Weights	Con50 vs Con33			NK50- vs NK33-			NK50+ vs NK33+			NK33- vs Con33			NK33+ vs Con33		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
BodyWeight	1.00	1.05	1.10	0.94	0.98	1.04	0.97	1.02	1.07	0.96	1.01	1.06	0.96	1.01	1.06
growthRate	0.98	1.00	1.02	0.99	1.01	1.02	0.97	0.99	1.00	0.97	0.99	1.00	0.99	1.00	1.02
FeedMean	1.00	1.05	1.10	0.96	1.01	1.06	0.97	1.02	1.07	0.94	0.99	1.04	0.97	1.01	1.06
Haematology	**Con50 vs Con33**			**NK50- vs NK33-**			**NK50+ vs NK33+**			**NK33- vs Con33**			**NK33+ vs Con33**		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
WBC	0.82	0.96	1.14	0.87	1.03	1.22	0.88	1.04	1.23	0.85	1.01	1.19	0.84	0.99	1.17
RBC	0.98	1.00	1.02	0.97	0.99	1.01	0.95	0.97	0.99	0.98	1.00	1.02	1.00	1.02	1.04
HGB	0.96	0.99	1.01	0.96	0.98	1.01	0.98	1.01	1.03	0.98	1.00	1.02	0.97	0.99	1.01
HCT	0.98	1.00	1.02	0.97	0.99	1.01	0.98	1.00	1.02	0.99	1.01	1.03	0.98	1.00	1.02
MCV	0.98	1.00	1.01	0.98	1.00	1.01	1.01	1.03	1.04	0.99	1.00	1.02	0.97	0.98	1.00
MCH	0.96	0.99	1.01	0.97	1.00	1.02	1.01	1.04	1.06	0.98	1.00	1.02	0.95	0.97	0.99
MCHC	0.97	0.99	1.00	0.99	1.00	1.01	1.00	1.01	1.02	0.98	1.00	1.01	0.97	0.99	1.00
PLT	0.85	0.96	1.08	0.98	1.11	1.25	0.87	0.98	1.11	0.89	1.00	1.13	0.98	1.11	1.25
LYMR	0.95	0.99	1.03	0.95	0.98	1.02	1.00	1.04	1.08	1.00	1.03	1.07	0.93	0.97	1.01
LYMA	0.85	1.00	1.18	0.86	1.01	1.19	0.92	1.08	1.27	0.93	1.09	1.28	0.86	1.01	1.18
ClinChem	**Con50 vs Con33**			**NK50- vs NK33-**			**NK50+ vs NK33+**			**NK33- vs Con33**			**NK33+ vs Con33**		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
ALP	0.79	0.95	1.13	0.79	0.94	1.11	0.82	0.98	1.17	0.79	0.94	1.12	0.83	0.99	1.18
ALT	0.85	0.99	1.15	0.87	1.01	1.18	0.90	1.05	1.22	0.84	0.98	1.14	0.88	1.02	1.19
AST	0.81	0.98	1.20	0.88	1.07	1.30	0.86	1.05	1.28	0.78	0.95	1.15	0.82	1.00	1.21
BIL	0.88	0.99	1.12	0.91	1.02	1.15	0.94	1.06	1.19	0.89	1.01	1.14	0.91	1.03	1.16
ALB	0.94	0.98	1.02	0.96	1.00	1.05	0.96	1.00	1.05	0.95	1.00	1.04	0.96	1.00	1.04
TP	0.95	0.97	0.99	0.98	1.00	1.02	0.98	1.00	1.02	0.97	0.99	1.01	0.97	0.99	1.02
Glu	0.86	0.97	1.08	0.86	0.96	1.08	0.84	0.94	1.06	0.84	0.94	1.05	0.82	0.92	1.03
CHOL	0.90	0.99	1.09	0.97	1.07	1.18	0.97	1.07	1.18	0.88	0.97	1.07	0.89	0.98	1.08
TAG	0.87	1.07	1.30	0.79	0.96	1.17	0.87	1.07	1.30	0.97	1.18	1.45	0.88	1.08	1.31
Crea	0.82	0.92	1.04	0.89	1.01	1.14	0.90	1.01	1.14	0.85	0.96	1.08	0.81	0.91	1.02
Urea	0.82	0.91	1.01	0.88	0.98	1.09	0.83	0.92	1.02	0.80	0.89	0.99	0.88	0.98	1.09
cHGB	0.55	0.77	1.07	0.76	1.06	1.48	0.88	1.22	1.70	0.61	0.84	1.17	0.68	0.95	1.32
Ca	0.98	1.00	1.01	0.99	1.00	1.02	0.98	1.00	1.01	0.99	1.00	1.02	0.99	1.00	1.02
Cl	0.99	1.01	1.02	1.00	1.01	1.02	0.97	0.99	1.00	0.99	1.00	1.02	1.00	1.01	1.02
K	0.91	0.97	1.05	0.96	1.03	1.11	0.96	1.03	1.10	0.92	0.99	1.06	0.91	0.97	1.05
Na	0.99	1.00	1.01	1.00	1.01	1.02	0.98	0.99	1.00	0.99	1.00	1.01	0.99	1.00	1.01
P	0.82	0.92	1.04	0.80	0.90	1.01	0.82	0.92	1.03	0.92	1.03	1.15	0.91	1.02	1.15

Urine	Con50 vs Con33			NK50- vs NK33-			NK50+ vs NK33+			NK33- vs Con33			NK33+ vs Con33		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
uVol	0.86	1.15	1.53	0.88	1.17	1.57	0.89	1.19	1.60	0.82	1.10	1.47	0.76	1.02	1.36
uVolW	0.82	1.09	1.45	0.90	1.20	1.59	0.88	1.17	1.55	0.82	1.09	1.45	0.76	1.01	1.35
uLeu	0.66	1.00	1.51	0.54	0.81	1.23	0.47	0.71	1.07	0.58	0.87	1.32	0.71	1.07	1.62
uOsmoll	0.77	0.99	1.28	0.68	0.88	1.14	0.65	0.84	1.08	0.75	0.96	1.24	0.85	1.10	1.41
uKeton	0.79	1.39	2.43	0.43	0.74	1.30	0.61	1.07	1.87	0.96	1.68	2.93	0.69	1.21	2.11
upH	0.61	0.86	1.21	0.65	0.91	1.28	0.42	0.59	0.83	0.67	0.94	1.32	0.83	1.17	1.65
Organs	Con50 vs Con33			NK50- vs NK33-			NK50+ vs NK33+			NK33- vs Con33			NK33+ vs Con33		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
Kidney	0.92	0.99	1.05	0.97	1.03	1.10	0.90	0.96	1.02	0.92	0.98	1.04	1.00	1.06	1.13
Spleen	0.89	0.98	1.08	0.93	1.02	1.12	0.92	1.01	1.11	0.91	1.00	1.09	0.91	1.00	1.10
Liver	0.93	0.97	1.00	0.98	1.02	1.06	0.96	1.00	1.04	0.94	0.97	1.01	0.94	0.97	1.01
AdrenGl	0.91	1.01	1.12	0.92	1.02	1.13	0.94	1.04	1.15	0.89	0.99	1.10	0.88	0.98	1.08
Heart	0.92	0.98	1.03	1.01	1.07	1.13	0.93	0.98	1.04	0.90	0.95	1.00	0.96	1.02	1.08
Thymus	0.88	1.01	1.15	1.00	1.14	1.31	0.85	0.97	1.11	0.79	0.90	1.02	0.87	0.99	1.13
Testis	0.89	0.95	1.01	0.98	1.05	1.12	0.97	1.03	1.10	0.92	0.98	1.04	0.90	0.96	1.02
Epididymis	0.88	0.95	1.03	0.99	1.07	1.15	0.93	1.01	1.09	0.88	0.96	1.03	0.92	0.99	1.07
Brain	0.92	0.97	1.02	0.97	1.03	1.08	0.93	0.98	1.03	0.95	1.00	1.05	0.95	1.00	1.05
Immunology	Con50 vs Con33			NK50- vs NK33-			NK50+ vs NK33+			NK33- vs Con33			NK33+ vs Con33		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
Monocytes	0.94	1.03	1.13	0.91	1.00	1.10	0.87	0.95	1.05	0.97	1.06	1.17	1.02	1.11	1.22
Granulocytes	0.96	1.01	1.06	0.95	1.00	1.05	0.92	0.97	1.02	0.95	1.01	1.06	0.97	1.01	1.07
RespirBurst	0.96	1.04	1.13	0.95	1.03	1.12	0.93	1.01	1.09	0.91	0.99	1.08	0.93	1.00	1.08
Con	0.21	0.49	1.13	0.23	0.55	1.32	0.27	0.66	1.58	0.45	1.10	2.67	0.32	0.74	1.72
PHA	0.33	0.71	1.50	0.26	0.58	1.26	0.32	0.70	1.52	0.60	1.34	2.96	0.45	0.94	2.00
PWM	0.50	0.89	1.59	0.43	0.79	1.45	0.35	0.64	1.17	0.72	1.34	2.47	0.66	1.18	2.10
Med3d	0.34	0.72	1.51	0.32	0.70	1.51	0.31	0.67	1.45	0.52	1.15	2.52	0.40	0.85	1.77
IprConA	0.48	0.68	0.96	0.54	0.78	1.13	0.68	0.98	1.41	0.66	0.95	1.39	0.62	0.88	1.25
IprPHA	0.64	0.98	1.50	0.53	0.83	1.29	0.66	1.04	1.62	0.74	1.16	1.83	0.73	1.12	1.71
IprPWM	0.84	1.24	1.81	0.76	1.13	1.68	0.64	0.95	1.41	0.78	1.16	1.74	0.95	1.39	2.03
G4c1	0.29	0.65	1.46	0.32	0.73	1.68	0.42	0.97	2.24	0.47	1.11	2.59	0.33	0.73	1.62
G4c2	0.25	0.64	1.67	0.18	0.48	1.31	0.41	1.10	2.98	0.52	1.42	3.91	0.25	0.64	1.66
G4c3	0.23	0.56	1.35	0.18	0.45	1.12	0.46	1.14	2.86	0.43	1.10	2.79	0.28	0.68	1.64
NG2c1	0.28	0.64	1.46	0.25	0.59	1.40	0.44	1.04	2.46	0.55	1.32	3.17	0.37	0.83	1.90
NG2c2	0.28	0.75	2.00	0.19	0.52	1.43	0.37	1.03	2.84	0.48	1.34	3.78	0.28	0.75	1.99
NG2c3	0.23	0.56	1.40	0.15	0.38	0.99	0.41	1.07	2.77	0.49	1.29	3.38	0.29	0.72	1.80
A6c1	0.21	0.52	1.29	0.18	0.45	1.15	0.41	1.06	2.71	0.45	1.18	3.07	0.25	0.61	1.50
A6c2	0.22	0.60	1.60	0.18	0.50	1.40	0.45	1.25	3.46	0.38	1.07	3.03	0.25	0.66	1.75
A6c3	0.20	0.50	1.25	0.17	0.43	1.11	0.45	1.15	2.95	0.38	0.98	2.56	0.27	0.65	1.62
Med6d	0.20	0.57	1.69	0.14	0.43	1.32	0.36	1.11	3.42	0.37	1.17	3.67	0.22	0.66	1.92
IprG4c1	0.73	1.14	1.77	1.08	1.70	2.69	0.55	0.87	1.38	0.59	0.94	1.50	0.72	1.11	1.72
IprG4c2	0.82	1.12	1.52	0.82	1.13	1.56	0.72	0.99	1.37	0.88	1.22	1.68	0.72	0.98	1.33
IprG4c3	0.63	0.97	1.50	0.67	1.05	1.64	0.65	1.03	1.61	0.59	0.94	1.48	0.68	1.04	1.60
IprNG2c1	0.76	1.11	1.61	0.94	1.38	2.04	0.63	0.93	1.38	0.76	1.13	1.67	0.88	1.27	1.84
IprNG2c2	0.94	1.31	1.83	0.85	1.21	1.70	0.65	0.92	1.30	0.81	1.15	1.63	0.82	1.14	1.59

	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
IprNG2c3	0.69	0.98	1.39	0.62	0.90	1.29	0.67	0.96	1.39	0.76	1.10	1.59	0.78	1.10	1.57
IprA6c1	0.63	0.91	1.31	0.72	1.05	1.53	0.65	0.95	1.38	0.69	1.01	1.48	0.65	0.93	1.33
IprA6c2	0.72	1.05	1.53	0.79	1.18	1.75	0.75	1.12	1.66	0.61	0.91	1.37	0.69	1.00	1.46
IprA6c3	0.61	0.88	1.27	0.69	1.01	1.49	0.70	1.03	1.52	0.57	0.84	1.24	0.69	1.00	1.45
CellPhenotype	Con50 vs Con33			NK50- vs NK33-			NK50+ vs NK33+			NK33- vs Con33			NK33+ vs Con33		
Males	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
sp3	0.78	0.98	1.24	0.80	1.01	1.29	0.92	1.17	1.49	0.79	1.00	1.28	0.72	0.90	1.13
sp3-4	0.68	0.91	1.22	0.77	1.04	1.41	0.91	1.24	1.68	0.68	0.92	1.25	0.61	0.82	1.10
sp3-8	0.55	0.80	1.15	0.61	0.90	1.31	0.83	1.21	1.77	0.65	0.96	1.41	0.63	0.91	1.31
sp3-45	0.93	1.11	1.33	0.95	1.14	1.37	0.82	0.98	1.18	0.83	1.00	1.21	0.91	1.09	1.30
sp3-161	0.90	1.09	1.33	0.87	1.07	1.31	0.74	0.91	1.12	0.78	0.96	1.18	0.89	1.09	1.32
ln3	0.84	0.98	1.15	0.91	1.08	1.27	0.85	1.01	1.19	0.79	0.94	1.11	0.83	0.97	1.14
ln3-4	0.81	0.95	1.12	0.97	1.14	1.35	0.85	1.01	1.20	0.75	0.89	1.06	0.81	0.95	1.12
ln3-8	0.76	0.92	1.12	0.71	0.87	1.06	0.83	1.02	1.24	0.89	1.09	1.34	0.80	0.97	1.17
ln3-45	0.72	0.95	1.26	0.70	0.94	1.26	0.75	1.01	1.35	0.74	1.00	1.35	0.71	0.94	1.24
ty3	0.97	1.16	1.39	0.82	0.99	1.20	0.83	1.00	1.21	0.93	1.13	1.37	0.89	1.07	1.28
ty3-4	0.98	1.15	1.35	0.84	0.99	1.17	0.82	0.97	1.15	0.95	1.13	1.34	0.92	1.08	1.26
ty3-8	0.97	1.20	1.49	0.83	1.04	1.29	0.73	0.91	1.14	0.86	1.07	1.34	0.93	1.15	1.43
bm3	0.32	**0.52**	0.83	0.56	0.92	1.52	0.36	**0.59**	0.97	0.36	**0.60**	0.99	0.50	0.81	1.30
bm3-45	0.88	0.96	1.05	0.90	0.98	1.08	1.01	**1.11**	1.22	0.93	1.02	1.13	0.86	0.94	1.03

Table 13 **95% Confidence interval plus estimate for the ratio Δ of the GMO feeds with 50% maize inclusion rate versus the corresponding non-GM control feed for females. Ratios with corresponding 95/99% intervals that do not encompass the value 1 are coloured red/gold; this is equivalent to a significant difference according to a 5/1% t-test.**

Weights	NK11-/50 vs Con50			NK50- vs Con50			NK11+/50 vs Con50			NK50+ vs Con50		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
BodyWeight	0.95	1.01	1.07	0.95	1.01	1.07	1.00	**1.06**	1.12	0.94	1.00	1.06
growthRate	1.00	1.03	1.06	1.00	**1.03**	1.06	0.98	1.01	1.04	0.98	1.01	1.04
FeedMean	0.96	1.02	1.08	0.96	1.02	1.08	1.05	**1.11**	1.18	0.98	1.04	1.10
Haematology	NK11-/50 vs Con50			NK50- vs Con50			NK11+/50 vs Con50			NK50+ vs Con50		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
WBC	0.90	1.09	1.32	0.83	1.01	1.22	0.88	1.07	1.29	0.81	0.98	1.18
RBC	0.98	1.01	1.03	0.99	1.02	1.04	0.98	1.01	1.03	0.98	1.01	1.03
HGB	0.97	1.00	1.03	0.98	1.01	1.04	0.99	1.02	1.05	0.97	1.00	1.03
HCT	0.97	1.00	1.02	0.98	1.01	1.04	0.99	1.01	1.04	0.97	1.00	1.03
MCV	0.97	0.99	1.00	0.98	0.99	1.01	0.99	1.01	1.02	0.98	0.99	1.01
MCH	0.97	0.99	1.01	0.97	0.99	1.01	0.99	1.01	1.03	0.97	0.99	1.01
MCHC	0.99	1.00	1.02	0.98	1.00	1.01	0.99	1.01	1.02	0.99	1.00	1.01
PLT	0.94	1.02	1.11	0.87	0.94	1.02	0.85	0.93	1.01	0.89	0.96	1.04
LYMR	0.93	0.98	1.04	0.95	1.00	1.06	0.92	0.98	1.03	0.95	1.00	1.06
LYMA	0.87	1.07	1.32	0.82	1.01	1.25	0.85	1.05	1.29	0.79	0.98	1.20
ClinChem	NK11-/50 vs Con50			NK50- vs Con50			NK11+/50 vs Con50			NK50+ vs Con50		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
ALP	0.74	0.91	1.11	0.81	0.98	1.20	0.78	0.95	1.16	0.86	1.05	1.28
ALT	0.82	0.99	1.19	0.85	1.02	1.23	0.83	1.00	1.21	0.85	1.02	1.23
AST	0.86	1.05	1.30	0.89	1.10	1.35	0.83	1.02	1.26	0.83	1.03	1.26

	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
BIL	0.91	1.23	1.64	0.80	1.08	1.44	0.93	1.24	1.67	0.84	1.13	1.52
ALB	0.93	0.97	1.01	0.97	1.01	1.06	0.94	0.98	1.02	0.93	0.97	1.01
TP	0.95	0.99	1.03	0.98	1.02	1.06	0.95	0.99	1.03	0.95	0.99	1.03
Glu	0.81	0.93	1.08	0.83	0.96	1.12	0.86	1.00	1.16	0.88	1.02	1.18
CHOL	0.79	0.95	1.14	0.81	0.98	1.18	0.78	0.93	1.13	0.68	0.82	0.99
TAG	0.78	0.95	1.17	0.75	0.92	1.13	0.76	0.93	1.14	0.66	0.81	0.99
Crea	0.94	1.04	1.15	0.94	1.03	1.14	0.93	1.03	1.14	0.84	0.93	1.03
Urea	0.98	1.08	1.20	0.89	0.99	1.09	0.97	1.07	1.19	0.89	0.98	1.09
cHGB	0.73	1.09	1.61	0.70	1.04	1.53	0.74	1.10	1.62	0.74	1.09	1.62
Ca	0.97	0.99	1.00	0.99	1.00	1.02	0.97	0.99	1.00	0.97	0.98	1.00
Cl	0.98	0.99	1.01	0.98	1.00	1.01	0.98	0.99	1.01	0.98	0.99	1.01
K	0.93	1.02	1.12	0.89	0.98	1.07	0.90	0.99	1.08	0.90	0.98	1.08
Na	0.99	1.00	1.00	0.99	1.00	1.01	0.99	1.00	1.01	0.99	1.00	1.00
P	0.84	1.00	1.19	0.86	1.03	1.22	0.86	1.02	1.22	0.88	1.05	1.25
Urine	**NK11-/50 vs Con50**			**NK50- vs Con50**			**NK11+/50 vs Con50**			**NK50+ vs Con50**		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
uVol	0.79	1.04	1.36	0.65	0.85	1.11	0.63	0.83	1.09	0.74	0.97	1.28
uVolW	0.77	1.03	1.39	0.63	0.85	1.14	0.58	0.78	1.05	0.73	0.98	1.32
uLeu	0.78	1.15	1.70	0.89	1.32	1.95	0.72	1.07	1.58	0.91	1.35	2.00
uOsmoll	0.80	1.06	1.40	0.89	1.18	1.56	0.96	1.27	1.68	0.75	0.99	1.30
uKeton	0.66	0.89	1.20	0.78	1.05	1.42	0.69	0.93	1.26	0.70	0.95	1.28
upH	0.76	1.00	1.31	0.76	1.00	1.31	0.84	1.10	1.44	0.86	1.13	1.49
Organs	**NK11-/50 vs Con50**			**NK50- vs Con50**			**NK11+/50 vs Con50**			**NK50+ vs Con50**		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
Kidney	0.94	1.01	1.09	0.96	1.03	1.11	0.96	1.03	1.11	1.01	1.08	1.16
Spleen	0.93	1.02	1.12	0.98	1.08	1.19	0.94	1.04	1.14	0.95	1.05	1.15
Liver	0.95	1.00	1.06	0.97	1.02	1.08	0.93	0.98	1.03	0.94	0.99	1.04
AdrenGl	0.89	0.98	1.07	0.96	1.05	1.15	0.95	1.04	1.14	0.93	1.01	1.11
Heart	0.93	0.98	1.04	0.98	1.04	1.10	0.97	1.03	1.09	1.00	1.06	1.12
Thymus	0.89	1.00	1.12	0.87	0.98	1.10	0.77	0.87	0.98	0.82	0.93	1.04
Uterus	0.92	1.14	1.42	0.86	1.07	1.33	0.79	0.98	1.21	0.82	1.02	1.26
Ovary	0.89	0.99	1.10	0.89	1.00	1.11	0.90	1.00	1.11	1.03	1.15	1.28
Brain	0.94	1.00	1.06	0.94	1.00	1.07	0.91	0.96	1.02	0.95	1.01	1.08
Immunology	**NK11-/50 vs Con50**			**NK50- vs Con50**			**NK11+/50 vs Con50**			**NK50+ vs Con50**		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
Monocytes	0.87	0.99	1.13	0.85	0.97	1.09	0.86	0.98	1.11	0.78	0.89	1.02
Granulocytes	0.91	0.96	1.02	0.89	0.94	0.99	0.91	0.96	1.01	0.88	0.93	0.98
RespirBurst	0.89	0.96	1.03	0.87	0.94	1.01	0.89	0.96	1.03	0.86	0.93	1.00
Con	0.34	0.76	1.72	0.53	1.16	2.54	0.67	1.52	3.43	0.35	0.81	1.87
PHA	0.63	1.42	3.21	0.86	1.89	4.12	0.90	2.04	4.62	0.48	1.09	2.52
PWM	0.59	1.07	1.94	0.63	1.11	1.97	0.78	1.41	2.56	0.43	0.80	1.46
Med3d	0.42	0.74	1.29	0.74	1.26	2.14	0.78	1.36	2.37	0.35	0.62	1.10
IprConA	0.63	1.03	1.68	0.58	0.93	1.48	0.68	1.12	1.83	0.79	1.30	2.16
IprPHA	0.97	1.93	3.81	0.78	1.50	2.88	0.76	1.50	2.98	0.87	1.75	3.52
IprPWM	1.03	1.45	2.06	0.63	0.88	1.23	0.73	1.04	1.47	0.90	1.28	1.82
G4c1	0.54	0.90	1.50	0.64	1.04	1.70	1.15	1.92	3.21	0.63	1.06	1.78
G4c2	0.54	0.92	1.55	0.64	1.06	1.75	0.93	1.57	2.66	0.59	1.02	1.74
G4c3	0.63	1.06	1.76	0.66	1.07	1.75	0.80	1.34	2.24	0.55	0.92	1.55
NG2c1	0.63	1.03	1.69	0.63	1.02	1.64	1.05	1.73	2.84	0.62	1.03	1.72
NG2c2	0.57	1.00	1.77	0.53	0.92	1.59	0.85	1.51	2.66	0.56	1.00	1.79
NG2c3	0.62	1.05	1.79	0.60	0.99	1.65	0.77	1.31	2.23	0.55	0.94	1.62
A6c1	0.70	1.12	1.79	0.71	1.11	1.74	1.02	1.62	2.59	0.51	0.81	1.31
A6c2	0.86	1.45	2.46	0.78	1.29	2.14	0.95	1.61	2.72	0.65	1.10	1.89

	NK11-/50 vs Con50			NK50- vs Con50			NK11+/50 vs Con50			NK50+ vs Con50		
	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
A6c3	0.58	1.00	1.72	0.61	1.02	1.73	0.80	1.38	2.39	0.49	0.86	1.50
Med6d	0.54	0.94	1.64	0.57	0.97	1.65	0.73	1.28	2.23	0.43	0.75	1.33
IprG4c1	0.62	0.96	1.47	0.71	1.07	1.62	0.98	1.51	2.32	0.90	1.40	2.18
IprG4c2	0.66	0.97	1.43	0.76	1.09	1.58	0.84	1.23	1.81	0.91	1.35	2.00
IprG4c3	0.87	1.12	1.44	0.87	1.10	1.40	0.82	1.05	1.35	0.95	1.22	1.58
IprNG2c1	0.76	1.09	1.57	0.74	1.05	1.49	0.94	1.36	1.95	0.95	1.37	1.99
IprNG2c2	0.77	1.06	1.46	0.70	0.95	1.29	0.85	1.18	1.63	0.96	1.33	1.85
IprNG2c3	0.85	1.12	1.47	0.79	1.02	1.33	0.78	1.03	1.35	0.95	1.25	1.65
IprA6c1	0.93	1.19	1.52	0.91	1.15	1.45	1.00	1.27	1.62	0.84	1.08	1.38
IprA6c2	1.19	1.54	2.00	1.04	1.33	1.71	0.97	1.26	1.63	1.13	1.47	1.91
IprA6c3	0.77	1.06	1.45	0.78	1.06	1.43	0.79	1.08	1.49	0.83	1.15	1.58
CellPhenotype	**NK11-/50 vs Con50**			**NK50- vs Con50**			**NK11+/50 vs Con50**			**NK50+ vs Con50**		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
sp3	0.83	0.97	1.13	0.81	0.94	1.09	0.83	0.97	1.14	0.82	0.96	1.12
sp3-4	0.89	1.03	1.18	0.87	0.99	1.14	0.81	0.94	1.08	0.85	0.98	1.13
sp3-8	0.89	1.08	1.31	0.87	1.04	1.25	0.90	1.09	1.32	0.79	0.96	1.16
sp3-45	0.77	0.88	1.01	0.78	0.89	1.02	0.82	0.94	1.07	0.80	0.92	1.06
sp3-161	0.84	0.95	1.09	0.77	0.87	0.99	0.96	1.10	1.26	0.83	0.94	1.08
ln3	0.86	0.98	1.12	0.87	0.99	1.12	0.82	0.94	1.07	0.96	1.09	1.25
ln3-4	0.88	0.99	1.12	0.89	1.00	1.12	0.83	0.93	1.05	1.02	1.15	1.30
ln3-8	0.78	0.94	1.14	0.85	1.02	1.22	0.84	1.02	1.23	0.78	0.94	1.14
ln3-45	0.70	0.92	1.21	0.69	0.89	1.16	0.86	1.13	1.48	0.56	0.74	0.97
ty3	0.96	1.08	1.22	0.91	1.02	1.14	0.92	1.04	1.17	0.92	1.05	1.18
ty3-4	0.99	1.10	1.23	0.91	1.01	1.12	0.95	1.06	1.18	0.93	1.04	1.16
ty3-8	0.76	0.89	1.05	0.82	0.96	1.12	0.96	1.13	1.33	0.83	0.98	1.16
bm3	0.73	1.12	1.69	0.71	1.06	1.58	0.71	1.07	1.63	0.77	1.18	1.80
bm3-45	0.93	1.04	1.16	0.91	1.01	1.13	0.90	1.00	1.12	0.83	0.93	1.04

Table 14 95% Confidence interval plus estimate for the ratio Δ of the feeds with 50% maize inclusion rate versus the corresponding 33% inclusion rate for females, and for the GM 33% feeds versus the non-GM 33% feed. Ratios with corresponding 95/99% intervals that do not encompass the value 1 are coloured red/gold; this is equivalent to a significant difference according to a 5/1% t-test.

Weights	Con50 vs Con33			NK50- vs NK33-			NK50+ vs NK33+			NK33- vs Con33			NK33+ vs Con33		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
BodyWeight	0.91	0.96	1.02	0.92	0.98	1.04	0.93	0.99	1.04	0.94	1.00	1.06	0.92	0.98	1.04
growthRate	0.95	0.98	1.01	0.99	1.02	1.05	0.96	0.99	1.02	0.96	0.99	1.02	0.97	1.00	1.03
FeedMean	0.91	0.96	1.02	0.91	0.97	1.02	0.96	1.01	1.07	0.95	1.01	1.07	0.93	0.98	1.04
Haematology	Con50 vs Con33			NK50- vs NK33-			NK50+ vs NK33+			NK33- vs Con33			NK33+ vs Con33		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
WBC	0.82	0.99	1.19	0.80	0.97	1.18	0.83	1.00	1.21	0.84	1.02	1.23	0.79	0.96	1.16
RBC	0.98	1.00	1.02	0.99	1.01	1.04	0.96	0.98	1.01	0.98	1.01	1.03	1.00	1.03	1.05
HGB	0.98	1.01	1.03	0.99	1.02	1.05	0.97	0.99	1.02	0.97	0.99	1.02	0.98	1.01	1.04
HCT	0.98	1.00	1.03	0.98	1.01	1.04	0.97	0.99	1.02	0.98	1.00	1.03	0.98	1.01	1.03
MCV	0.99	1.00	1.02	0.98	1.00	1.01	0.99	1.01	1.03	0.98	1.00	1.01	0.97	0.98	1.00
MCH	0.99	1.01	1.03	0.98	1.00	1.02	0.99	1.01	1.03	0.98	1.00	1.02	0.96	0.98	1.00
MCHC	0.99	1.00	1.02	0.98	1.00	1.01	0.99	1.00	1.02	0.99	1.00	1.02	0.99	1.00	1.02
PLT	0.93	1.00	1.09	0.89	0.97	1.05	0.84	0.92	1.00	0.90	0.98	1.06	0.97	1.05	1.15

	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
LYMR	0.97	1.02	1.08	0.95	1.00	1.06	0.97	1.02	1.08	0.96	1.02	1.07	0.95	1.00	1.06
LYMA	0.81	1.00	1.23	0.79	0.98	1.21	0.83	1.02	1.26	0.84	1.04	1.28	0.78	0.96	1.18

ClinChem	Con50 vs Con33			NK50- vs NK33-			NK50+ vs NK33+			NK33- vs Con33			NK33+ vs Con33		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
ALP	0.82	1.00	1.23	0.73	0.89	1.08	0.92	1.12	1.37	0.91	1.11	1.36	0.77	0.94	1.15
ALT	0.80	0.97	1.16	0.87	1.04	1.26	1.03	1.24	1.50	0.79	0.95	1.14	0.66	0.79	0.96
AST	0.75	0.92	1.13	0.89	1.10	1.35	0.75	0.93	1.14	0.75	0.92	1.13	0.83	1.02	1.25
BIL	0.70	0.94	1.27	0.69	0.93	1.25	0.64	0.86	1.15	0.81	1.09	1.47	0.93	1.24	1.67
ALB	0.95	0.99	1.04	0.93	0.97	1.01	0.91	0.96	1.00	0.99	1.04	1.09	0.96	1.01	1.05
TP	0.94	0.98	1.02	0.94	0.98	1.02	0.94	0.98	1.02	0.98	1.02	1.07	0.96	1.00	1.04
Glu	0.92	1.07	1.24	0.84	0.97	1.13	0.92	1.06	1.24	0.91	1.06	1.23	0.88	1.02	1.19
CHOL	0.75	0.90	1.09	0.68	0.82	0.99	0.68	0.82	0.99	0.89	1.07	1.29	0.75	0.90	1.09
TAG	0.77	0.94	1.15	0.70	0.86	1.05	0.67	0.81	1.00	0.82	1.01	1.23	0.76	0.94	1.15
Crea	0.94	1.03	1.14	0.97	1.07	1.18	0.82	0.91	1.00	0.91	1.00	1.10	0.96	1.06	1.17
Urea	0.98	1.08	1.19	0.89	0.99	1.09	0.90	0.99	1.10	0.98	1.08	1.19	0.97	1.07	1.19
cHGB	0.72	1.06	1.57	0.79	1.16	1.72	0.67	1.01	1.52	0.64	0.94	1.40	0.76	1.14	1.72
Ca	0.98	1.00	1.02	0.97	0.99	1.01	0.99	1.00	1.02	1.00	1.01	1.03	0.96	0.98	1.00
Cl	1.01	1.02	1.04	0.99	1.00	1.02	0.99	1.01	1.02	1.00	1.02	1.03	0.99	1.01	1.02
K	0.93	1.02	1.12	0.89	0.97	1.06	0.88	0.97	1.06	0.94	1.03	1.13	0.95	1.04	1.14
Na	0.99	1.00	1.01	0.99	1.00	1.01	0.99	1.00	1.01	1.00	1.01	1.01	0.99	1.00	1.01
P	0.85	1.01	1.20	0.76	0.90	1.07	0.76	0.91	1.08	0.96	1.15	1.36	0.98	1.17	1.39

Urine	Con50 vs Con33			NK50- vs NK33-			NK50+ vs NK33+			NK33- vs Con33			NK33+ vs Con33		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
uVol	0.98	1.28	1.68	0.56	0.73	0.96	0.75	0.99	1.29	1.13	1.49	1.95	0.97	1.27	1.66
uVolW	0.99	1.33	1.79	0.56	0.76	1.02	0.75	1.00	1.35	1.11	1.49	2.00	0.96	1.30	1.74
uLeu	0.59	0.87	1.29	0.83	1.23	1.82	0.91	1.35	2.00	0.63	0.93	1.38	0.59	0.87	1.29
uOsmoll	0.60	0.80	1.05	0.95	1.25	1.66	0.75	0.99	1.30	0.57	0.75	0.99	0.60	0.80	1.05
uKeton	0.84	1.13	1.53	0.82	1.11	1.50	0.85	1.15	1.55	0.79	1.07	1.45	0.69	0.93	1.26
upH	0.69	0.91	1.20	0.54	0.71	0.93	0.63	0.83	1.09	0.98	1.28	1.69	0.95	1.24	1.63

Organs	Con50 vs Con33			NK50- vs NK33-			NK50+ vs NK33+			NK33- vs Con33			NK33+ vs Con33		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
Kidney	0.94	1.01	1.08	0.97	1.04	1.12	0.99	1.07	1.14	0.93	1.00	1.07	0.95	1.02	1.09
Spleen	0.91	1.00	1.11	0.95	1.04	1.15	0.98	1.08	1.18	0.95	1.04	1.15	0.89	0.98	1.08
Liver	0.93	0.98	1.04	0.93	0.98	1.03	0.96	1.01	1.06	0.98	1.03	1.09	0.92	0.97	1.02
AdrenGl	0.88	0.97	1.06	0.97	1.06	1.16	0.91	1.00	1.09	0.87	0.95	1.04	0.89	0.98	1.07
Heart	0.92	0.97	1.03	0.98	1.04	1.10	1.01	1.07	1.13	0.91	0.97	1.02	0.90	0.96	1.01
Thymus	0.91	1.02	1.15	0.93	1.05	1.18	0.87	0.98	1.10	0.85	0.95	1.07	0.86	0.97	1.09
Uterus	0.80	1.00	1.24	0.82	1.02	1.26	0.80	0.99	1.23	0.84	1.05	1.30	0.82	1.02	1.27
Ovary	0.88	0.98	1.09	0.97	1.07	1.19	1.08	1.20	1.33	0.82	0.91	1.01	0.84	0.94	1.04
Brain	1.00	1.06	1.12	0.97	1.03	1.10	0.98	1.04	1.11	0.97	1.03	1.09	0.97	1.03	1.10

Immunology	Con50 vs Con33			NK33- vs Con33			NK33+ vs Con33			NK50- vs NK33-			NK50+ vs NK33+		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
Monocytes	0.86	0.98	1.11	0.83	0.95	1.08	0.80	0.91	1.04	0.87	0.99	1.14	0.85	0.96	1.09
Granulocytes	0.96	1.01	1.06	0.92	0.97	1.03	0.91	0.97	1.02	0.92	0.98	1.03	0.93	0.97	1.03
RespirBurst	0.97	1.04	1.11	0.93	1.00	1.08	0.95	1.02	1.10	0.90	0.97	1.05	0.88	0.94	1.01
Con	0.38	0.84	1.84	0.71	1.60	3.60	0.50	1.15	2.63	0.27	0.61	1.41	0.27	0.60	1.31

	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
PHA	0.26	0.56	1.23	0.80	1.81	4.07	0.57	1.32	3.04	0.25	0.58	1.34	0.21	0.46	1.01
PWM	0.47	0.83	1.47	0.73	1.31	2.38	0.58	1.07	1.96	0.38	0.70	1.29	0.35	0.62	1.09
Med3d	0.76	1.30	2.22	0.97	1.69	2.95	0.52	0.92	1.62	0.55	0.96	1.70	0.52	0.88	1.51
IprConA	0.40	0.65	1.04	0.58	0.95	1.55	0.76	1.25	2.07	0.38	0.64	1.05	0.42	0.68	1.08
IprPHA	0.22	0.43	0.83	0.54	1.07	2.11	0.72	1.44	2.90	0.30	0.61	1.22	0.27	0.52	1.01
IprPWM	0.46	0.64	0.89	0.55	0.78	1.10	0.82	1.17	1.67	0.51	0.73	1.04	0.50	0.70	0.98
G4c1	0.68	1.12	1.82	0.76	1.26	2.10	0.68	1.14	1.92	0.55	0.92	1.55	0.63	1.03	1.68
G4c2	0.68	1.12	1.87	0.68	1.15	1.94	0.65	1.12	1.92	0.61	1.04	1.77	0.62	1.02	1.69
G4c3	0.66	1.08	1.76	0.69	1.14	1.90	0.56	0.94	1.59	0.60	1.01	1.70	0.64	1.05	1.72
NG2c1	0.64	1.03	1.66	0.68	1.11	1.82	0.66	1.10	1.83	0.57	0.94	1.57	0.60	0.96	1.55
NG2c2	0.66	1.15	1.98	0.54	0.95	1.68	0.58	1.04	1.86	0.62	1.11	1.98	0.64	1.10	1.91
NG2c3	0.67	1.11	1.85	0.56	0.96	1.63	0.53	0.91	1.57	0.67	1.15	1.98	0.69	1.15	1.92
A6c1	0.68	1.07	1.67	0.60	0.96	1.53	0.43	0.69	1.11	0.77	1.23	1.99	0.80	1.26	1.97
A6c2	0.62	1.03	1.70	0.54	0.92	1.55	0.48	0.82	1.40	0.84	1.44	2.47	0.84	1.39	2.31
A6c3	0.71	1.20	2.02	0.56	0.97	1.67	0.52	0.91	1.59	0.72	1.26	2.20	0.67	1.13	1.91
Med6d	0.70	1.20	2.05	0.60	1.05	1.83	0.48	0.85	1.50	0.63	1.11	1.96	0.63	1.07	1.82
IprG4c1	0.61	0.93	1.40	0.78	1.20	1.85	0.87	1.35	2.09	0.53	0.83	1.29	0.64	0.97	1.46
IprG4c2	0.65	0.93	1.35	0.74	1.09	1.60	0.89	1.32	1.96	0.63	0.93	1.38	0.66	0.96	1.38
IprG4c3	0.70	0.89	1.14	0.85	1.09	1.39	0.86	1.11	1.44	0.70	0.91	1.17	0.77	0.98	1.25
IprNG2c1	0.60	0.85	1.21	0.74	1.06	1.52	0.90	1.30	1.88	0.59	0.85	1.23	0.64	0.90	1.28
IprNG2c2	0.70	0.95	1.30	0.66	0.91	1.25	0.88	1.23	1.71	0.72	1.00	1.39	0.76	1.03	1.41
IprNG2c3	0.71	0.92	1.20	0.70	0.91	1.20	0.81	1.07	1.42	0.78	1.03	1.37	0.83	1.08	1.40
IprA6c1	0.70	0.89	1.12	0.72	0.91	1.16	0.63	0.81	1.04	0.87	1.11	1.43	0.93	1.18	1.49
IprA6c2	0.67	0.85	1.09	0.68	0.88	1.13	0.74	0.96	1.25	1.00	1.30	1.69	1.02	1.30	1.67
IprA6c3	0.74	1.00	1.35	0.68	0.92	1.26	0.78	1.08	1.48	0.83	1.14	1.57	0.78	1.06	1.44
CellPhenotype	**Con50 vs Con33**			**NK50- vs NK33-**			**NK50+ vs NK33+**			**NK33- vs Con33**			**NK33+ vs Con33**		
Females	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp	low	ratio	upp
sp3	0.91	1.06	1.23	0.98	1.15	1.34	0.88	1.03	1.21	0.74	0.87	1.02	0.85	0.99	1.15
sp3-4	0.85	0.97	1.11	0.96	1.11	1.27	0.88	1.02	1.18	0.76	0.87	1.01	0.81	0.93	1.07
sp3-8	0.78	0.94	1.14	0.91	1.10	1.33	0.83	1.01	1.23	0.74	0.90	1.09	0.74	0.89	1.07
sp3-45	0.99	1.13	1.29	0.86	0.99	1.13	0.90	1.04	1.19	0.89	1.02	1.17	0.89	1.01	1.15
sp3-161	0.89	1.01	1.15	0.77	0.88	1.00	0.85	0.97	1.11	0.88	1.01	1.15	0.87	0.98	1.12
ln3	0.86	0.98	1.11	0.92	1.04	1.19	0.99	1.13	1.29	0.81	0.92	1.06	0.83	0.95	1.07
ln3-4	0.87	0.97	1.09	0.90	1.01	1.14	1.06	1.20	1.35	0.85	0.96	1.08	0.83	0.93	1.04
ln3-8	0.81	0.97	1.17	0.97	1.17	1.41	0.80	0.97	1.17	0.70	0.85	1.02	0.79	0.95	1.14
ln3-45	1.01	1.31	1.70	0.65	0.85	1.12	0.50	0.66	0.87	1.04	1.37	1.82	1.13	1.47	1.91
ty3	0.79	0.89	1.00	0.81	0.91	1.02	0.85	0.96	1.08	0.89	1.00	1.13	0.87	0.97	1.09
ty3-4	0.81	0.90	1.00	0.84	0.94	1.05	0.84	0.94	1.05	0.86	0.96	1.08	0.89	0.99	1.10
ty3-8	0.89	1.04	1.21	0.75	0.88	1.03	0.85	1.00	1.18	0.96	1.13	1.33	0.87	1.02	1.19
bm3	0.53	0.79	1.19	0.89	1.35	2.05	0.58	0.88	1.35	0.40	0.62	0.95	0.71	1.06	1.58
bm3-45	0.96	1.07	1.19	0.88	0.98	1.10	0.91	1.01	1.13	0.83	0.93	1.04	0.95	1.06	1.18
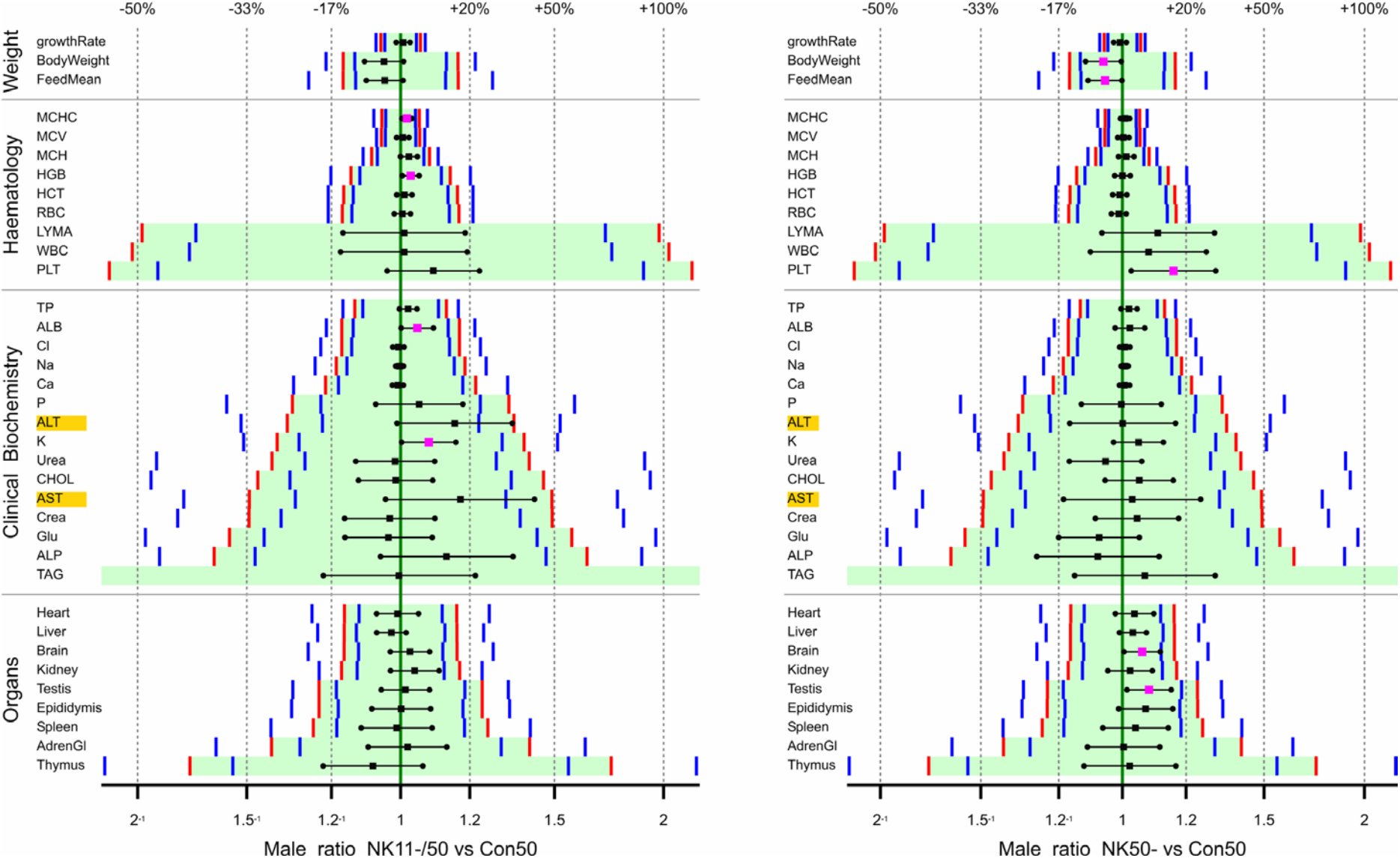
Figure 10 Confidence intervals for the ratio of NK11-/50 and NK50- vs the Con50 feed for males with intervals for equivalence limits (see text).
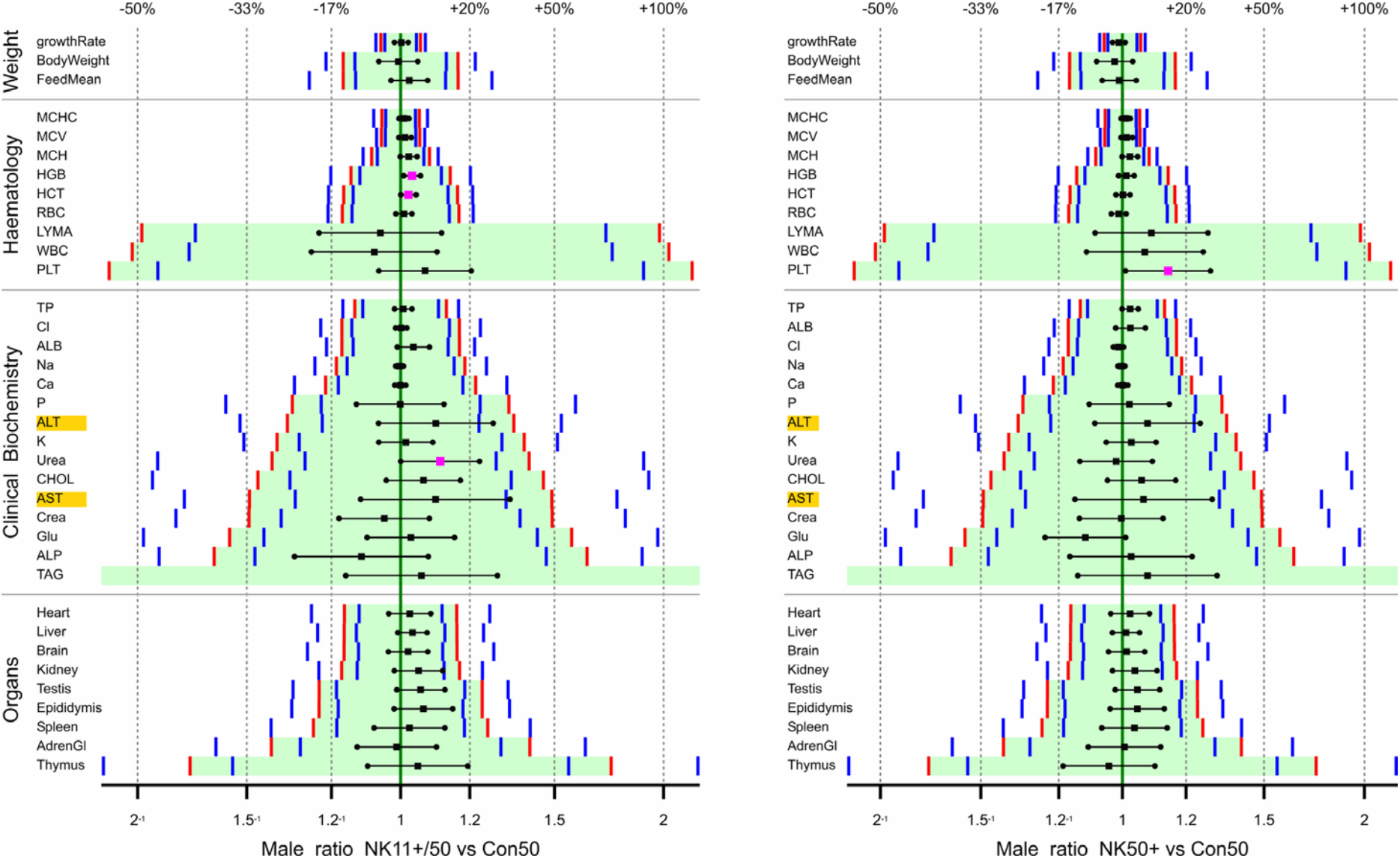
Figure 11 Confidence intervals for the ratio of NK11+/50 and NK50+ vs the Con50 feed for males with intervals for equivalence limits (see text).
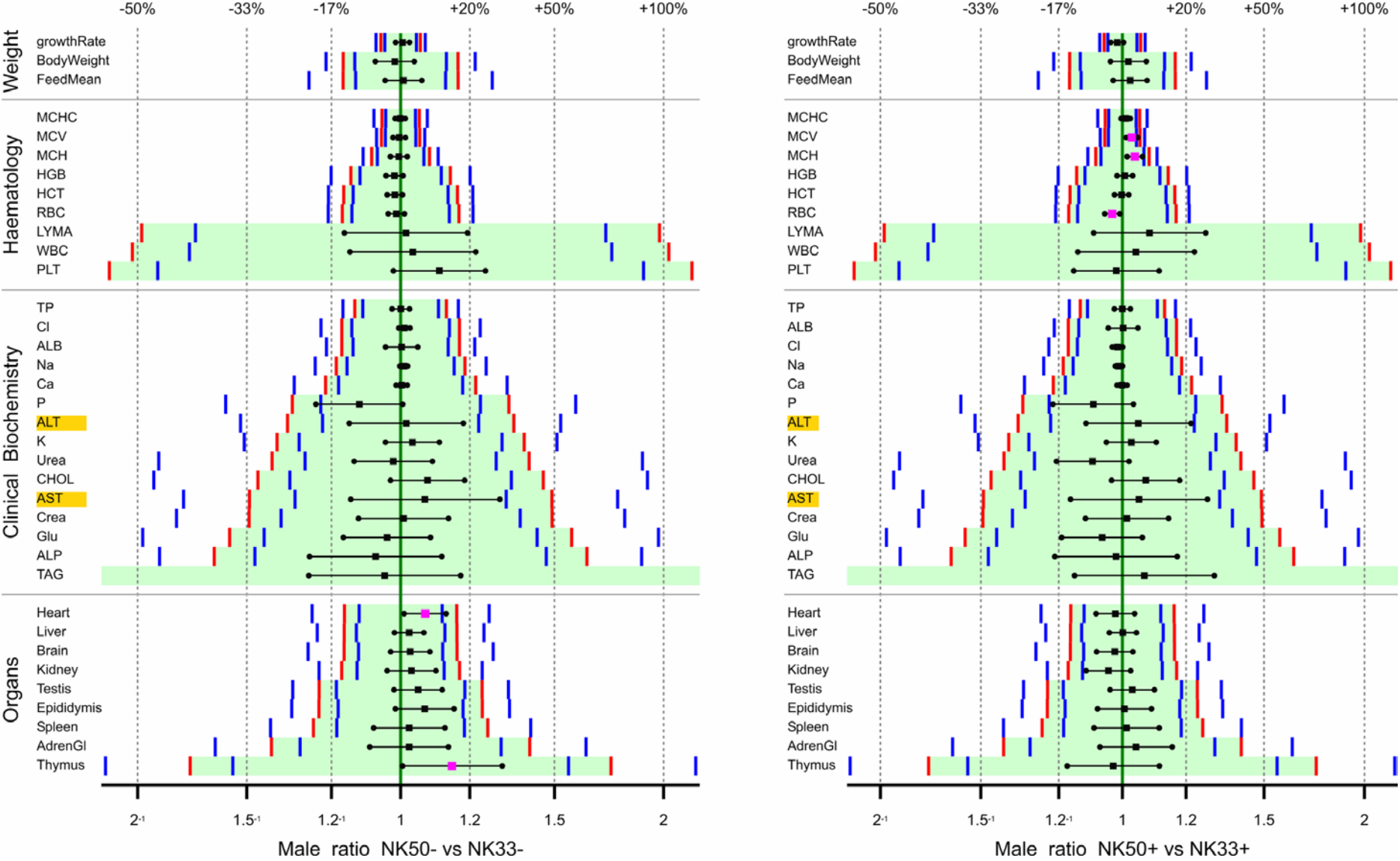
Figure 12 Confidence intervals for the ratio of NK50- and NK50+ vs the corresponding 33% feed for males with intervals for equivalence limits (see text).
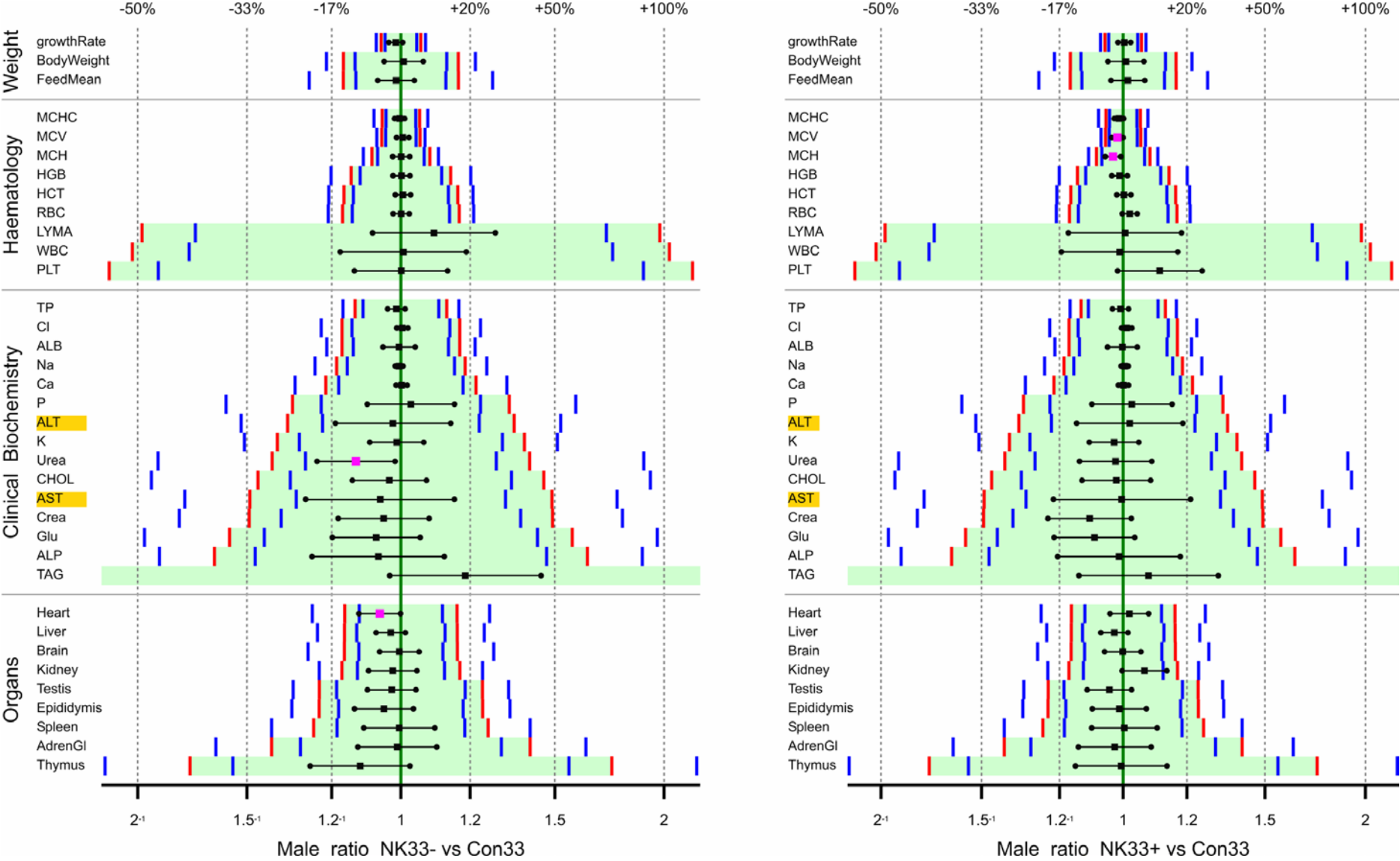
Figure 13 Confidence intervals for the ratio of NK33- and NK33+ vs the Con33 feed for males with intervals for equivalence limits (see text).
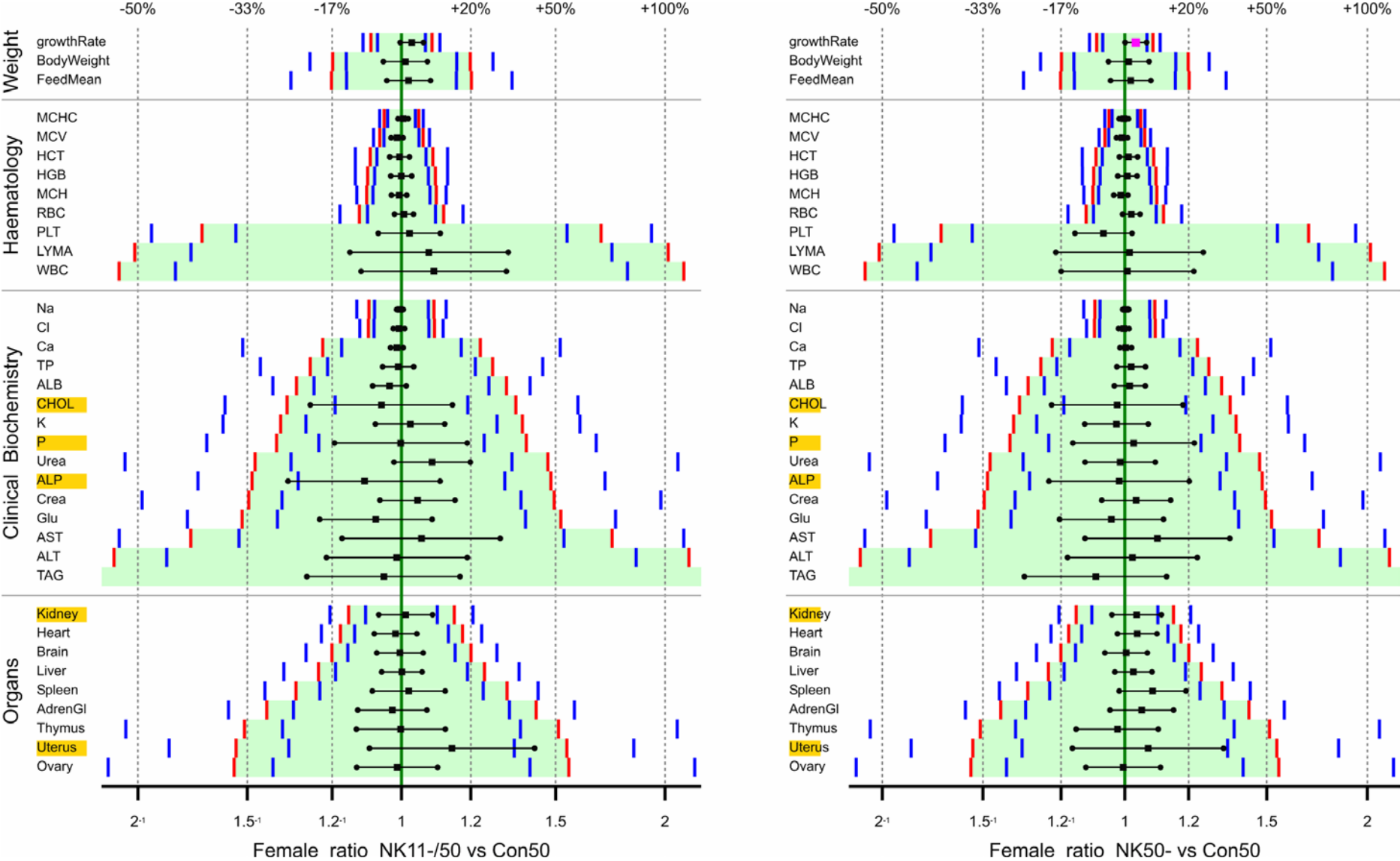
Figure 14 Confidence intervals for the ratio of NK11-/50 and NK50- vs the Con50 feed for females with intervals for equivalence limits (see text).
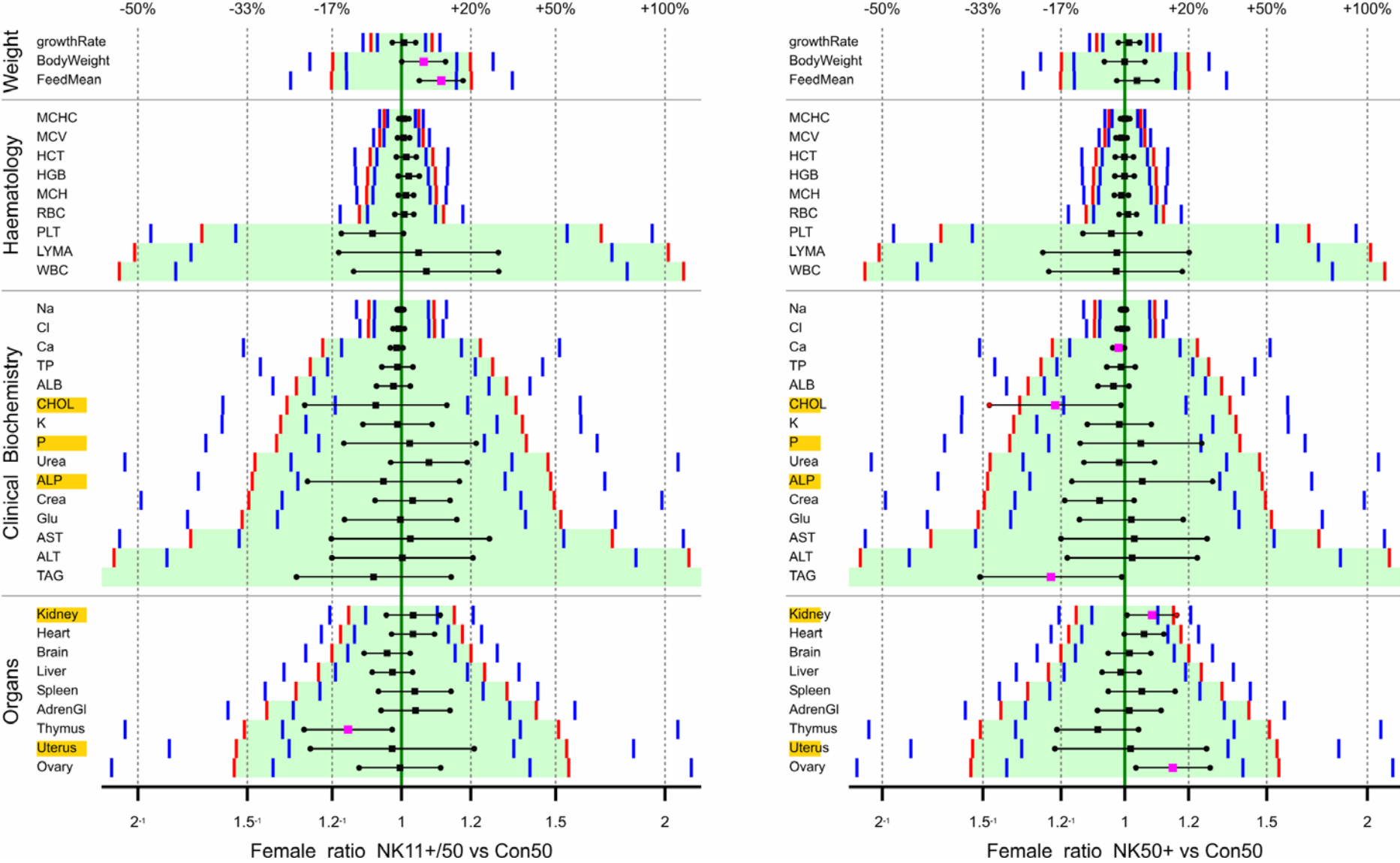
Figure 15 Confidence intervals for the ratio of NK11+/50 and NK50+ vs the Con50 feed for females with intervals for equivalence limits (see text).
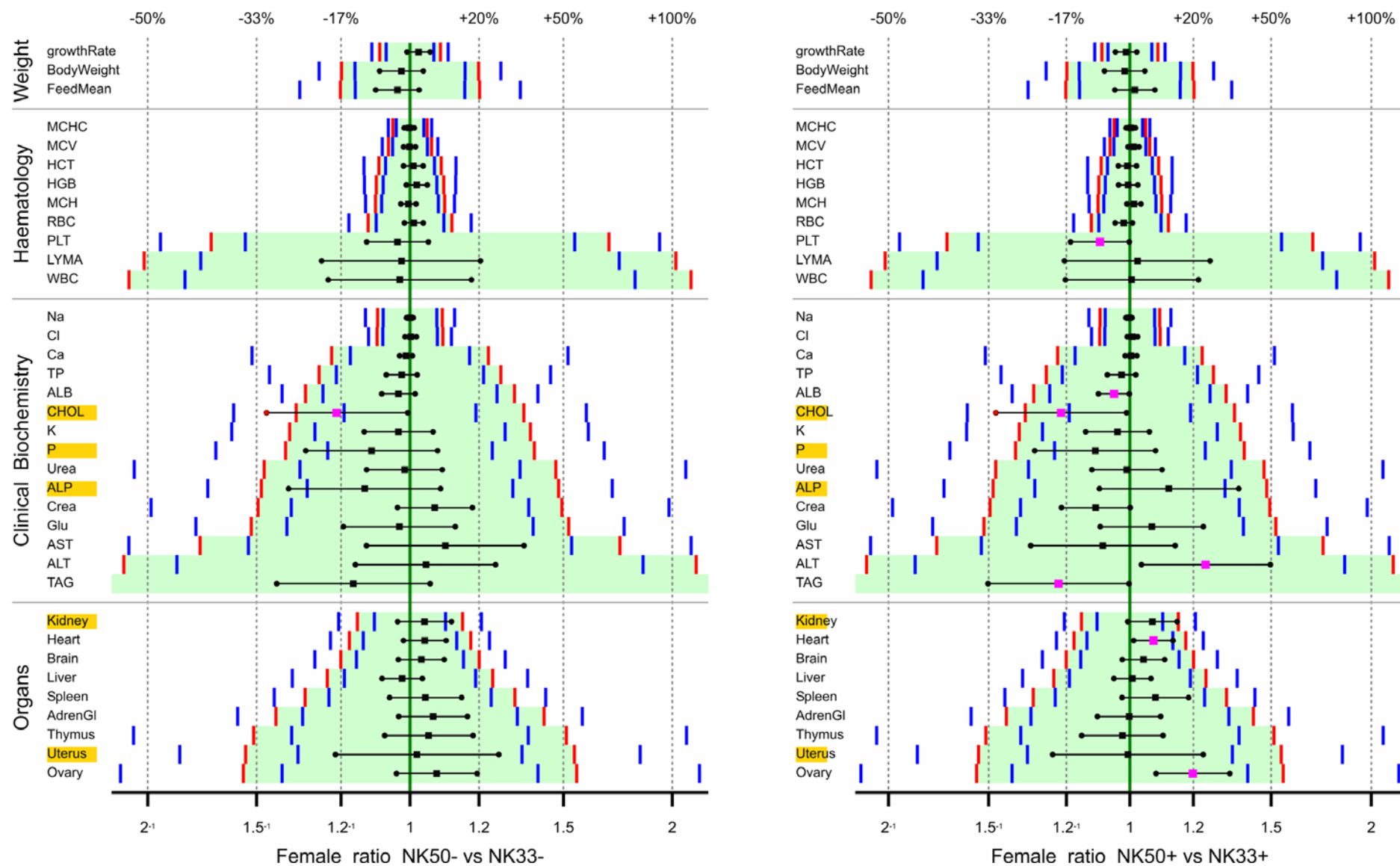
Figure 16 Confidence intervals for the ratio of NK50- and NK50+ vs corresponding 33% feed for females with intervals for equivalence limits (see text).
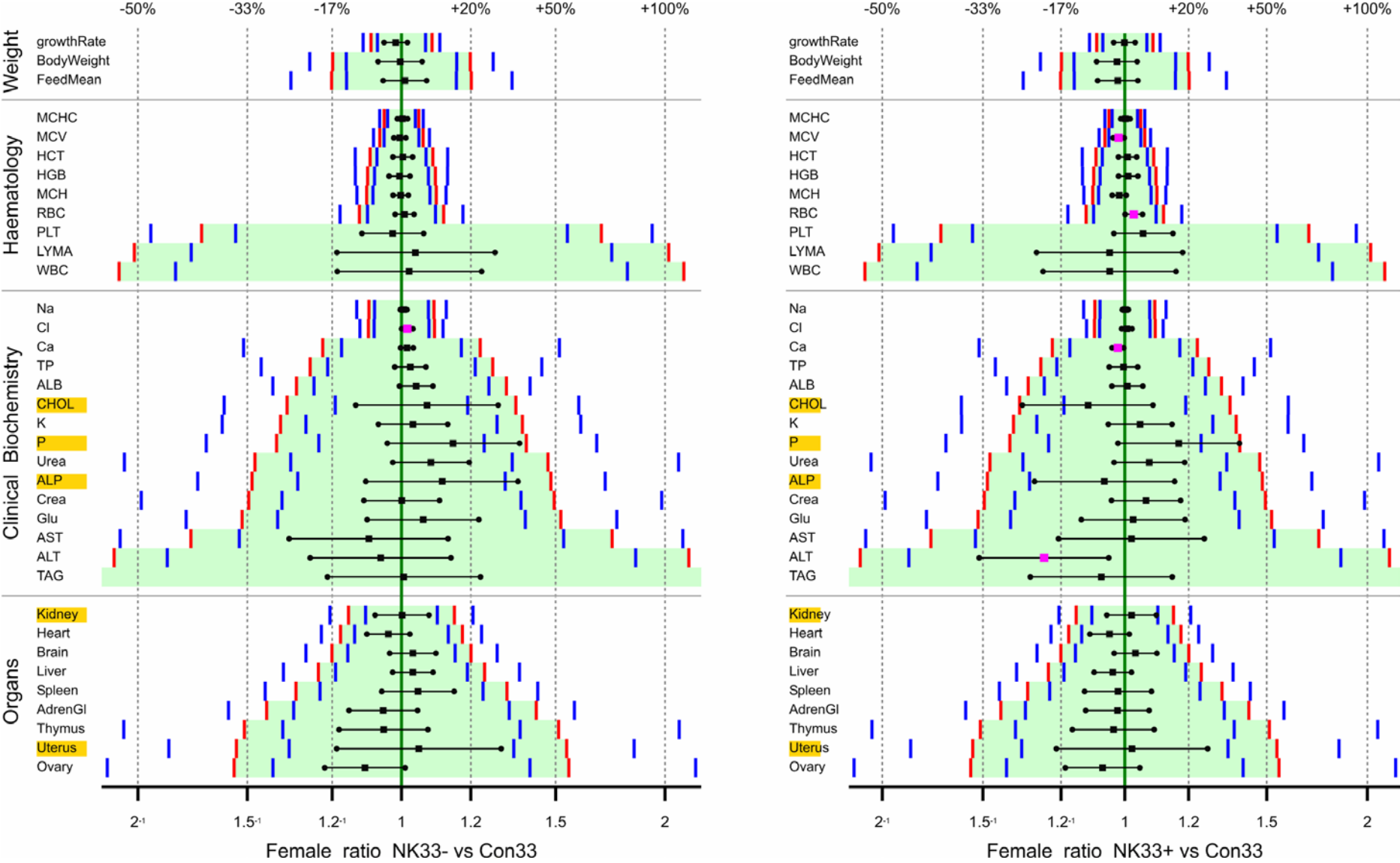
Figure 17 Confidence intervals for the ratio of NK33- and NK33+ vs the Con33 feed for females with intervals for equivalence limits (see text).
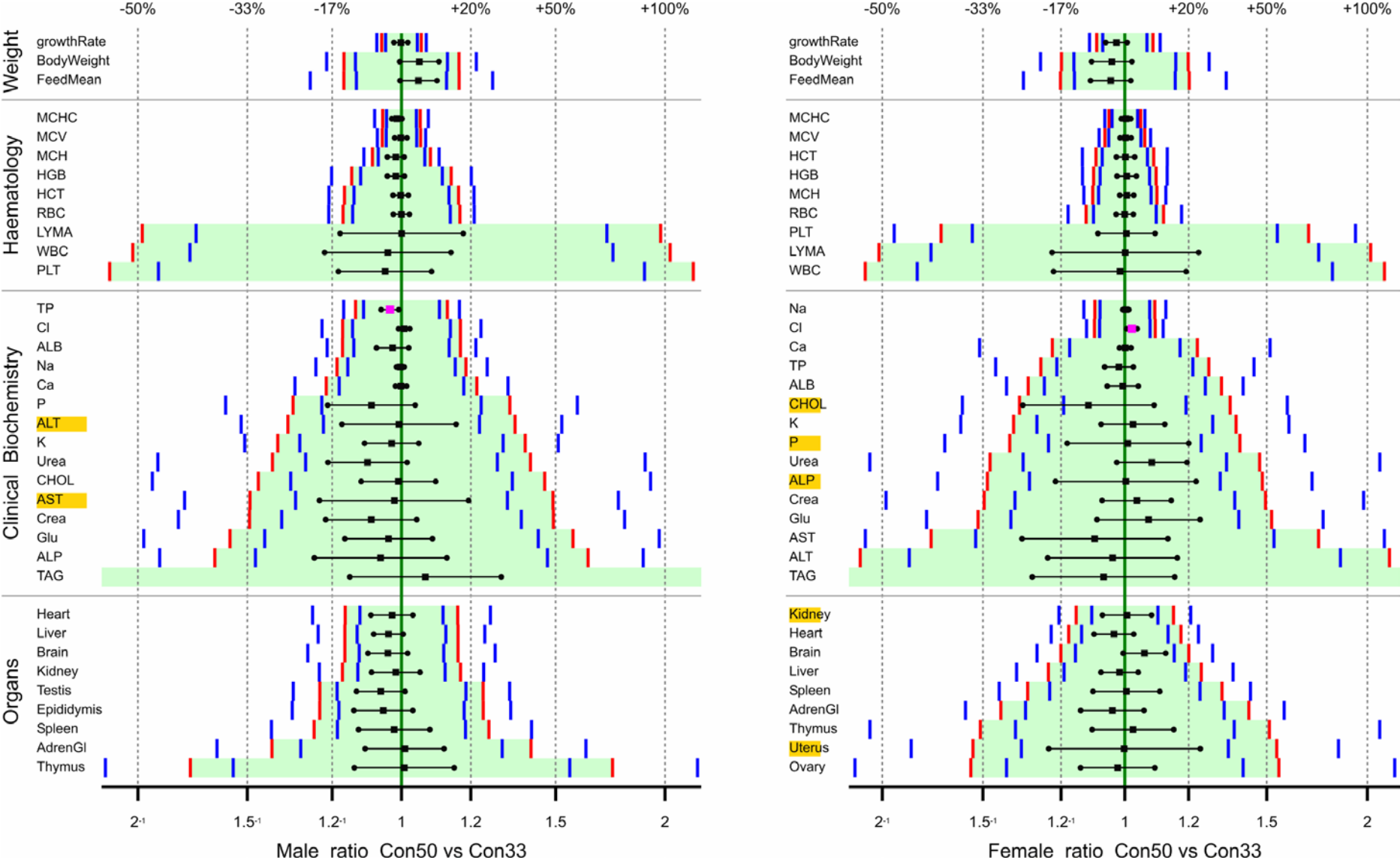
Figure 18 Confidence intervals for the ratio of the two control feeds for males and females with intervals for the equivalence limits (see text).
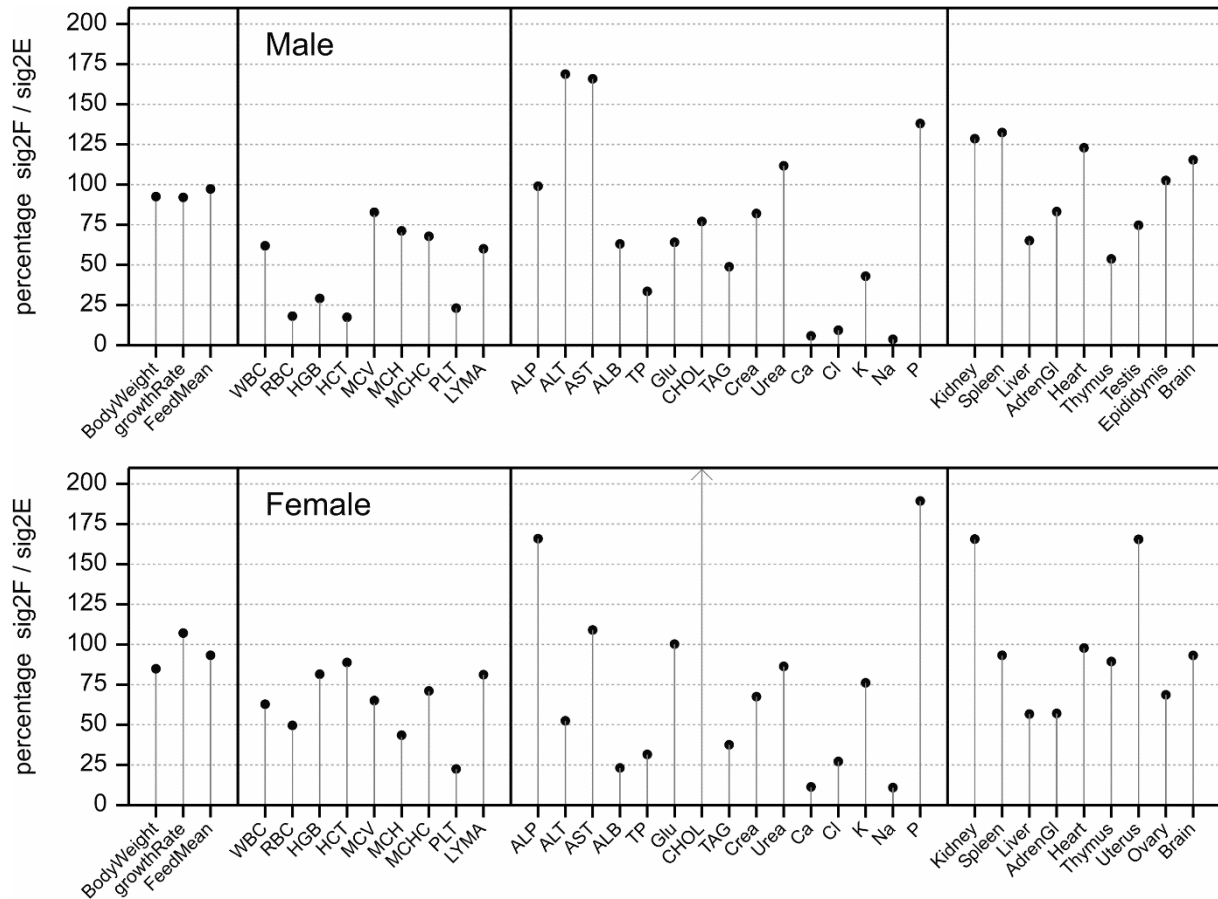
Figure 19 Residual variance (sig2F or σ_F^2) in the current G-TwYST B study as a percentage of the residual variance (sig2E or σ_E^2) in the historical GRACE studies for males (top panel) and females (bottom panel).

4.2   Equivalence testing using target effect sizes

4.2.1   Method

For a limited number of variables Hong *et al* (2017) use what they call targeted effect sizes for the purpose of statistical power analysis for a rat sub-chronic feeding study. Although they warn that these effect sizes should not be considered synonymous with biologically or toxicologically relevant effects, these targeted effect sizes were used for equivalence testing. The targeted effect sizes for nine variables that are also relevant in G-TwYST are given in Table 15 along with the implied limits on the log-ratio scale which are used in the equivalence test. Note the asymmetry in these limits: for a targeted effect size of +/- 30%, +30% corresponds to a factor 1.3 which is 0.262 on the log scale, while -30% corresponds to a factor 0.7 which equals -0.357 on the log scale. Denoting the limits on the log-ratio scale as δ_{low} and δ_{upp}, the two-sided non-equivalence null hypothesis reads, with Δ the ratio of the mean of a GMO feed and the mean of the control feed:

$$H_0: \quad \log(\Delta) < \delta_{low} \quad \text{or} \quad \log(\Delta) > \delta_{upp}$$

$$H_1: \quad \delta_{low} \leq \log(\Delta) \leq \delta_{upp}$$

This was tested by means of the TOST approach of Schuirmann (1987) at the 5% level which is equivalent to checking whether the 10% confidence interval for $\log(\Delta)$ lies completely within the

interval $(\delta_{low}, \delta_{upp})$ composed of the equivalence limits. For one-sided tests the same confidence interval can be used where only one of the confidence bounds is relevant.

Table 15   Targeted effect sizes from Table 1 in Hong *et al* (2017) along with their implied lower and upper limits on the ratio scale and on the log-ratio scale.

Name in Hong *et al* (2017)	G-TwYST Name	Targeted effect size	Ratio scale		Log-ratio scale	
			Lower	Upper	Lower	Upper
Body weight; final non-fasted	BodyWeight	- 10%	0.90	-	-0.105	-
Leukocyte (WBC) count	WBC	+/- 30%	0.70	1.30	-0.357	0.262
Lymphocyte (ALYM) count	LYMA	+/- 30%	0.70	1.30	-0.357	0.262
Alkaline phosphatase (ALKP)	ALP	+ 100%	-	2.00	-	0.693
Creatinine (CREA)	Krea	+ 50%	-	1.50	-	0.405
Blood urea nitrogen (BUN)	Urea	+ 50%	-	1.50	-	0.405
Cholesterol (CHOL)	CHOL	+ 200%	-	3.00	-	1.099
Kidney, % body weight	Kidney	+ 25%	-	1.25	-	0.223
Liver, % body weight	Liver	+ 25%	-	1.25	-	0.223

4.2.2   Results

The confidence intervals for the 9 variables in Table 15, as well as the equivalence limits are given in Figure 20 for males and in Figure 21 for females. In all cases the null hypothesis of non-equivalence is rejected in favour of equivalence with generally small p-values (Table 16).

Table 16   P-values of equivalence tests for the ratio of two means using targeted effect sizes of Hong et al (2017), see Table 15, as equivalence limits. P-values smaller than 0.01/0.05 have a gold/yellow background.

Males Variable	NK11-/50 Con50	NK50- Con50	NK11+/50 Con50	NK50+ Con50	Con50 Con33	NK50- NK33-	NK50+ NK33+	NK33- Con33	NK33+ Con33
BodyWeight	0.010	0.027	0.000	0.001	0.000	0.000	0.000	0.000	0.000
WBC	0.002	0.014	0.001	0.010	0.000	0.004	0.005	0.002	0.001
LYMA	0.001	0.025	0.000	0.015	0.001	0.002	0.013	0.017	0.001
ALP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Krea	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Urea	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CHOL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Kidney	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Liver	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Females Variable	NK11-/50 Con50	NK50- Con50	NK11+/50 Con50	NK50+ Con50	Con50 Con33	NK50- NK33-	NK50+ NK33+	NK33- Con33	NK33+ Con33
BodyWeight	0.000	0.000	0.000	0.000	0.011	0.003	0.001	0.000	0.003
WBC	0.034	0.005	0.021	0.002	0.003	0.002	0.004	0.007	0.001
LYMA	0.037	0.010	0.021	0.004	0.008	0.004	0.013	0.018	0.002
ALP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Krea	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Urea	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CHOL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Kidney	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Liver	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Figure 20 90% confidence intervals for the ratio of two means for selected variables for males along with equivalence intervals defined by targeted effect sizes of Hong et al (2017).
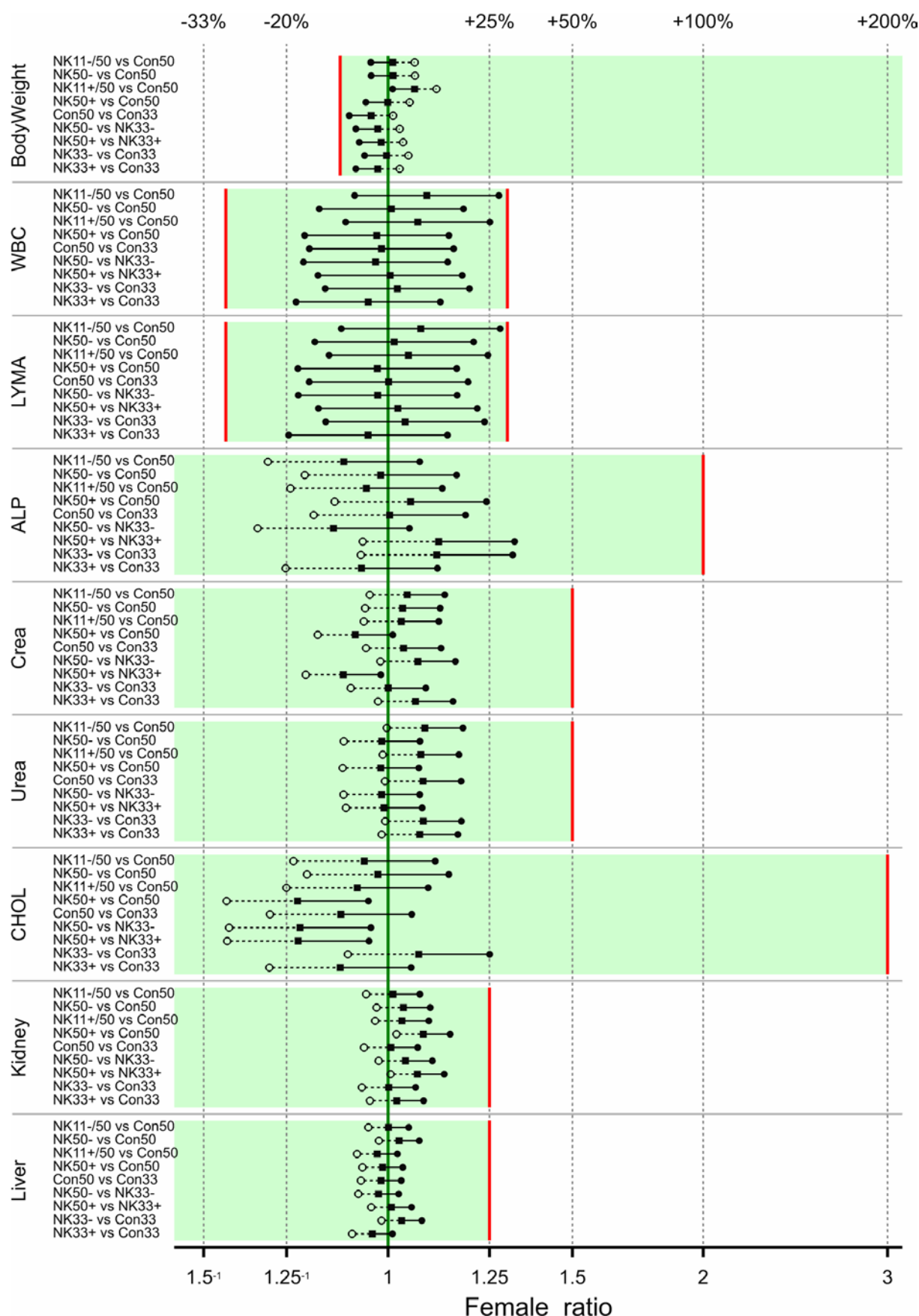
Figure 21 90% confidence intervals for the ratio of two means for selected variables for females along with equivalence intervals defined by targeted effect sizes of Hong et al (2017).

4.3   Classical statistical analysis

4.3.1   Method

G-TwYST study C is based on OECD guidance 408 on repeated dose 90-day oral toxicity studies in rodents (OECD 1998), EFSA guidance complementing the OECD guidance for whole food/feed studies (EFSA 2011b), and additional EFSA clarifications (EFSA 2013, 2014). OECD guidance 408 (OECD 1998) requires numerical results to be evaluated by an appropriate and acceptable statistical method, but gives no further guidance on statistical analysis. More detailed guidance, although strictly meant for chronic and carcinogenicity studies, is provided in chapter 4 of OECD guidance document 116 (OECD 2012), which describes a flowchart for statistical analysis methods (reproduced in Figure 22).

Figure 1 A statistical decision tree, summarising common statistical procedures used for the analysis of data in long-term toxicology studies.
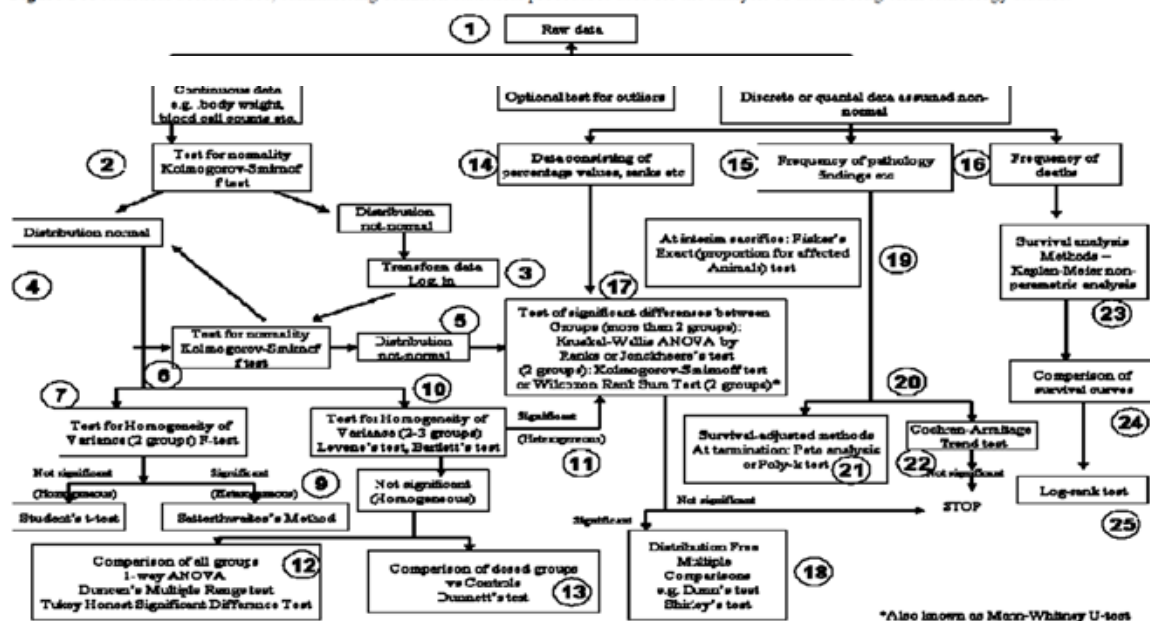
Figure 22   Classical approach to statistical analysis of data in long-term toxicity studies (copied from OECD 2012).

EFSA (2011b) gives further guidance, such as considering cage as the experimental unit, and including block in the model for data from a randomised block design (as is the case for the G-TwYST study).

In the current section we apply classical statistical methods for continuous data in line with these OECD and EFSA approaches, and very similar to the approaches followed in the GRACE project (Schmidt and Schmidtke 2014, Schmidt *et al* 2015ab).

A classical analysis of variance was performed on the cage means after log transforming the data. This was done in the statistical program R. The R-script which analyses a single response variable is given in Appendix 10; Appendix 11 contains an example dataset for the R-script. The classical analysis involves:

- Analysis of variance according to the randomized block design employing the model "Block + Treatment" where Treatment defines the eight feeding groups. The model was fitted by means of linear regression, using the lm() function in R, because this takes proper account of any missing values. The usual summary statistics are saved as well as estimates for the nine

primary comparisons. Standard errors, t-values and p-values of the differences were also saved. These are all calculated using the pooled ANOVA residual standard error which has 49 degrees of freedom whenever measurements are available for 8 cages per feeding group (Table 3).

- The ANOVA p-values do not take account of multiple comparisons between the feeds. Therefore Dunnett's test was performed separately for two sets of comparisons. The first set compared the four GM feeds with maize inclusion rate 33% with the non-GM control feed Con33, and the second set compared the two GM feeds NK50- and NK50+ to the corresponding non-GM control feed Con50. The first set thus contains four simultaneous comparisons, while the second set contains two simultaneous comparisons. Dunnett's test is performed by means of the glht() function in the multcomp R-package.

- The residuals of the analysis of variance are checked for normality using the Kolmogorov-Smirnov test as well as the Shapiro-Wilk test for normality. These tests are only approximate since the residuals are not independent. The p-value of the Kolmogorov-Smirnov test is not reported since it is almost always larger that the p-value of the Shapiro-Wilk test, moreover for variables where it is smaller, the p-value is far from significant. Note that the ANOVA residuals were already assessed by means of a normal probability plot (Appendix 5) and a plot of residuals versus fitted values (Appendix 6).

- The non-parametric Wilcoxon signed rank test is used to test for a difference for each of the nine comparisons. Note that this test only uses data of two feeds and that the test employs the within block difference between the two feeds. This test is nor useful for the endpoints in the Immunology and CellPhenotype groups because these consist of non-aligned missing values. The exact p-value of the test is calculated by means of the wilcox.test() function in R.

- The non-parametric Friedman test, which is applicable to a randomized block design, is used to test for overall differences between the eight feeds. Results of this test are not reported.

- Homogeneity of variance is assessed by means of Bartlett's test and by means of Levene's test both using the mean and the median. These test do not take blocking into account and basically compare the within feed variances. Note that homogeneity of variance was already assessed by means of a plot of residuals versus fitted values (Appendix 6). The p-value of the Levene test with the median is not reported since it is almost always larger that the p-value of the Levene test with the mean, and for variables where it is smaller the p-value is far from significant. Note that both analysis of variance and non-parametric tests require homogeneity of variance.

- Finally, for each feeding group separately, normality was assessed by means of the Kolmogorov-Smirnov test and the Shapiro-Wilk test. The p-value of the Kolmogorov-Smirnov test is always larger than 5% and is thus not reported.

4.3.2 Results

Table 17 (males) and Table 18 (females) present the results of t-tests, of Dunnett's tests and of Wilcoxon tests for the 88 variables divided in seven groups. Note that Dunnett's test is only performed for comparisons between GM 50% feeds and Con 50, and between GM 33% feeds and Con33. Ordinary letters indicate a significant difference at the 5% level, while a capital letter indicates significance at the 1% level. Exact p-values for all tests are given in Appendix 12. For ease of interpretation means on the original scale are given, rather than means on the log-transformed scale. Note however that 95% confidence intervals on the ratio scale are given in Table 11 to Table 14. The

colouring in these latter tables corresponds to the letters t/d and T/D in Table 17 and Table 18, since both are based on significance of t-tests at the 5% and 1% level.

For males a difference was significant by at least one of the tests at the 5% level in 48 cases (6.1% of the 792 tests). On their own Dunnett's test resulted in 5 significant differences (0.6%), the t-test resulted in 38 significant differences (4.8%), and Wilcoxon's test resulted in 25 significant differences (6.2% of 405 tests, since Wilcoxon is not performed for Immunology and CellPhenotype endpoints). For females a difference was significant by at least one of the tests at the 5% level in 62 cases (7.8%). On their own Dunnett's test resulted in 12 significant differences (1.5%), the t-test resulted in 52 significant differences (6.6%), and Wilcoxon's test resulted in 20 significant differences (4.9%).

Results of the Shapiro-Wilks test for normality and Bartlett's and Levene's test for homogeneity of variance are given in Appendix 13. The non-normality as indicated by the Shapiro-Wilks test for separate feeding groups is frequently significant. The graphs of cage means on the log scale in Appendix 4 indicate that significance of non-normality is mostly due to one outlying observation in a feeding group.

Table 17   Means per feeding group and results of statistical tests for nine comparisons for male rats. Significant difference are marked, with red background colouring, as follows: *D*: P<0.01 by Dunnett-test, *d*: P<0.05 by Dunnett-test, *T*: P<0.01 by t-test but not by Dunnett-test, *t*: P<0.05 by t-test but not by Dunnett-test, *W*: P<0.01 by Wilcoxon signed rank test, *w*: P<0.05 by Wilcoxon signed rank test. See text.

Males	Means								NK11- Con50	NK50- Con50	NK11+ Con50	NK50+ Con50	Con50 Con33	NK50- NK33-	NK50+ NK33+	NK33- Con33	NK33+ Con33
Weights	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+									
BodyWeight	462.0	443.0	437.5	459.0	452.9	441.2	444.3	444.2		tw			w				
growthRate	0.166	0.172	0.159	0.168	0.156	0.167	0.154	0.171									
FeedMean	2.769	2.655	2.633	2.833	2.745	2.648	2.615	2.683		tw							
Males	Means								NK11- Con50	NK50- Con50	NK11+ Con50	NK50+ Con50	Con50 Con33	NK50- NK33-	NK50+ NK33+	NK33- Con33	NK33+ Con33
Haematology	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+									
WBC	7.981	8.012	8.562	7.406	8.462	8.363	8.238	8.219									
RBC	8.630	8.673	8.539	8.707	8.538	8.631	8.636	8.793							Tw		w
HGB	15.72	16.14	15.71	16.19	15.89	15.95	15.97	15.78	t		dTW						
HCT	46.84	47.31	46.46	47.79	46.89	46.91	47.16	47.02			tw						
MCV	54.28	54.58	54.42	54.92	54.95	54.37	54.62	53.48							Tw		t
MCH	18.22	18.62	18.42	18.62	18.61	18.50	18.51	17.96							Tw		d
MCHC	33.58	34.13	33.82	33.88	33.89	33.99	33.87	33.57	t								
PLT	736.7	792.2	858.8	776.2	828.9	770.2	762.6	843.1		tw		tw					
LYMR	74.46	74.49	76.30	75.71	75.79	75.25	77.89	72.99							t		
LYMA	5.919	5.975	6.525	5.612	6.400	5.944	6.406	5.994									
Males	Means								NK11- Con50	NK50- Con50	NK11+ Con50	NK50+ Con50	Con50 Con33	NK50- NK33-	NK50+ NK33+	NK33- Con33	NK33+ Con33
ClinChem	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+									
ALP	1.283	1.471	1.193	1.201	1.333	1.386	1.294	1.349									
ALT	0.536	0.658	0.533	0.639	0.574	0.533	0.524	0.546									
AST	2.366	2.916	2.456	2.665	2.571	2.424	2.276	2.392									
BIL	7.469	8.919	7.856	8.044	8.206	7.500	7.587	7.719	t								
ALB	37.46	39.29	38.27	38.99	38.36	38.38	38.19	38.29	t								
TP	64.17	65.49	65.39	64.63	65.64	66.17	65.36	65.66		W		w	t				
Glu	5.578	5.449	5.224	5.699	5.027	5.743	5.395	5.292									
CHOL	2.133	2.110	2.234	2.252	2.262	2.151	2.079	2.114									
TAG	1.132	1.118	1.178	1.184	1.201	1.068	1.275	1.122									
Crea	33.41	32.31	34.90	32.23	33.32	36.18	34.38	33.28									
Urea	4.958	4.912	4.691	5.501	4.859	5.455	4.838	5.307			tw					d	

	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+	NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
cHGB	66.4	138.2	88.9	106.1	107.1	87.0	73.9	88.7	d		tW						
Ca	2.388	2.371	2.403	2.386	2.391	2.391	2.396	2.393									
Cl	100.1	99.6	100.8	100.3	99.0	99.4	99.8	100.4							w		w
K	4.438	4.888	4.662	4.500	4.556	4.556	4.512	4.431	t								
Na	143.6	143.2	144.6	143.2	143.2	144.1	143.4	144.7		w							
P	1.999	2.114	1.993	2.025	2.043	2.192	2.224	2.216							w		
Males					**Means**				NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
Urine	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+	Con50	Con50	Con50	Con50	Con33	NK33-	NK33+	Con33	Con33
uVol	23.75	23.00	25.19	25.31	25.50	18.88	21.12	20.75									
uVolW	5.306	5.476	6.141	5.747	5.801	4.533	4.941	4.842									
uLeu	12.50	9.38	4.69	7.81	6.25	12.50	9.38	20.31									
uOsmoll	457.7	417.2	395.1	405.2	432.7	432.0	421.5	515.9									
uKeton	0.500	0.344	0.375	0.219	0.469	0.312	0.625	0.438									
upH	6.938	6.906	6.938	7.125	6.719	7.094	7.031	7.250							Tw		
Males					**Means**				NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
Organs	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+	Con50	Con50	Con50	Con50	Con33	NK33-	NK33+	Con33	Con33
Kidney	0.535	0.555	0.547	0.561	0.554	0.543	0.533	0.579									
Spleen	0.163	0.162	0.170	0.168	0.170	0.167	0.167	0.168									
Liver	2.272	2.219	2.342	2.345	2.296	2.352	2.289	2.293									
AdrenGl	0.0138	0.0140	0.0138	0.0135	0.0138	0.0136	0.0135	0.0133									
Heart	0.250	0.248	0.258	0.256	0.255	0.256	0.242	0.261						tw		tw	
Thymus	0.095	0.090	0.097	0.101	0.092	0.096	0.087	0.096						t			
Testis	0.819	0.830	0.883	0.863	0.855	0.864	0.846	0.833		tW	w						
Epididymis	0.280	0.280	0.298	0.296	0.291	0.293	0.280	0.290									
Brain	0.493	0.506	0.522	0.502	0.500	0.511	0.509	0.510		t		w					
Males					**Means**				NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
Immunology	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+	Con50	Con50	Con50	Con50	Con33	NK33-	NK33+	Con33	Con33
Monocytes	62.55	65.42	66.20	61.45	67.63	63.35	66.73	68.80									d
Granulocytes	82.43	80.28	82.76	81.91	81.46	82.73	83.88	84.03									
RespirBurst	76.01	79.79	72.31	76.65	76.74	74.16	73.03	74.99									
Con	87808	105440	135550	128899	98139	154631	175636	134747									
PHA	45620	50308	51155	68895	38421	59322	77656	51970									
PWM	31814	40579	54890	41618	31232	38623	52515	51542									
Med3d	1834	2281	3155	2156	1469	2628	3649	3175									

									NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
IprConA	49.24	52.23	47.33	63.45	62.08	62.09	50.34	63.17			t					t	
IprPHA	29.93	30.27	21.59	34.62	27.36	26.36	24.66	31.37									
IprPWM	23.87	22.27	24.25	24.66	23.89	17.33	16.88	24.34									
G4c1	1991	1855	2227	2233	1493	2210	3181	1694									
G4c2	2341	2218	2231	2435	2209	2666	3947	1729									
G4c3	2630	2171	2628	3259	3534	3266	4055	2119									
NG2c1	1979	2047	2445	2262	2116	2199	3649	1970									
NG2c2	2442	2130	2378	2545	2437	3043	4124	1959									
NG2c3	2417	2259	2450	2968	3948	3446	4620	2189								t	
A6c1	2330	2467	2300	2620	2161	3123	4264	2137									
A6c2	2449	2726	2195	2603	3049	3204	4156	2240									
A6c3	2316	2118	2197	2601	3881	3192	3517	2062									
Med6d	2287	1818	1950	1988	1941	2498	3493	1742									
IprG4c1	1.336	1.397	2.004	1.211	1.132	1.119	0.920	1.076								t	
IprG4c2	1.330	1.372	1.704	1.188	1.152	1.128	1.355	1.061									
IprG4c3	1.733	2.051	1.701	1.914	1.730	1.690	1.448	1.578									
IprNG2c1	1.287	1.423	1.903	1.469	1.331	1.100	1.080	1.239									
IprNG2c2	1.663	1.416	1.729	1.210	1.264	1.283	1.258	1.282									
IprNG2c3	1.774	1.821	1.637	1.787	1.811	1.682	1.581	1.693									
IprA6c1	1.356	1.701	1.601	1.571	1.361	1.458	1.288	1.231									
IprA6c2	1.560	1.486	1.565	1.293	1.904	1.398	1.253	1.402									
IprA6c3	1.527	1.841	1.378	1.568	1.775	1.652	1.303	1.535									

Males				Means					NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
CellPhenotype	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+									
sp3	45.88	42.09	46.60	44.42	46.16	44.70	44.96	41.80									
sp3-4	31.49	28.64	32.55	29.92	31.78	31.84	31.17	28.94									
sp3-8	12.84	12.93	13.43	13.35	14.35	14.17	14.99	14.21									
sp3-45	24.93	28.51	26.20	23.92	24.88	23.38	25.26	24.98									
sp3-161	7.871	8.196	7.379	7.983	7.915	7.896	7.125	8.088									
ln3	47.38	46.02	47.71	46.78	48.39	48.98	45.18	45.20									
ln3-4	34.55	35.13	36.20	34.74	36.30	36.89	32.20	33.63									
ln3-8	10.95	10.84	11.02	11.70	11.82	12.15	13.23	11.16									
ln3-45	29.68	33.54	27.75	31.48	32.60	35.33	31.30	31.64									
ty3	21.13	18.45	19.83	19.84	19.71	18.16	20.06	19.65									
ty3-4	17.54	15.45	17.10	16.77	16.37	15.41	17.69	16.96									

	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+	NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
ty3-8	11.28	9.45	9.70	11.30	9.48	9.44	9.11	10.27									
bm3	7.67	9.23	7.23	5.89	6.31	12.46	5.74	13.74			T				t	t	
bm3-45	53.94	57.23	60.15	57.86	59.27	57.75	66.49	60.77			t					t	

Table 18 **Means per feeding group and results of statistical tests for nine comparisons for female rats. Significant difference are marked, with red background colouring, as follows: *D*: P<0.01 by Dunnett-test, *d*: P<0.05 by Dunnett-test, *T*: P<0.01 by t-test but not by Dunnett-test, *t*: P<0.05 by t-test but not by Dunnett-test, *W*: P<0.01 by Wilcoxon signed rank test, *w*: P<0.05 by Wilcoxon signed rank test. See text.**

Females	Means								NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
Weights	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+									
BodyWeight	246.7	249.4	249.2	261.5	246.2	255.7	254.6	250.2			t						
growthRate	0.128	0.155	0.160	0.135	0.140	0.152	0.137	0.151		t							
FeedMean	1.829	1.864	1.857	2.030	1.893	1.901	1.919	1.863			Dw						
Females	Means								NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
Haematology	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+									
WBC	5.006	5.344	4.950	5.250	4.906	4.994	5.169	4.812									
RBC	7.636	7.684	7.781	7.689	7.702	7.636	7.699	7.836									tw
HGB	15.11	15.10	15.22	15.40	15.10	15.02	14.97	15.17									
HCT	43.96	43.73	44.44	44.51	43.92	43.87	44.04	44.22									
MCV	57.59	56.93	57.13	57.91	57.04	57.46	57.21	56.46									t
MCH	19.80	19.68	19.57	20.04	19.61	19.69	19.66	19.38									
MCHC	34.38	34.53	34.29	34.60	34.42	34.27	34.36	34.33									
PLT	787.4	807.2	745.3	733.9	760.4	784.6	773.0	823.7							tw		
LYMR	79.38	78.14	79.82	77.47	79.59	77.89	79.24	77.96									
LYMA	3.969	4.156	3.956	4.050	3.919	3.913	4.094	3.788									
Females	Means								NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
ClinChem	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+									
ALP	0.656	0.591	0.644	0.626	0.676	0.643	0.716	0.625									
ALT	0.481	0.484	0.494	0.484	0.497	0.499	0.475	0.431							t		d
AST	2.223	2.362	2.439	2.288	2.286	2.397	2.207	2.481									
BIL	6.24	8.65	6.61	8.97	8.05	6.50	8.01	10.42									
ALB	44.05	42.67	44.69	43.11	42.71	44.39	46.15	44.67							t		
TP	69.16	68.57	70.42	68.41	68.56	70.41	72.11	70.14									

	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+	NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
Glu	5.237	4.948	5.010	5.248	5.356	4.869	5.156	5.051									
CHOL	1.627	1.574	1.647	1.538	1.336	1.834	1.954	1.681				t		t	tw		
TAG	0.521	0.511	0.479	0.484	0.427	0.561	0.558	0.540				t		w	t		
Crea	36.54	38.46	37.68	37.65	34.01	35.37	35.33	37.59							w		
Urea	5.925	6.471	5.866	6.364	5.851	5.485	5.959	5.899									
cHGB	73.2	97.9	77.5	109.6	100.7	67.2	65.4	100.5									
Ca	2.459	2.429	2.465	2.428	2.416	2.456	2.492	2.408			w	tW					d
Cl	102.7	102.0	102.4	101.9	101.9	100.6	102.2	101.2					T			t	
K	4.188	4.306	4.094	4.175	4.150	4.087	4.244	4.319									
Na	143.8	143.2	144.2	143.4	143.3	143.4	144.2	143.6									
P	1.647	1.634	1.637	1.660	1.709	1.622	1.841	1.874									

Females	Means								NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
Urine	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+									
uVol	19.94	21.06	18.12	16.50	19.94	15.31	22.56	20.06						w	t	DW	w
uVolW	8.494	9.110	7.731	6.728	8.588	6.380	9.381	8.494						w		dTW	w
uLeu	0.00	3.12	9.38	1.56	32.81	3.12	1.56	0.00									
uOsmoll	340.2	383.5	434.8	429.0	340.5	422.6	321.6	347.2			w					tw	
uKeton	0.125	0.031	0.188	0.094	0.063	0.031	0.063	0.000									
upH	6.438	6.438	6.437	6.531	6.563	6.531	6.781	6.750							t		

Females	Means								NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
Organs	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+									
Kidney	0.581	0.589	0.600	0.601	0.628	0.577	0.579	0.588			t						
Spleen	0.214	0.220	0.233	0.222	0.226	0.213	0.223	0.209									
Liver	2.400	2.413	2.461	2.346	2.377	2.437	2.514	2.354									
AdrenGl	0.0300	0.0293	0.0315	0.0312	0.0305	0.0312	0.0298	0.0304									
Heart	0.302	0.297	0.313	0.312	0.320	0.313	0.301	0.298							t		
Thymus	0.134	0.134	0.131	0.116	0.124	0.131	0.126	0.127			t	w					
Uterus	0.259	0.298	0.273	0.249	0.257	0.253	0.270	0.262									
Ovary	0.0398	0.0391	0.0396	0.0399	0.0458	0.0403	0.0371	0.0389					dw		TW		
Brain	0.855	0.853	0.858	0.823	0.868	0.807	0.832	0.833						w			

Females Immunology	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+	NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
				Means													
Monocytes	63.81	65.83	59.79	62.54	56.88	64.35	63.04	61.33									
Granulocytes	80.73	78.87	73.77	76.39	74.40	78.88	76.98	76.86		t		d					
RespirBurst	81.03	79.61	73.12	77.10	74.88	76.76	74.44	73.35									
Con	75692	68670	81048	117206	54584	78625	62550	60367									
PHA	26868	30960	34023	53806	15683	32723	26036	22252									
PWM	20924	26486	25090	36709	14985	24881	18383	20029									
Med3d	1975	1923	3125	3244	1654	1768	1558	1652									
IprConA	34.23	31.90	28.30	32.90	36.59	42.50	34.95	34.83									
IprPHA	12.74	17.58	15.47	15.90	15.18	23.62	14.43	14.41					t				
IprPWM	11.06	15.10	9.47	10.60	12.97	15.92	12.48	12.81	t				t				t
G4c1	2319	2175	2536	3478	1990	1886	1894	2242			d						
G4c2	2535	2535	2826	3409	2435	2165	2371	2591									
G4c3	2775	2586	2779	3122	2334	2170	2474	2798									
NG2c1	2299	2158	2465	3432	1931	1956	2216	2385			t						
NG2c2	2559	2577	2882	3309	2357	2182	2643	2811									
NG2c3	2674	2593	2939	3274	2499	2151	2691	2795									
A6c1	2444	2544	2862	3288	1858	2182	2657	2905			t						
A6c2	1980	2729	2699	3204	2596	1967	2722	2808									
A6c3	2417	2337	2724	3162	2161	1945	2439	2488									
Med6d	2572	2451	2480	2663	1697	1910	2177	2307									
IprG4c1	0.953	0.927	1.076	1.431	1.317	1.001	0.993	1.016									
IprG4c2	1.100	1.130	1.319	1.343	1.502	1.182	1.302	1.225									
IprG4c3	1.169	1.264	1.383	1.206	1.416	1.265	1.240	1.290									
IprNG2c1	1.001	0.976	1.112	1.374	1.309	1.101	1.074	1.107									
IprNG2c2	1.193	1.205	1.223	1.353	1.630	1.273	1.306	1.484									
IprNG2c3	1.354	1.261	1.326	1.275	1.729	1.390	1.421	1.475									
IprA6c1	1.076	1.228	1.262	1.421	1.225	1.235	1.341	1.486									
IprA6c2	0.995	1.384	1.420	1.267	1.668	1.296	1.484	1.564	D	t		dT				t	t
IprA6c3	1.084	1.119	1.241	1.256	1.487	1.268	1.322	1.238									

Females CellPhenotype	Con50	NK11-	NK50-	NK11+	NK50+	Con33	NK33-	NK33+	NK11-Con50	NK50-Con50	NK11+Con50	NK50+Con50	Con50Con33	NK50-NK33-	NK50+NK33+	NK33-Con33	NK33+Con33
				Means													
sp3	50.67	52.00	50.95	52.82	58.57	52.68	44.47	46.88									
sp3-4	25.56	26.71	26.21	24.80	26.51	27.22	22.61	24.82									

sp3-8	10.90	12.31	12.83	12.17	12.07	12.82	11.25	11.08										
sp3-45	32.09	28.32	27.89	31.03	29.75	28.18	28.59	28.04										
sp3-161	7.300	6.685	6.296	7.625	6.415	6.917	7.290	7.158		t								
ln3	53.23	50.50	55.07	49.37	56.36	53.07	51.70	52.01										
ln3-4	38.12	36.77	39.83	35.59	43.17	38.55	38.98	36.75				t			T		
ln3-8	14.12	13.11	15.19	14.09	12.66	14.02	12.98	14.21										
ln3-45	27.80	23.97	24.66	33.53	20.23	21.04	28.28	31.38				t	t		T	d	dT
ty3	17.22	18.05	18.35	18.84	18.49	18.77	19.44	19.43										
ty3-4	15.48	16.88	16.47	17.31	16.78	17.09	16.92	17.71					t				
ty3-8	8.596	7.970	7.900	9.950	8.660	8.333	8.900	8.783										
bm3	11.20	13.93	16.30	15.78	16.72	18.03	7.93	16.07								t	
bm3-45	62.30	62.56	60.67	59.74	53.90	55.61	62.79	55.79										

4.4   Standardised effect sizes

4.4.1   Method

EFSA (2011b) defines the standardised effect size (SES) as the effect size measured in SD units, where SD is the standard deviation among experimental units. We will assume that in a randomised block, experiment like the current G-TwYST study, SD refers to the pooled residual variation. The use of SES in EFSA (2011b) was in the context of determination of sample size and power: '*If experience from previous toxicity tests shows an effect size of, say, one SD or less is of little toxicological relevance then this can be used to determine sample size in new situations*' (EFSA 2011b). Zeljenková *et al* (2014) followed this example and, without further toxicological motivation, '*assumed that an SES of 1.0 SD or less is unlikely to be of toxicological importance*'. Consequently, all results of the GRACE studies have been reported as confidence intervals on the SES scale (Zeljenková *et al*, 2014, 2016, Schmidt *et al* 2015, 2017). In this section the same SES graphs are calculated for comparability between GRACE and G-TwYST.

Standardized effect sizes (SES), again using the residual standard error with 49 degrees of freedom whenever 8 cages are available for an endpoint, and their exact 95% confidence intervals were calculated. This employed the conf.limits.nct() function in the MBESS R-package, see section 3 in Kelley (2007). Note that, since the calculated SES confidence interval is exact, the SES interval does not contain zero if and only if the p-value of the t-test is smaller than 5%.

4.4.2   Results

SES intervals were calculated for all 88 variables, and these are given in Figure 23 to Figure 31. Only very few intervals (26 out of 1584, or 1.6%) are within the (-1,1) SD interval. Intervals are only inside the (-1,1) SD interval for those endpoints which are measured in all 8 cages per feeding group, and for which the difference on the log-scale is almost zero. Examples of endpoints for males in Figure 23 which are (just) inside the SD limits are uVol when comparing NK11-/50 versus Con50, and HGB, ALT and upH when comparing NK50- versus Con50.
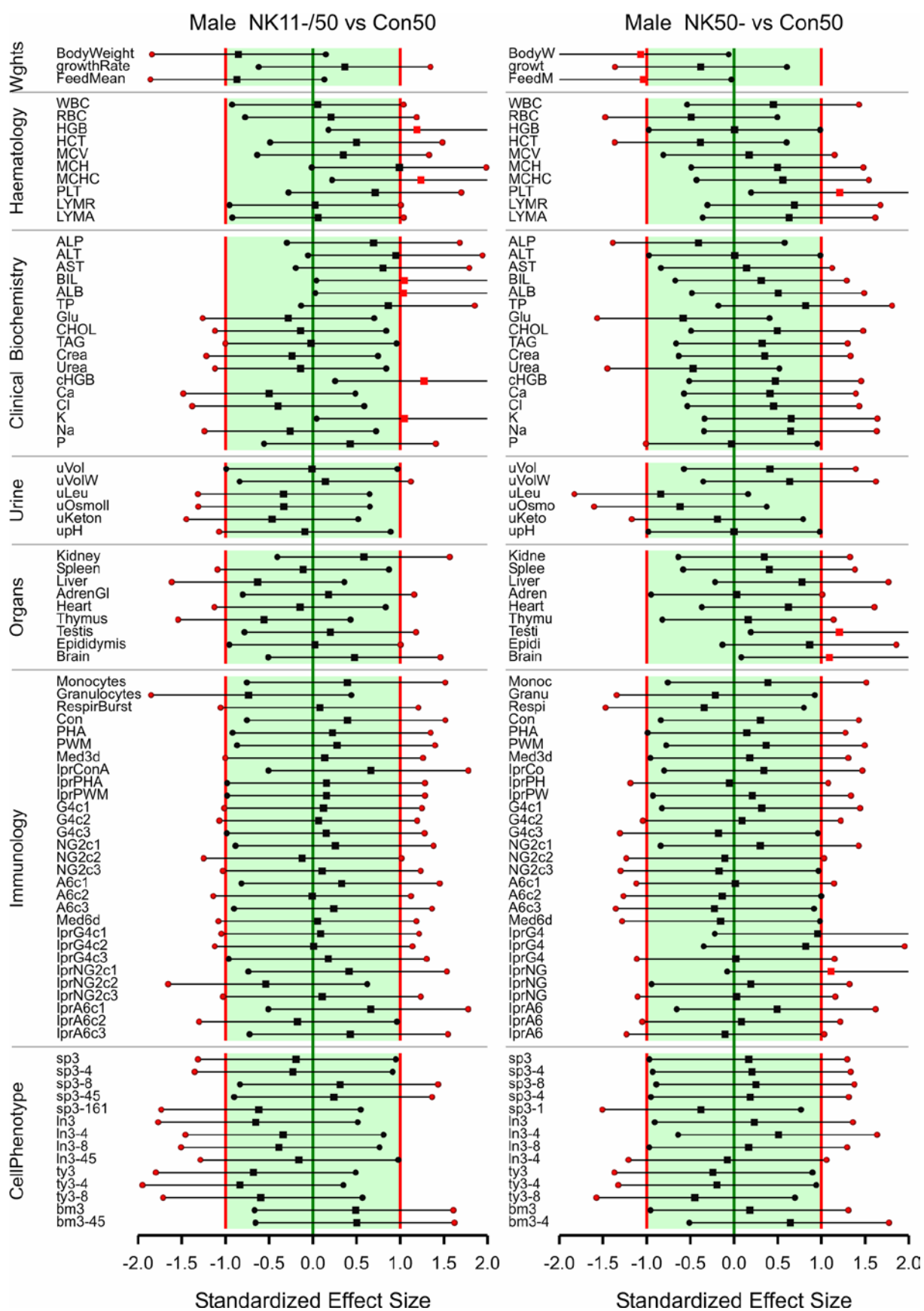
Figure 23 Confidence intervals for Standardized Effect Sized (SES) for male rats for GMO feeds NK11-/50 and NK50- versus the non-GM control feed Con50.
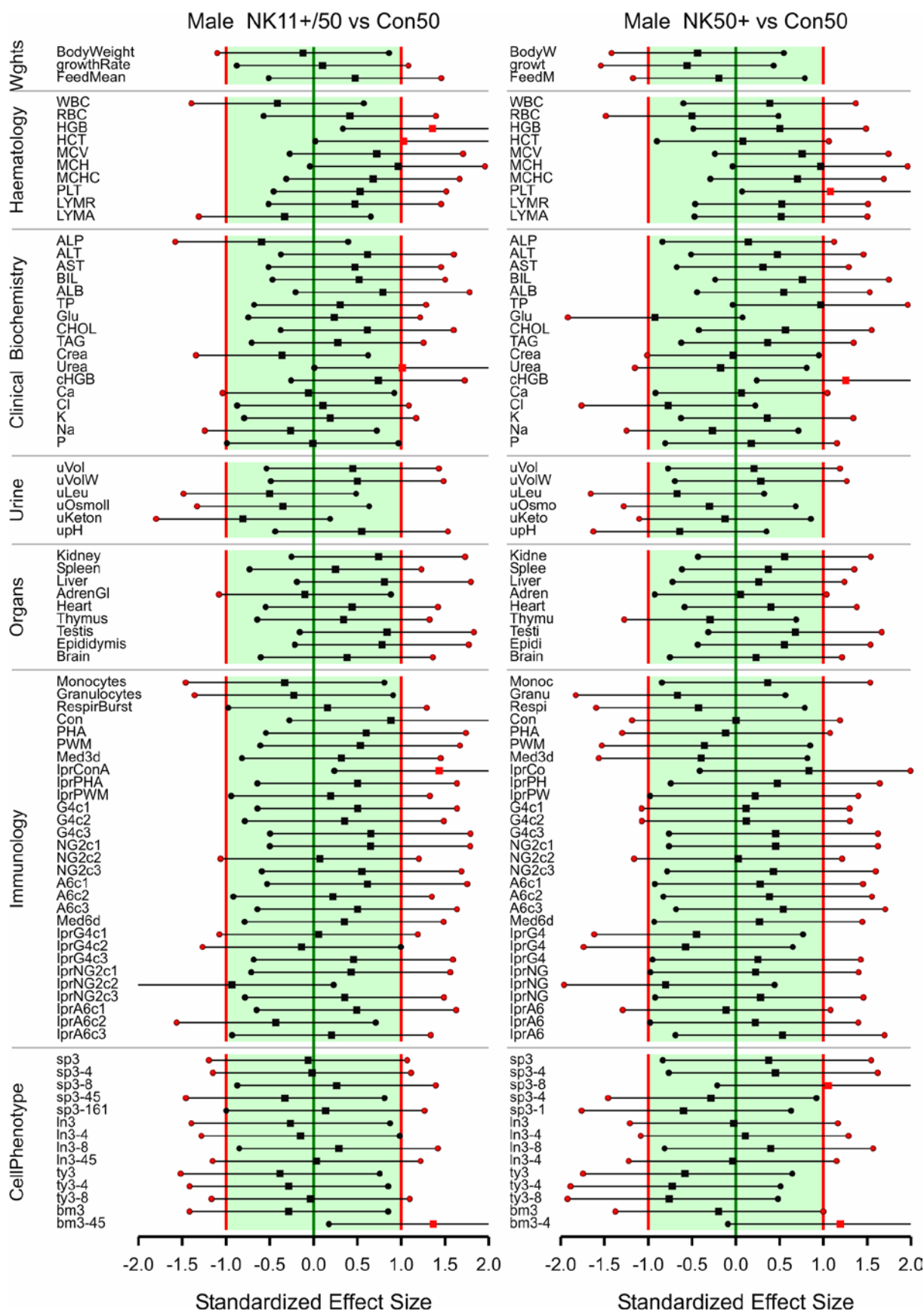
Figure 24 Confidence intervals for Standardized Effect Sized (SES) for male rats for GMO feeds NK11+/50 and NK50+ versus the non-GM control feed Con50.

Figure 25 Confidence intervals for Standardized Effect Sized (SES) for male rats for GMO feeds NK50- versus NK33- and for NK50+ versus NK33+.

Figure 26 Confidence intervals for Standardized Effect Sized (SES) for male rats for GMO feeds NK33- and NK33+ versus the non-GM control feed Con33.

Figure 27 Confidence intervals for Standardized Effect Sized (SES) for female rats for GMO feeds NK11-/50 and NK50- versus the non-GM control feed Con50.

Figure 28 Confidence intervals for Standardized Effect Sized (SES) for female rats for GMO feeds NK11+/50 and NK50+ versus the non-GM control feed Con50.
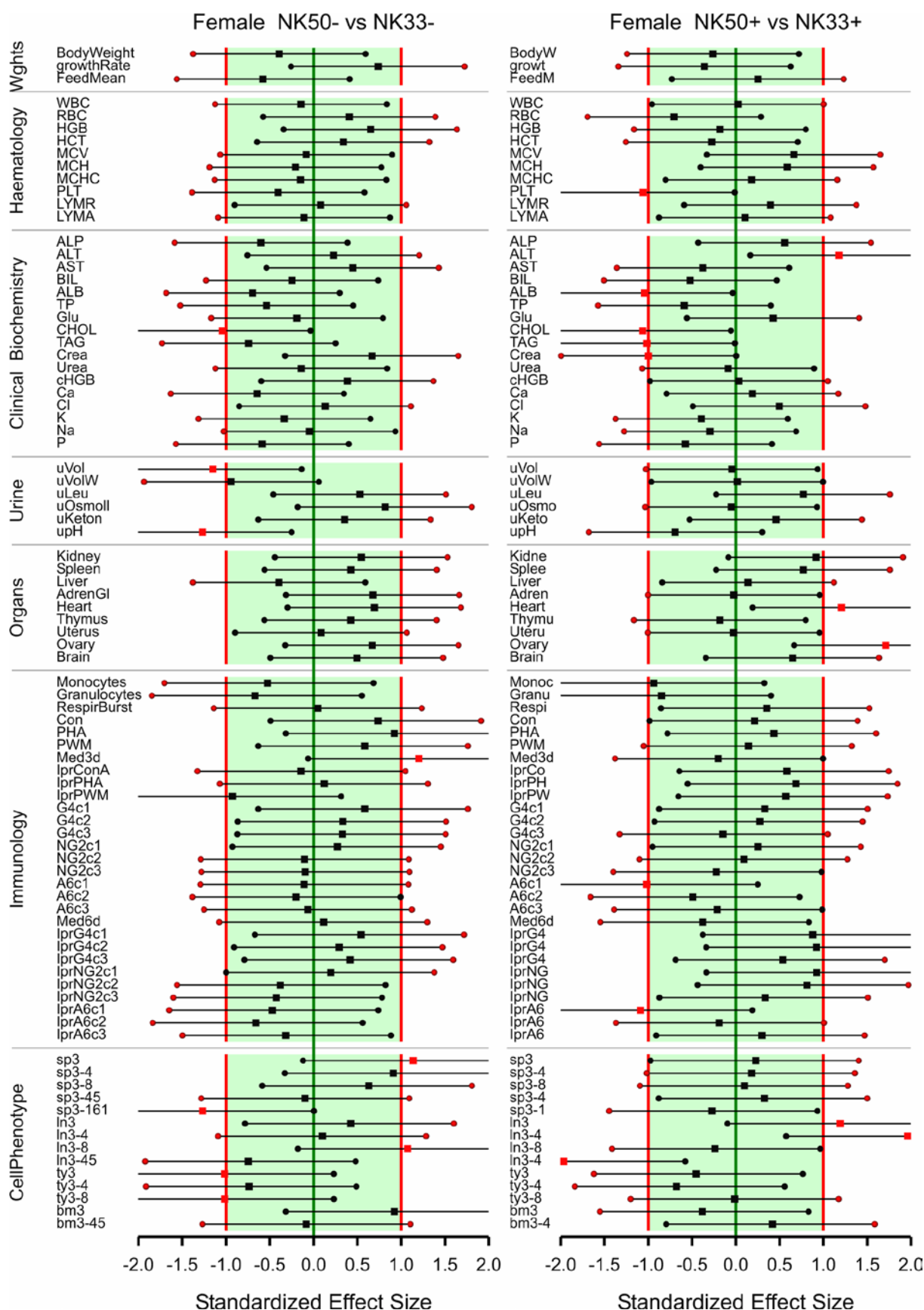
Figure 29 Confidence intervals for Standardized Effect Sized (SES) for female rats for GMO feeds NK50- versus NK33- and for NK50+ versus NK33+.
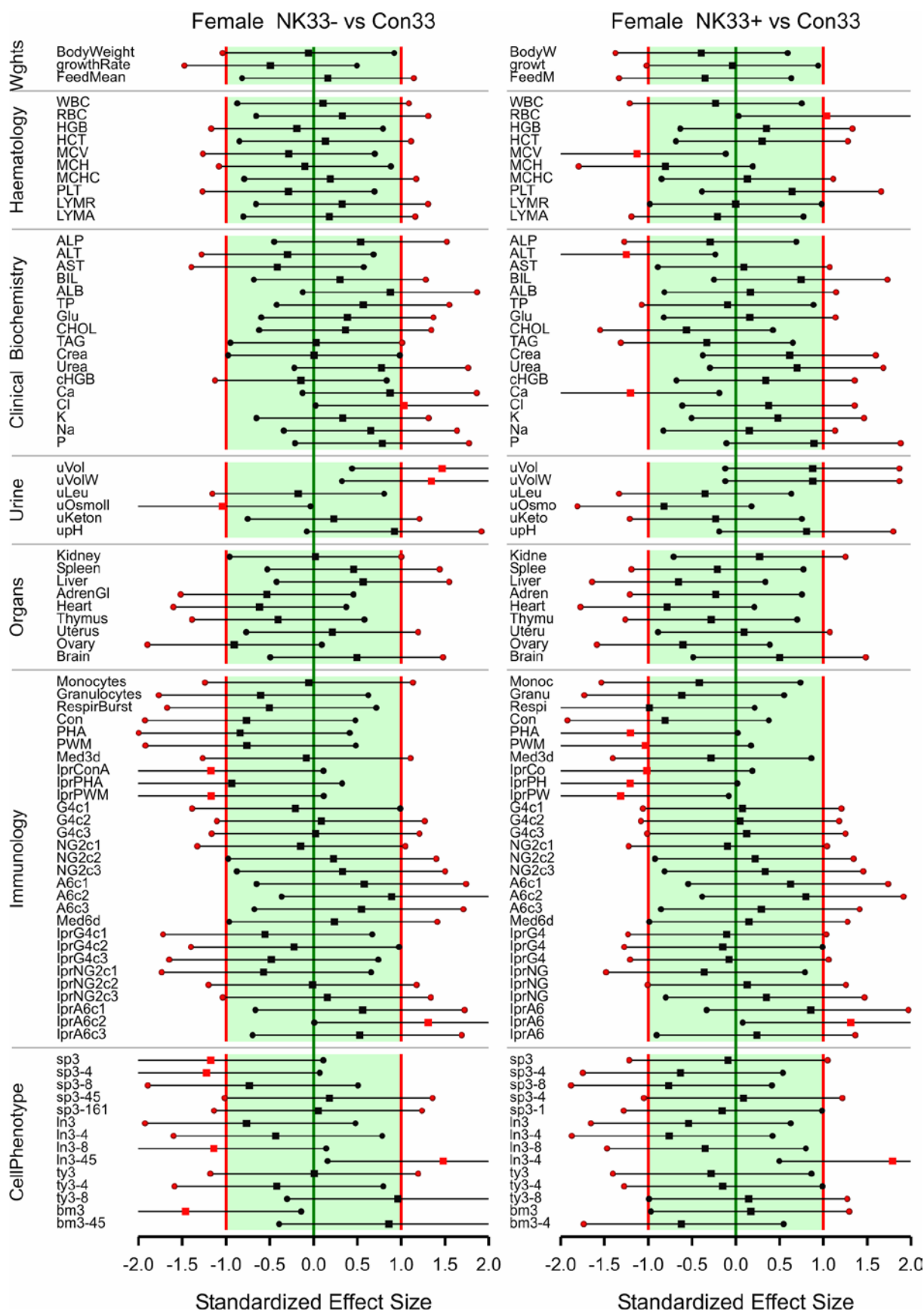
Figure 30 Confidence intervals for Standardized Effect Sized (SES) for female rats for GMO feeds NK33- and NK33+ versus the non-GM control feed Con33.
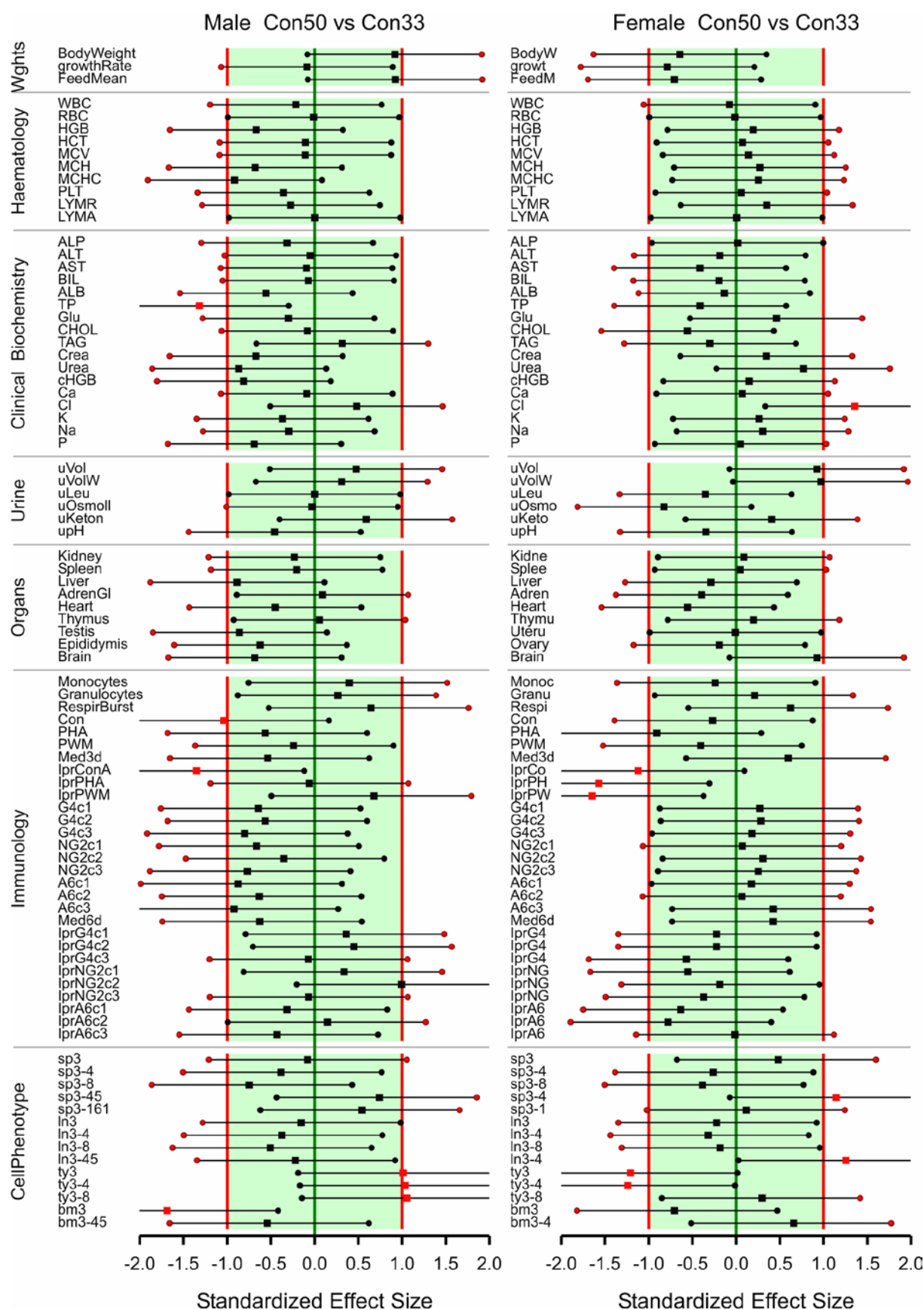
Figure 31 Confidence intervals for Standardized Effect Sized (SES) for male and female rats for the non-GM control feed Con50 versus the non-GM control feed Con33.

4.5   Factorial analysis

4.5.1   Method

The treatment structure of Study C can be considered as a 2 × 3 factorial with additional treatments NK11-/50 and NK11+/50 as in Table 19.

Table 19   Diets organized as a 2 × 3 factorial with treatments MaizeRate with 2 levels (33% and 50%) and MaizeType with three levels (Control, NK- and NK+), with additional treatments NK11-/50 and NK11+/50.

MaizeRate / MaizeType	Control	NK-	NK+	Additional treatments	
33% Maize	Con33	NK33-	NK33+	-	-
50% Maize	Con50	NK50-	NK50+	NK11-/50	NK11+/50

The factorial setup enables testing whether there is a significant interaction between MaizeRate and MaizeType, and , if not, whether there are significant main effects for MaizeRate and MaizeType. Of special interest is the main MaizeRate effect because this study was specifically setup to see whether an inclusion rate of 50% is different from an inclusion rate of 33%. The main effect MaizeType has two degrees of freedom which can be split up in one degree of freedom for the difference between NK- and NK+ (this comparison will be called MaizeType.RoundUp), and one degree of freedom for the difference between the mean of NK- and NK+ and the control feed (which will be called MaizeType.GM). The latter comparison is only relevant when the first comparison is not significant. Similarly, the interaction between MaizeRate and MaizeType can be split up in the same way. This gives rise to the independent contrasts for the 2 × 3 factorial part in Table 20.

Table 20   Contrasts for the 2 × 3 factorial defined in Table 19.

Contrast (abbreviation in Results tables)	Con50	NK50-	NK50+	Con33	NK33-	NK33+
MaizeRate (Rate)	1	1	1	-1	-1	-1
MaizeType.GM (Type1)	2	-1	-1	2	-1	-1
MaizeType.RoundUp (Type2)	0	-1	1	0	-1	1
MaizeRate.MaizeType.GM (Int1)	2	-1	-1	-2	1	1
MaizeRate.MaizeType.RoundUp (Int2)	0	-1	1	0	1	-1

The following sequence of testing is followed:
1. In case MaizeRate.MaizeType.RoundUp is significant the ratio of NK50+ versus NK50- is significantly different from the ratio of NK33+ versus NK33-, and therefore both ratios, denoted as 50.RoundUp and 33.RoundUp, are reported. Results for all other comparisons are not reported.
2. In case the MaizeRate.MaizeType.RoundUp is not significant, then the ratios 50.RoundUp and 33.RoundUp are set to missing. Furthermore
    a. if also MaizeRate.MaizeType.GM is significant, then the ratio of the mean of NK50- and NK50+ versus Con50 is reported, as well as the ratio of the mean of NK33- and NK33+ versus Con30. These are denoted as 50.GM and 30.GM. Results for the ratios MaizeRate and MaizeType.GM are set to missing.

b. if also MaizeType.RoundUp is significant the ratio NK+ versus NK- is reported, where NK+ is the mean of NK50+ and NK33+, and similarly NK-. Results for the ratio MaizeType.GM are set to missing.

3.   In case both interactions MaizeRate.MaizeType.RoundUp and MaizeRate.MaizeType.GM are not significant, the main effect MaizeRate is reported whenever it is significant. Moreover MaizeType.RoundUp is reported (as in 2b) whenever it is significant, and MaizeType.GM is reported when it is significant and MaizeType.RoundUp is not significant.

Due to missing values and unbalance for the endpoints in the Immunology and CellPhenotype group, the contrast given in Table 20 are not independent and therefore this factorial approach is not followed for those endpoints.

4.5.2 Results

P-values for testing the five contrasts are given in Table 21, while significant effects according to the testing sequence are given in Table 22

Table 21   P values for significance of contrasts obtained for the factorial model. Type1 / Type2 denote contrasts MaizeType.GM / MaizeType.RoundUp, while Int1 / Int2 denote the interaction contrasts MaizeRate.MaizeType.GM / MaizeRate.MaizeType.RoundUp. P-values smaller than 0.01/0.05 have a gold/yellow background.

Variable	Males					Females				
Weights	Rate	Type1	Type2	Int1	Int2	Rate	Type1	Type2	Int1	Int2
BodyWeight	0.277	0.328	0.371	0.147	0.380	0.140	0.825	0.452	0.607	0.854
growthRate	0.467	0.225	0.296	0.764	0.126	0.638	0.472	0.788	0.117	0.127
FeedMean	0.083	0.323	0.057	0.318	0.670	0.238	0.563	0.775	0.378	0.246
Variable	Males					Females				
Haematology	Rate	Type1	Type2	Int1	Int2	Rate	Type1	Type2	Int1	Int2
WBC	0.807	0.509	0.817	0.486	0.955	0.821	0.861	0.475	0.980	0.813
RBC	0.030	0.945	0.243	0.127	0.232	0.720	0.050	0.673	0.827	0.121
HGB	0.214	0.934	0.952	0.459	0.149	0.447	0.748	0.728	0.951	0.244
HCT	0.265	0.964	0.680	0.591	0.373	0.874	0.524	0.689	0.948	0.389
MCV	0.128	0.873	0.306	0.182	0.010	0.411	0.044	0.191	0.811	0.295
MCH	0.411	0.890	0.220	0.029	0.013	0.456	0.113	0.389	0.892	0.266
MCHC	0.735	0.953	0.468	0.051	0.261	0.746	0.890	0.765	0.699	0.644
PLT	0.693	0.013	0.309	0.253	0.169	0.111	0.479	0.094	0.203	0.360
LYMR	0.724	0.293	0.008	0.362	0.027	0.346	0.732	0.634	0.853	0.657
LYMA	0.513	0.163	0.380	0.646	0.576	1.000	0.949	0.424	0.988	0.765
Variable	Males					Females				
ClinChem	Rate	Type1	Type2	Int1	Int2	Rate	Type1	Type2	Int1	Int2
ALP	0.360	0.586	0.252	0.907	0.699	0.976	0.738	0.482	0.950	0.107
ALT	0.687	0.703	0.306	0.686	0.773	0.166	0.287	0.184	0.153	0.185
AST	0.585	0.902	0.554	0.540	0.901	0.694	0.835	0.795	0.465	0.249
BIL	0.508	0.277	0.402	0.519	0.673	0.271	0.168	0.391	0.760	0.699
ALB	0.610	0.474	0.891	0.324	0.981	0.035	0.616	0.016	0.237	0.628
TP	0.138	0.452	0.640	0.036	0.962	0.081	0.601	0.058	0.807	0.942
Glu	0.202	0.027	0.487	0.857	0.790	0.425	0.740	0.821	0.579	0.388
CHOL	0.131	0.658	0.799	0.204	0.957	0.003	0.261	0.011	0.423	0.975
TAG	0.625	0.128	0.535	0.668	0.460	0.021	0.157	0.164	0.350	0.701
Crea	0.566	0.479	0.257	0.222	0.952	0.989	0.864	0.528	0.406	0.022
Urea	0.035	0.109	0.088	0.559	0.367	0.535	0.342	0.887	0.155	0.940

cHGB	0.977	0.397	0.114	0.055	0.548	0.512	0.675	0.381	0.919	0.621
Ca	0.967	0.599	0.552	0.804	0.702	0.658	0.311	0.000	0.629	0.243
Cl	0.826	0.621	0.261	0.311	0.024	0.026	0.556	0.187	0.096	0.608
K	0.618	0.691	0.472	0.215	0.914	0.593	0.759	0.734	0.313	0.936
Na	0.589	0.781	0.982	0.735	0.013	0.965	0.592	0.085	0.441	0.727
P	0.009	0.631	0.790	0.808	0.759	0.204	0.095	0.751	0.307	0.987
Variable			**Males**					**Females**		
Urine	Rate	Type1	Type2	Int1	Int2	Rate	Type1	Type2	Int1	Int2
uVol	0.064	0.406	0.512	0.863	0.933	0.756	0.186	0.915	0.016	0.125
uVolW	0.089	0.302	0.387	0.643	0.901	0.961	0.202	0.972	0.024	0.181
uLeu	0.128	0.177	0.348	0.279	0.638	0.279	0.443	0.878	0.108	0.733
uOsmoll	0.163	0.572	0.245	0.357	0.780	0.943	0.290	0.549	0.055	0.224
uKeton	0.836	0.437	0.463	0.201	0.358	0.166	1.000	0.256	1.000	0.883
upH	0.011	0.766	1.000	0.458	0.076	0.010	0.080	0.627	0.305	0.419
Variable			**Males**					**Females**		
Organs	Rate	Type1	Type2	Int1	Int2	Rate	Type1	Type2	Int1	Int2
Kidney	0.637	0.219	0.038	0.817	0.132	0.080	0.133	0.224	0.299	0.602
Spleen	0.849	0.536	0.943	0.525	0.872	0.157	0.200	0.167	0.374	0.627
Liver	0.756	0.799	0.495	0.057	0.438	0.531	0.907	0.010	0.793	0.453
AdrenGl	0.432	0.843	0.871	0.736	0.817	0.766	0.946	0.900	0.245	0.327
Heart	0.681	0.788	0.127	0.168	0.034	0.127	0.866	0.805	0.018	0.474
Thymus	0.309	0.423	0.651	0.558	0.087	0.614	0.216	0.613	0.897	0.394
Testis	0.715	0.479	0.282	0.022	0.691	-	-	-	-	-
Epididymis	0.747	0.574	0.856	0.086	0.292	-	-	-	-	-
Uterus	-	-	-	-	-	0.955	0.576	0.623	0.950	0.878
Ovary	-	-	-	-	-	0.015	0.837	0.024	0.028	0.147
Brain	0.474	0.326	0.258	0.248	0.203	0.021	0.301	0.814	0.566	0.829

Table 22   **Ratios for significant contrasts for males and females according to the testing sequence for the factorial model (see text).**

Male	**Interaction effects**				**Main effects**		
Variable	50.Roundup	33.Roundup	50.GM	33.GM	Roundup	GM	Rate
RBC	-	-	-	-	-	-	0.99
MCV	1.01	0.98	-	-	-	-	-
MCH	1.01	0.97	-	-	-	-	-
PLT	-	-	-	-	-	1.10	-
LYMR	0.99	0.94	-	-	-	-	-
TP	-	-	1.02	0.99	-	-	-
Glu	-	-	-	-	-	0.92	-
Urea	-	-	-	-	-	-	0.94
Cl	0.98	1.01	-	-	-	-	-
Na	0.99	1.01	-	-	-	-	-
P	-	-	-	-	-	-	0.91
upH	-	-	-	-	-	-	0.77
Kidney	-	-	-	-	1.05	-	-
Heart	0.99	1.08	-	-	-	-	-
Testis	-	-	1.06	0.97	-	-	-

Female	Interaction effects				Main effects		
Variable	50.Roundup	33.Roundup	50.GM	33.GM	Roundup	GM	Rate
MCV	-	-	-	-	-	0.99	-
ALB	-	-	-	-	0.96	-	0.97
CHOL	-	-	-	-	0.84	-	0.85
TAG	-	-	-	-	-	-	0.87
Crea	0.90	1.06	-	-	-	-	-
Ca	-	-	-	-	0.97	-	-
Cl	-	-	-	-	-	-	1.01
uVol	-	-	0.91	1.37	-	-	-
uVolW	-	-	0.91	1.39	-	-	-
upH	-	-	-	-	-	-	0.81
Liver	-	-	-	-	0.95	-	-
Heart	-	-	1.05	0.96	-	-	-
Ovary	-	-	1.07	0.92	1.09	-	-
Brain	-	-	-	-	-	-	1.04

4.6   Correlation analysis

4.6.1   Method

For single variables the difference between two feeding groups is quantified by the ratio of the responses. These can then be compared to given limits (as in Figure 20 and Figure 21) or to limits calculated from historical data (as in Figure 10 - Figure 18), or rescaled, as in Figure 6 -Figure 9.

For a toxicological interpretation it may be helpful to see results for variables that simultaneously relate to the same pathological endpoint. Bivariate plots were prepared showing the patterns for each pair for three variables related to liver disorder (relative liver weight, ALP, CHOL) and three variables related to kidney disorder (relative kidney weight, Urea, Crea). The eight points in each graph are based on the cage means in the eight blocks of the study.

For comparison, the proposed target effect sizes of Hong *et al* (2017), see Table 15, are included in the plots as horizontal and vertical lines (together with lines at ratio 1 for reference). It can be noted that similar plots could have been made using the equivalence limit scaled differences (ELSDs) as presented in Figure 6 - Figure 9.

4.6.2   Results

The correlation plots for three liver-related and three kidney-related variables in males and females are shown in Figure 32 - Figure 39. In most cases there appears to be no clear correlation with liver or kidney weights. The following four correlation are significant: (1) A significant positive correlation for liver weight versus CHOL for NK33+ versus Con33 in males (corr = 0.74, p-value = 0.03, Figure 33); (2) A significant negative correlation between liver weight and CHOL for NK50+ versus Con50 in females (corr=-0.81, p-value=0.01, Figure 36); (3) A significant positive correlation for kidney weight versus Urea for NK50+ versus NK33+ in females (corr = 0.77, p-value = 0.02, Figure 39). In this case there are also significant correlations between Kidney and Crea, and between Urea and Crea; (4) A significant negative correlation for kidney weight versus Crea for NK33- versus Con33 in females (corr = 0.-71, p-value = 0.05, Figure 39).
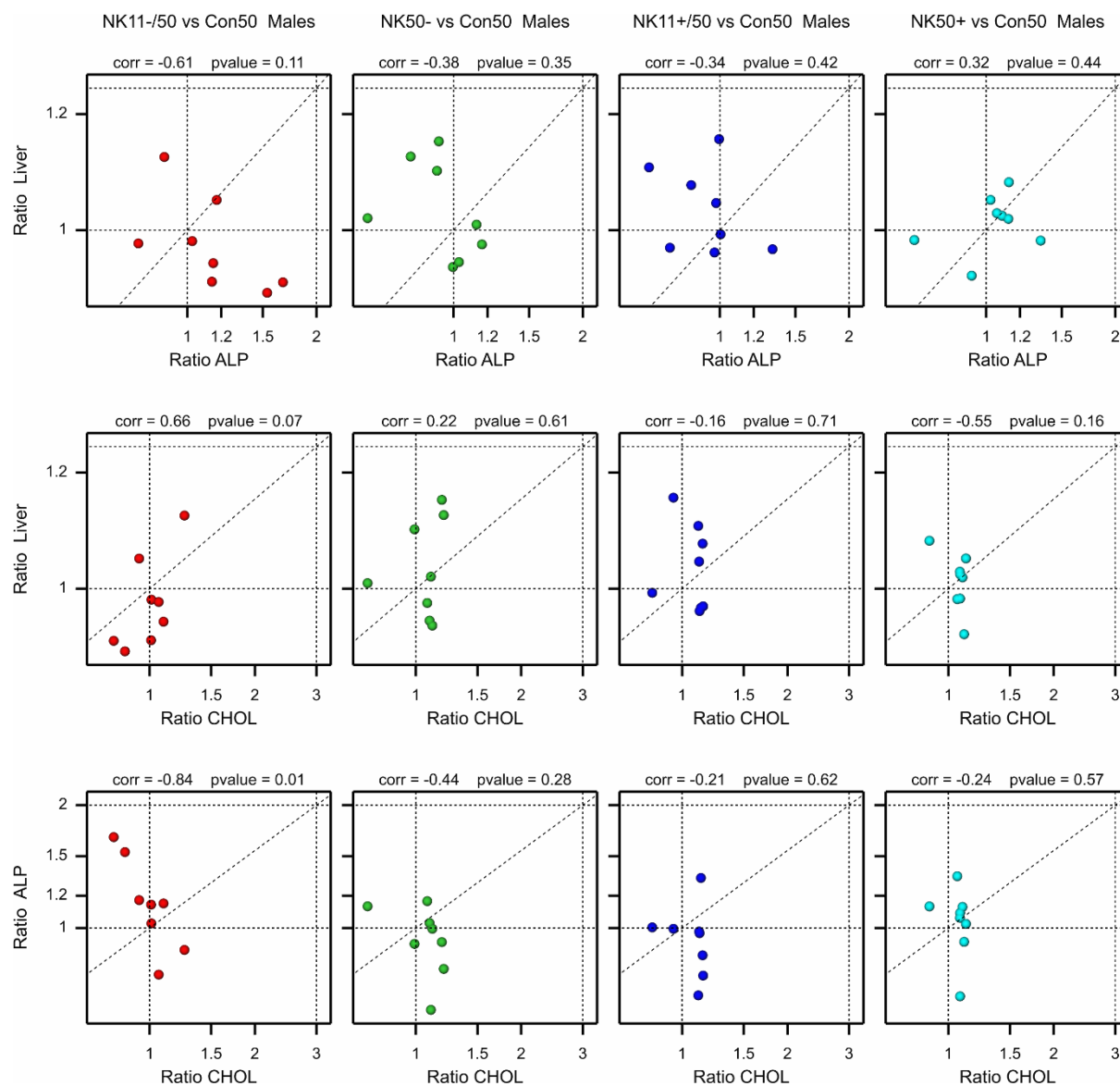
Figure 32 Pairwise results for variables with set target effect sizes related to liver damage in males. Points represent the ratio of the cage means for the comparison given at the top of each column. Horizontal and vertical lines represent a ratio of 1 and the target effect sizes from Hong *et al* (2017).
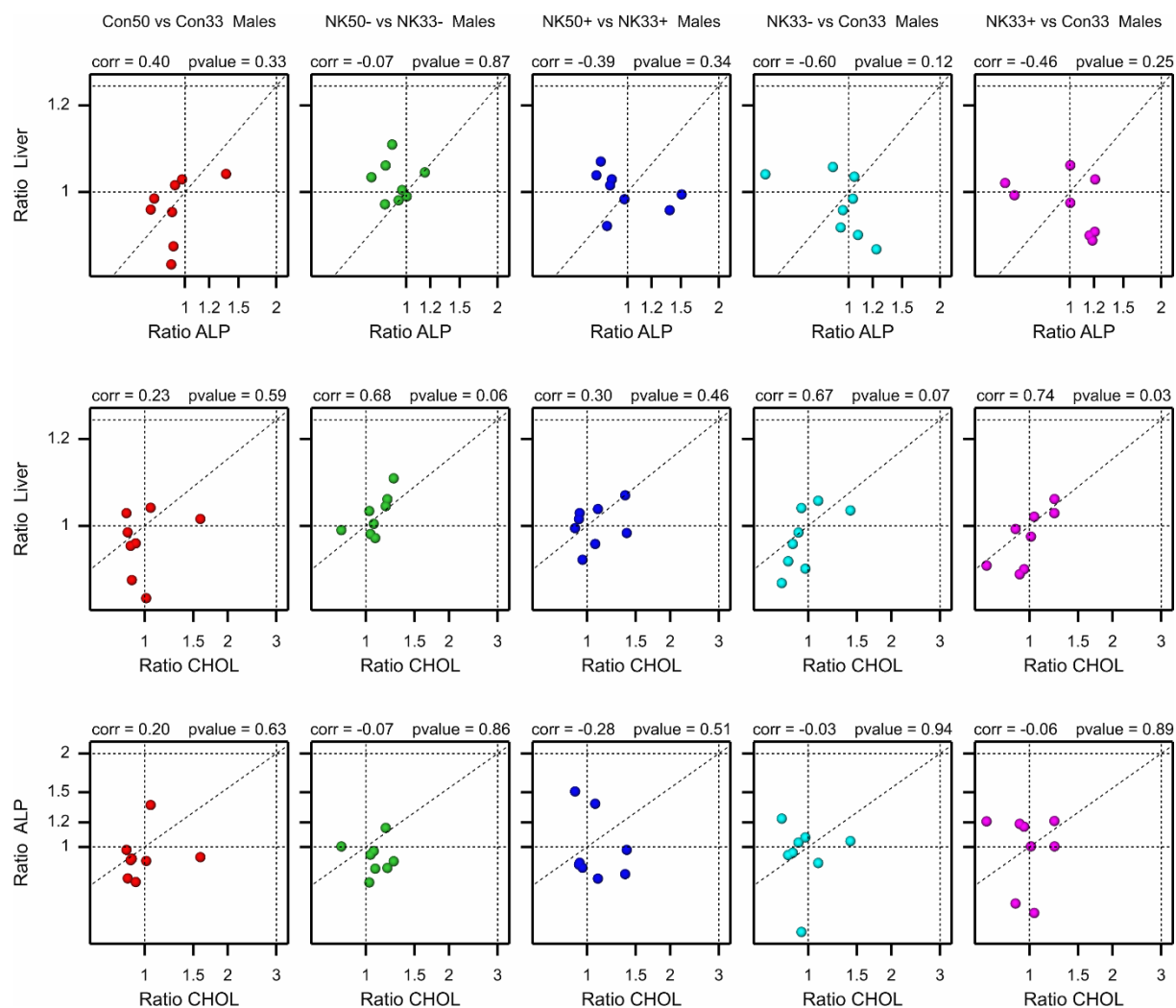
Figure 33 Pairwise results for variables with set target effect sizes related to liver damage in males. Points represent the ratio of the cage means for the comparison given at the top of each column. Horizontal and vertical lines represent a ratio of 1 and the target effect sizes from Hong *et al* (2017).
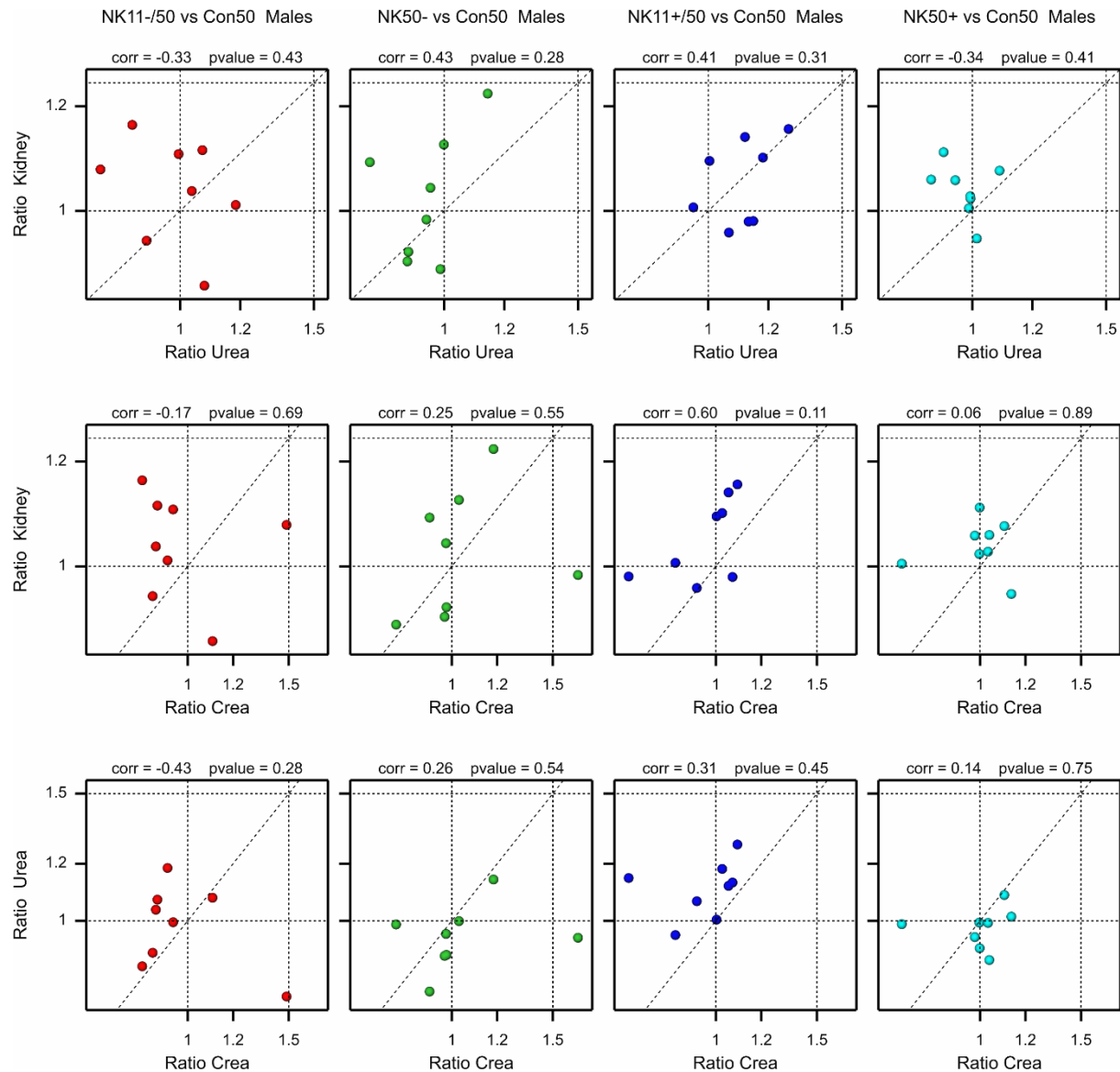
Figure 34 Pairwise results for variables with set target effect sizes related to kidney damage in males. Points represent the ratio of the cage means for the comparison given at the top of each column. Horizontal and vertical lines represent a ratio of 1 and the target effect sizes from Hong *et al* (2017).
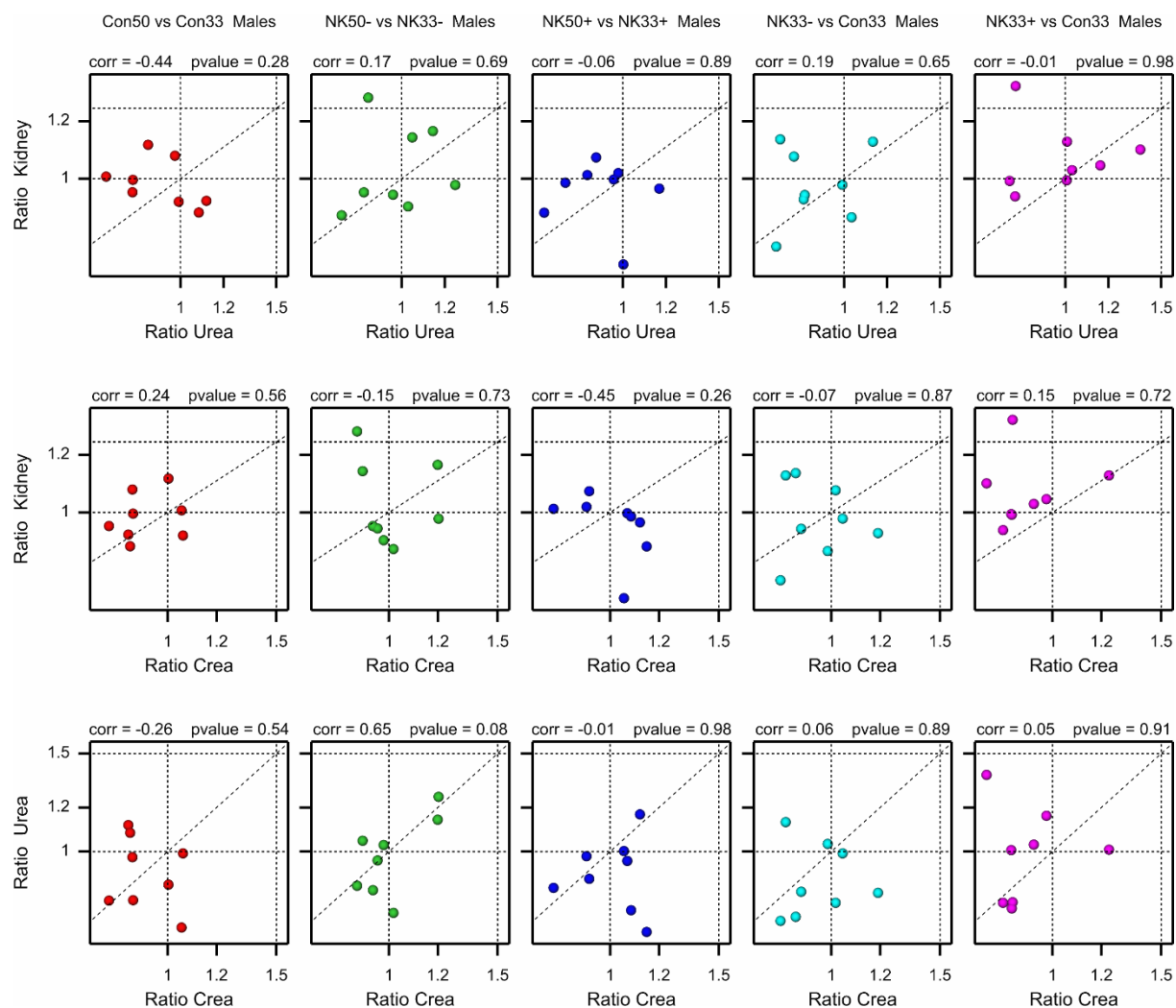
Figure 35 Pairwise results for variables with set target effect sizes related to kidney damage in males. Points represent the ratio of the cage means for the comparison given at the top of each column. Horizontal and vertical lines represent a ratio of 1 and the target effect sizes from Hong *et al* (2017).
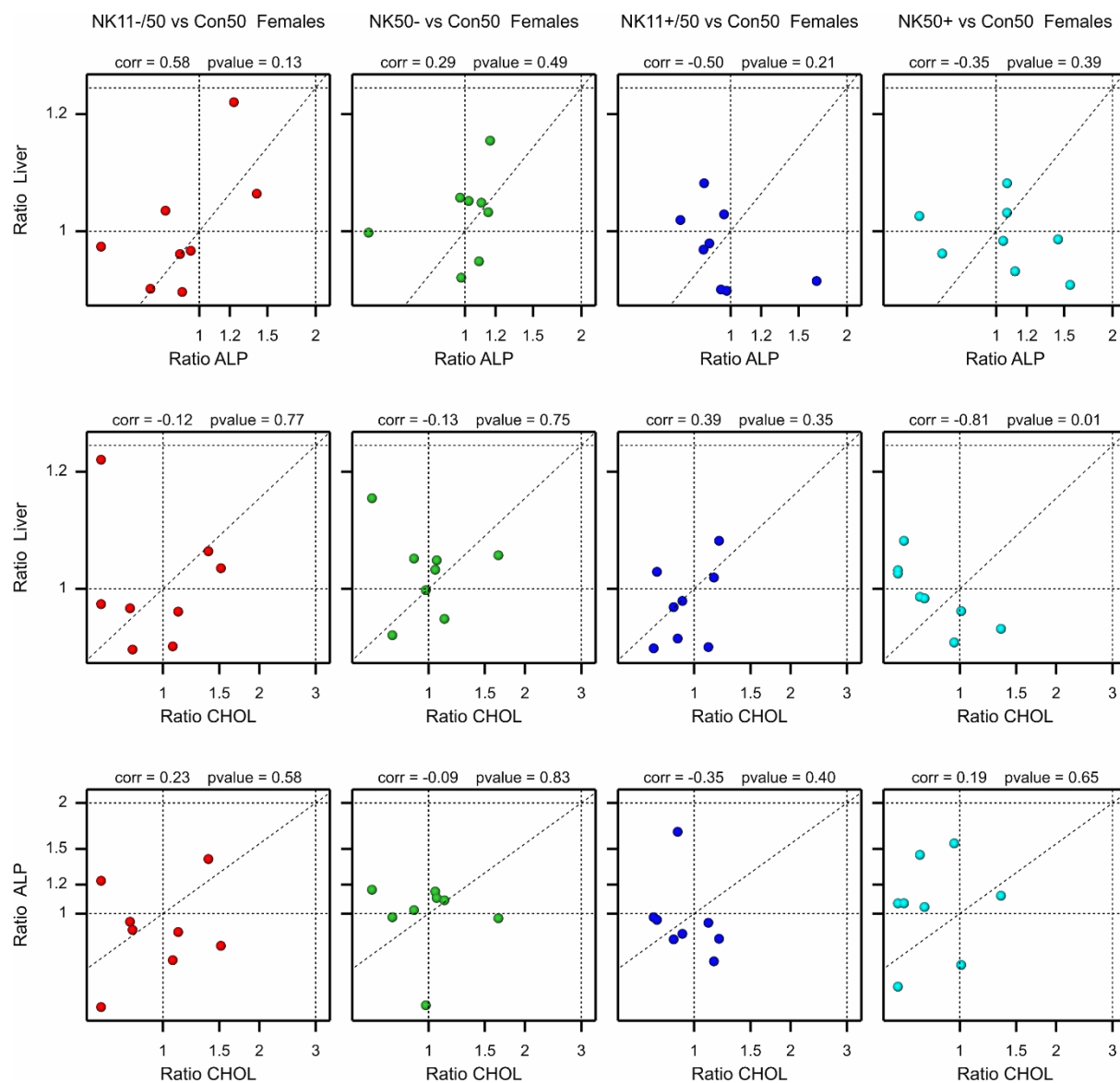
Figure 36 Pairwise results for variables with set target effect sizes related to liver damage in females. Points represent the ratio of the cage means for the comparison given at the top of each column. Horizontal and vertical lines represent a ratio of 1 and the target effect sizes from Hong *et al* (2017).
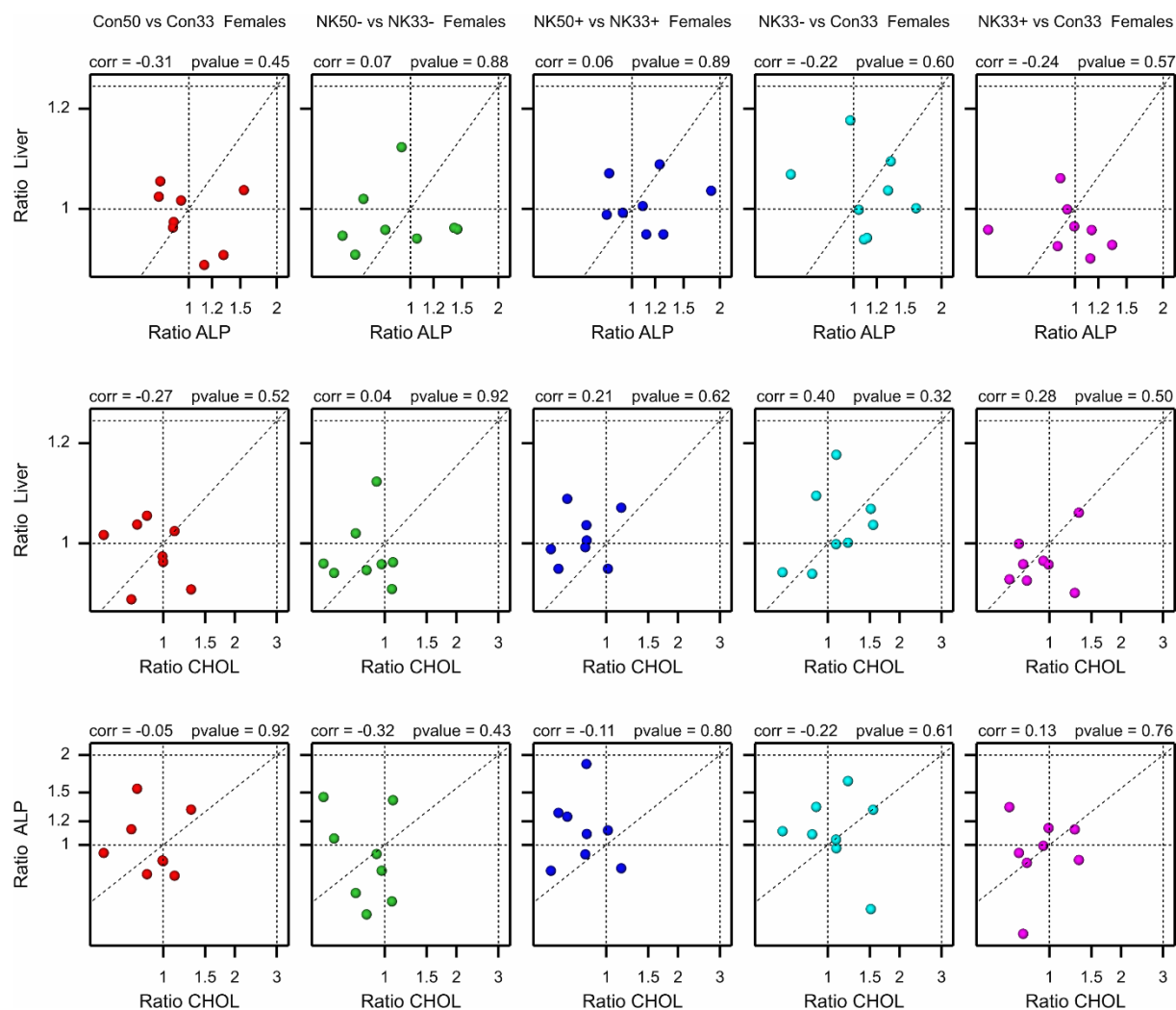
Figure 37 Pairwise results for variables with set target effect sizes related to liver damage in females. Points represent the ratio of the cage means for the comparison given at the top of each column. Horizontal and vertical lines represent a ratio of 1 and the target effect sizes from Hong *et al* (2017).
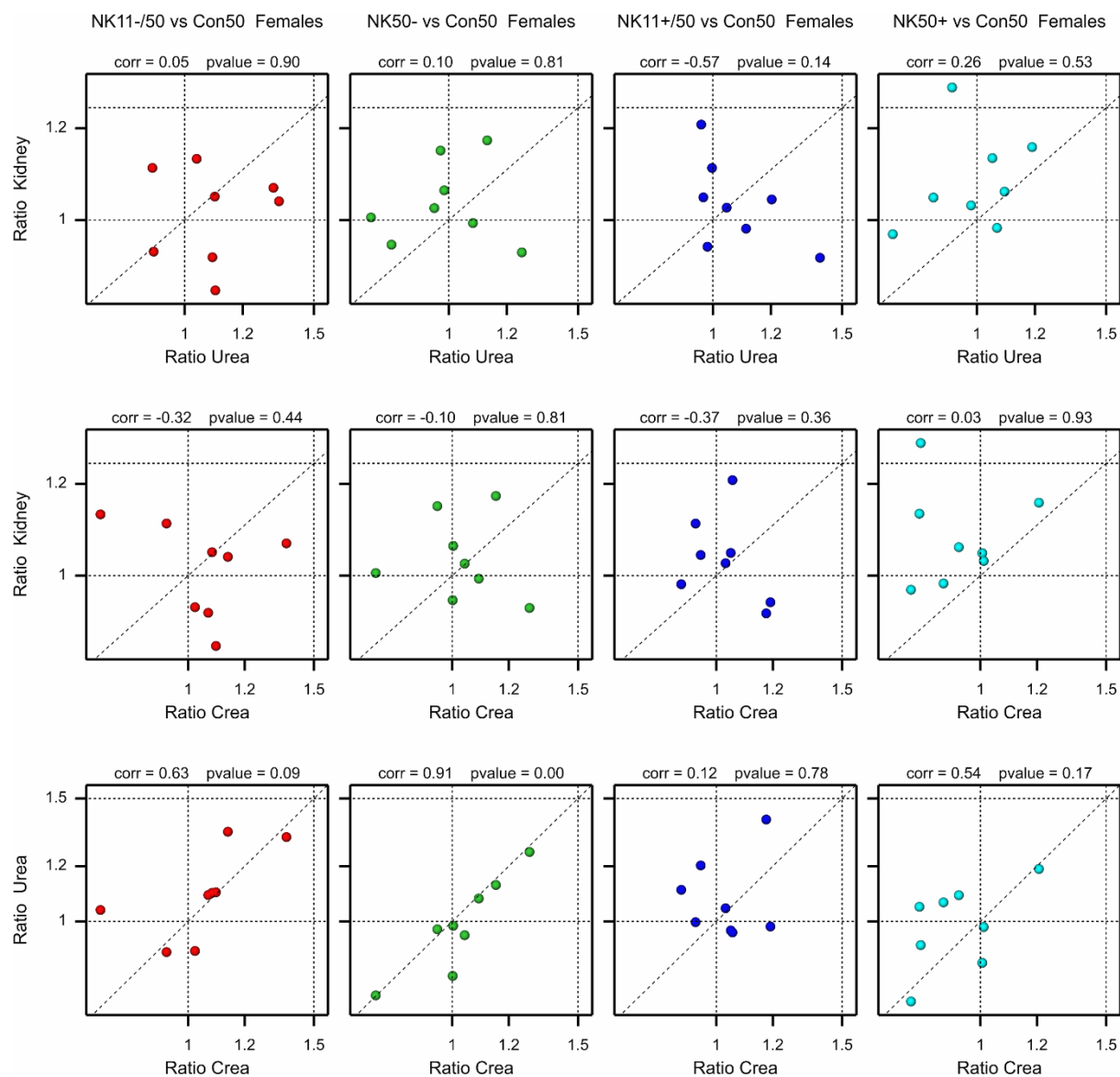
Figure 38 Pairwise results for variables with set target effect sizes related to kidney damage in females. Points represent the ratio of the cage means for the comparison given at the top of each column. Horizontal and vertical lines represent a ratio of 1 and the target effect sizes from Hong *et al* (2017).
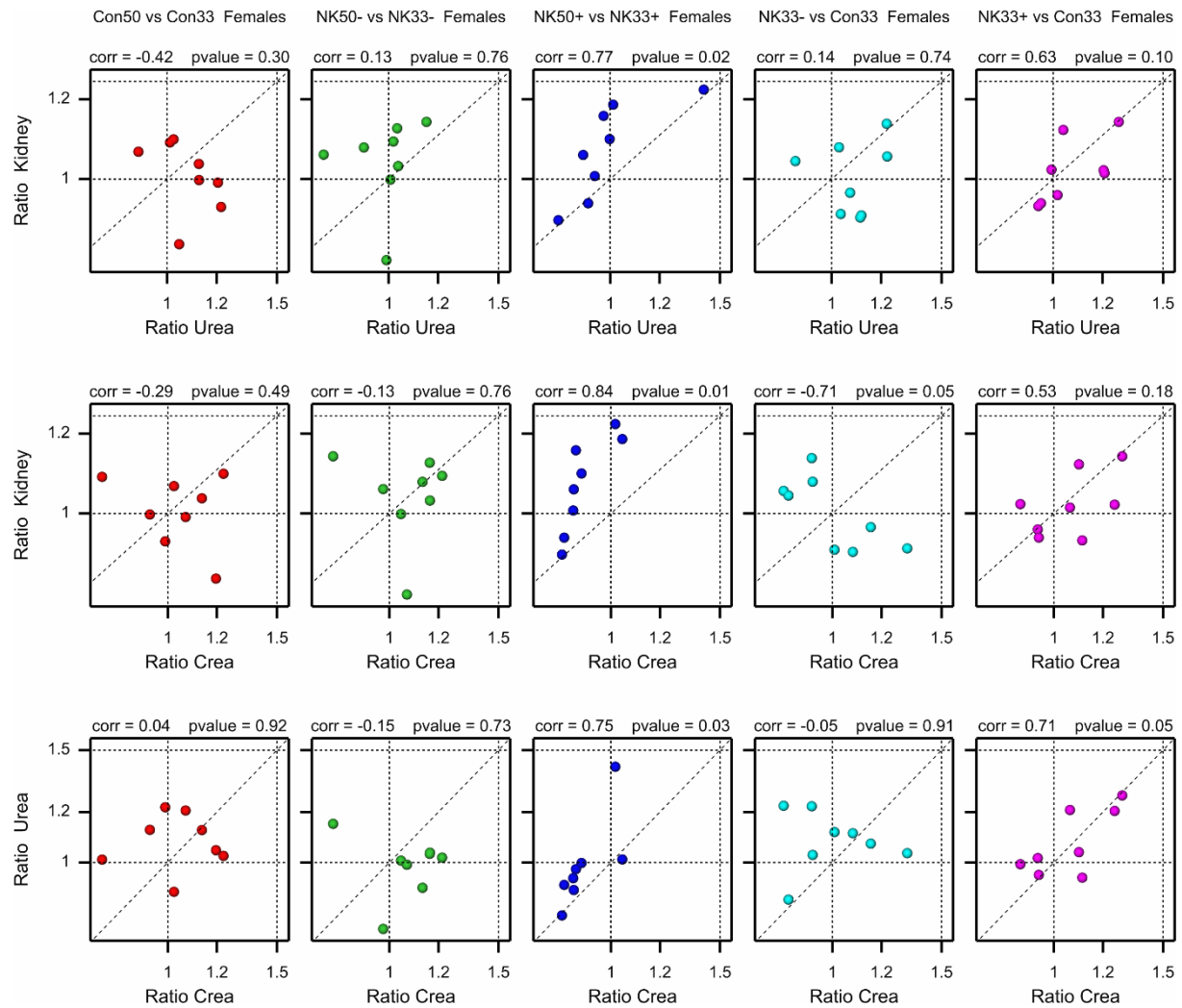
Figure 39 Pairwise results for variables with set target effect sizes related to kidney damage in females. Points represent the ratio of the cage means for the comparison given at the top of each column. Horizontal and vertical lines represent a ratio of 1 and the target effect sizes from Hong *et al* (2017).

4.7   Post-hoc power analysis comparing the 33% and 50% inclusion rates

One of the main question of this study was whether a trial with 50% maize inclusion rate has a larger power for finding differences between GM feeds and the corresponding non-GM feed, than a trial with 33% maize inclusion rate. Given the design of the experiment and the number of replications, the power of any trial is dependent on (1) the difference that is relevant, and (2) the residual standard error.

With respect to the difference, when there would indeed be a difference between the GM and non-GM feeding groups, higher inclusion rates might give rise to larger differences between groups. In Figure 40 the ratio of the mean of the two NK50 feeds (i.e. NK50- and NK50+) and the Con50 feed is expressed as a percentage of the ratio of the mean of the two NK33 feeds (i.e. NK33- and NK33+) and the Con30 feed, using the ratios in Table 11 to Table 14. For endpoints with a significant difference between the NK50 feeds or between the NK33 feeds, the mean of the NK50 feeds or the mean of the NK33 feeds might be uninformative, and therefore such ratios are denoted by a red circle. In general the observed effect for the NK50 feeds is similar to the observed effect for the NK33 feeds with notable exceptions for variables in the Urine, Immunology and CellPhenotype groups.

in Figure 41 the residual standard error of an ANOVA on the five 50% feeds only is compared to the residual standard error of an ANOVA on the three 33% feeds only. This reveals that the residual standard error for the 50% feeds is larger for almost all Immunology variables and also for the CellPhenotype variables in females. For the other categories the pattern is somewhat mixed, although all-in-all the residual standard error for the 50% feeds seems somewhat larger than for the 33% feeds.

Since the power of a statistical test depends on the ratio of the true difference and the residual standard error, the ratio of the values in Figure 40 (which displays the differences) and the values in Figure 41 (which displays the residual standard error) is displayed in Figure 42. Positive values would then imply a larger power of the statistical test for the 50% feeds as compared to the 33% feeds, and negative values would indicate smaller power. Again the pattern of positive and negative values is mixed and can be different for males and females (see e.g. the CellPhenotype variables). Only for the Immunology variables it is clear that a trial with 50% feeds will have lower power than a trial with 33% feeds.

Conclusion: There is no evidence that a trial with 50% maize inclusion rates has generally larger power, as compared to a trial with 33% inclusion rates, in finding differences between a GM feed and a control non-GM feed. For the Immunology endpoints the power of a 50% trial is likely to be lower due to the increased residual standard error.
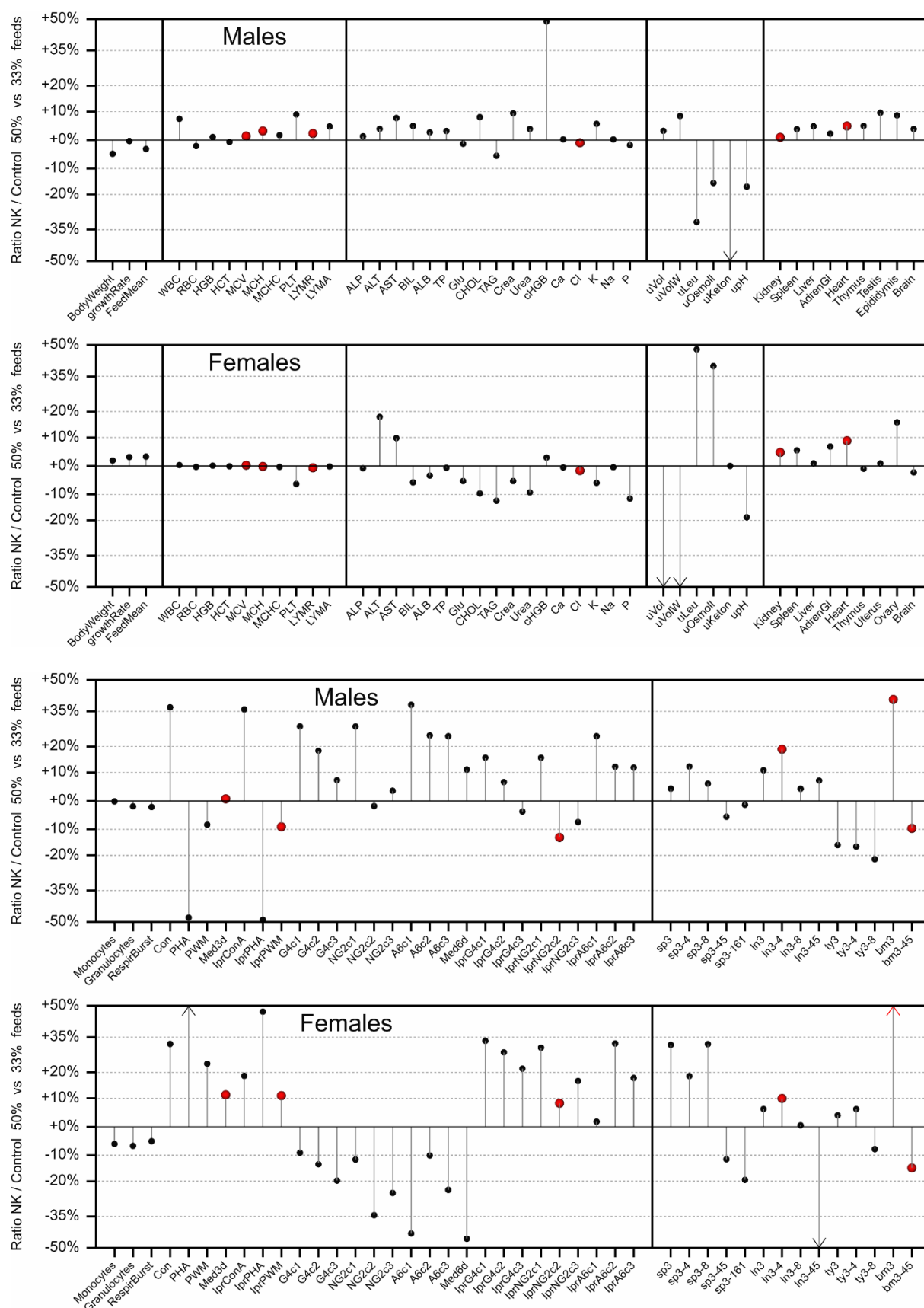
Figure 40 Observed ratio between NK50 feeds and the Con50 feed compared to the ratio between NK33 feeds and the Con33 feed. The red dots are for endpoints with a significant difference between NK50+ and NK50- and/or between NK33+ and NK33-.
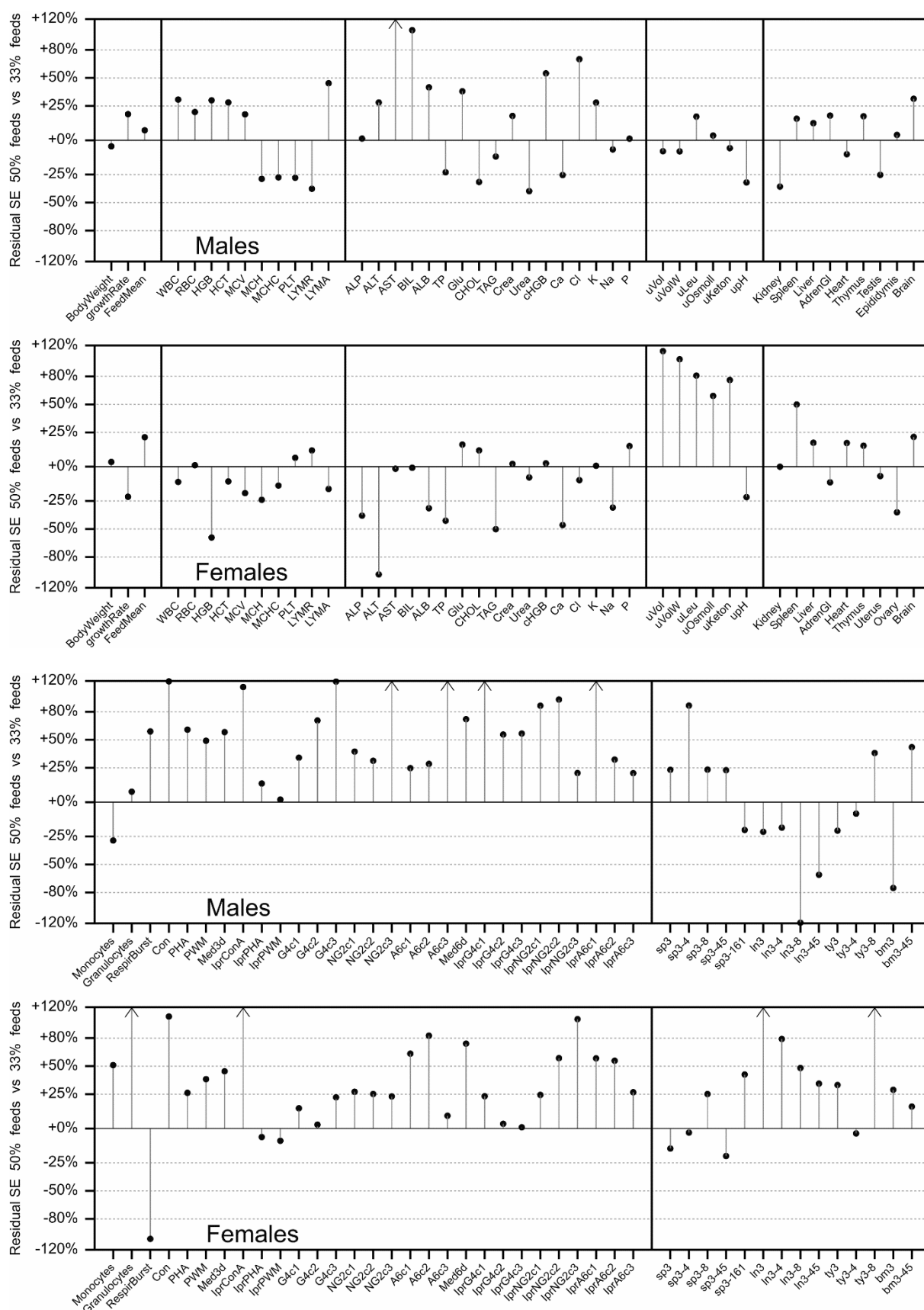
Figure 41 Residual standard error of an ANOVA for the five 50% feeds compared to the residual standard error of an ANOVA for the three 33% feeds.
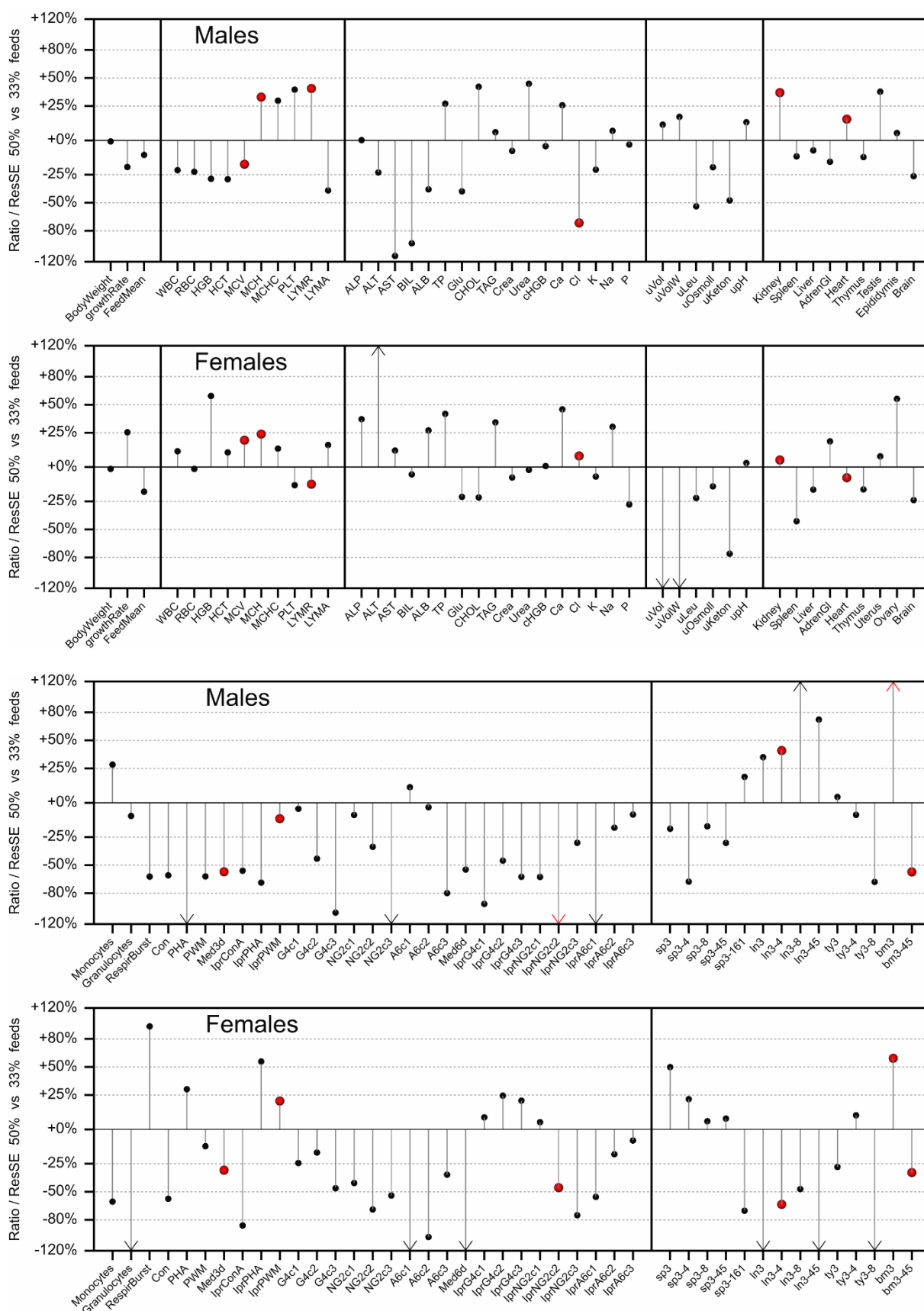
Figure 42 Observed ratio divided by the residual standard error for the 50% feeds as compared to the observed ratio divided by the residual standard error for the 33% feeds.

5   Summary and discussion

In this report the data from G-TwYST study C have been analysed following six approaches. For comparisons between a GM feeding group and the control feed, or between the two control groups with 33% or 50% maize inclusion rate, for a single variable, these approaches were two forms of equivalence analysis (4.1, 4.2), the traditional approach focusing on significant differences (4.3), and the standardised effect size (SES) approach (4.4). In addition, a factorial analysis (0) and a correlation analysis (4.6) allowed some limited forms of integration over dose groups or variables, respectively.

Among the two forms of equivalence analysis, the approach with given external equivalence limits (4.2) is the most simple one. It could be preferred if toxicologists were able to set external equivalence limits for all relevant variables based on their expert knowledge. In the current report it was applied to nine variables, for which Hong *et al* (2017) recently proposed targeted effect sizes. Obviously, the uncertainty in setting these targeted effect sizes is not accounted for in the equivalence analysis using these fixed limits.

However, external equivalence limits are often not available, and toxicologists notice many uncertainties about the impact of toxicological effects. Moreover, they find it often difficult to come to a conclusion on such equivalence limits. For such cases, the equivalence analysis which bases equivalence on historical non-GM data (4.1) may be an attractive alternative. This approach assumes that test facilities perform whole-food studies with rodents on a routine basis, such that variations between non-GM foods and between experimental units which are seen in historical studies have a relevance for the current study. In the current report, the approach could be applied to 36 variables, which were also observed in five preceding studies in the same test facility in the GRACE project. Van der Voet *et al* (2017) discusses this new method which was developed in the G-TwYST project.

Given tentative settings for regulatory parameters, equivalence was established in 100% of cases (162/162) for the approach with external equivalence limits and for 99% of cases (643/648) for the approach based on the historical GRACE data. The regulatory parameters are the testing level (set at 5%) for all approaches, and two parameters for the new G-TwYST equivalence approach: the desired power (set at 95%) and the minimum sample size per group (set at 8 experimental units). Note that test results could be different if these parameters were chosen differently.

In the five cases where equivalence was not established by means of the statistical test, the median estimate was still in the equivalence region. Therefore, in the terminology of EFSA (2011a), these cases are still classified as 'equivalence more likely than lack of equivalence'.

As noted by EFSA (2011b), separate analysis of many endpoints, most of which are not expected to differ between treatment groups, results in a large number of statistical tests. This will lead to the issue of multiple testing (multiplicity). The proportion of non-significant equivalence tests (0% or 1%) was lower than the nominal level of the tests (which was set at 5%). In this report, we have not tried to adapt equivalence tests for multiplicity. However, it should be pointed out that a recently proposed approach to adjusting for multiplicity based on the False Discovery Rate (FDR) is not appropriate. Hong *et al* (2017) used adjusted p values using the FDR method for multiplicity adjustment. This means that effectively most p values are much larger (indicating less significant differences) than in a standard unadjusted analysis. This may explain why they report that 'no treatment-related differences were observed', although there were some 150 continuous endpoints in total. This complete absence of statistically significant differences is very much at odds with what

is commonly found (e.g. in the GRACE, G-TwYST, and GMO90+ studies). Indeed, the absence of significant differences in Hong *et al* (2017) could be a direct consequence of using the FDR adjustment. It is doubtful whether the use of the FDR-correction makes sense in food safety testing (EFSA 2010). It controls false discoveries, and is therefore connected to difference testing, where false positives are considered as error of the first kind. i.e. one wants to have a small probability of erroneously reporting a difference. In the context of equivalence testing the statistical hypotheses are reversed, and false negatives are the error of the first kind, i.e. one wants to have a small probability of erroneously reporting equivalence. Consequently, the FDR concept is addressing the wrong type of error.

Classical analysis following OECD guidance is only focusing on finding differences, not equivalences. If performed using t-tests after applying an ANOVA model, there were 90 significant differences at the 5% significance level. This is 6% of the total number of comparisons (1584), and slightly higher than the nominal 5% level that could be expected. The scheme advocated by OECD contains several adaptations. First, a multiplicity correction by using Dunnett's test rather than the straight-forward t-test is proposed for the fact that four groups are compared at the same time to the control group. Thus, applying Dunnett's test the number of significant cases at the 5% significance level was reduced to 17 (1% of all comparisons, i.e. similar to the nominal error level). However, a multiplicity correction may be wrong for the same reason why the FDR method was wrong: if we are primarily interested in safety and equivalence, then the roles of the statistical hypotheses are reversed, and corrections as used in Dunnett's test address the wrong type of error.

OECD (2012) also proposes non-parametric tests in case of non-normality or heterogeneity of variance. For the current set of variables (176, i.e. 88 for males and 88 for females) 18 variables (10%) showed non-normality of ANOVA residuals in a Shapiro-Wilks test at the 5% level, while in a Shapiro-Wilks test at the 1% level 10 variables were significant (Appendix 13). 23 variables (13%) showed variance heterogeneity in a Bartlett's or Levene's test at the 5% level. Among all 1584 comparisons the non-parametric Wilcoxon's test resulted in 45 significant differences (3%); note that this tests also requires homogeneity of variance. Despite these findings, i.e. non-normality or variance heterogeneity, the normal probability plots (Appendix 5) and the plots of residuals versus fitted values (Appendix 6) were generally satisfactory. This suggests that parametric t-tests and Dunnett tests can safely be applied.

In this report confidence intervals were also expressed and plotted as Standardised Effect Size (SES), see EFSA (2011b), in order to allow a comparison with SES results for the preceding GRACE project (Schmidt and Schmidtke 2014, Schmidt *et al* 2015ab, 2016, 2017, Zeljenková *et al* 2014, 2016). SES, also known as Cohen's d, is often used in meta-analyses to show the results of different variables in the same plot. Reporting and graphically displaying effect sizes was described in Schmidt *et al* (2016) as a way 'to avoid the yes/no decision trap of statistical tests and to illustrate the size of effects in the context of biological relevance'. However, in the absence of clear limit values for biological relevance, these authors had to build on the arbitrary EFSA example, where effects of ± 1 SD were assumed to be unlikely to be of toxicological importance. Schmidt *et al* (2016) already concluded that the pooled standard deviation SD of individual measurements 'is a priori not expected to be directly related to biological relevance', and Schmidt *et al* (2017) warned that 'it should therefore be kept in mind that future decisions on relevant equivalence limits may influence the equivalence results'. The results of the current G-TwYST study, where 98% of all intervals extended outside the ± 1 SD limits, confirms

the pattern observed in GRACE and in G-TwYST studies A and B. Whereas, displaying the confidence intervals indeed gives a richer view on the results than just reporting yes/no decisions, the scale of the SES plots does not seem the best choice for equivalence assessments. As Hong *et al* (2017) remark, the value of SES to support data interpretation is limited. Alternatively scaled effect sizes, such as those presented in section 4.1 (Figure 6 - Figure 9) can be preferred, because the scaling factor (the equivalence limit) is based on data analysis of in this case historical data, rather than being an arbitrary value. It can be noted, however, that this approach was not available for the GRACE project, because of lack of historical data in the same test facility.

Factorial analyses for single variables allowed to consider effects pooled over more than two groups, thus providing more powerful tests for main effects in the absence of interaction. However, this approach was in the current work restricted to the testing of differences. In principle, it could be further developed for the equivalence tests.

Most statistical analyses in this report have considered variables one by one, collecting the results only in a joint table or plot for ease of interpretation. However, toxicologists often stress that effects should be judged together. Wherever a prior hypothesis exists that links multiple variables, these may sometimes be translated in a function of those variables. For example, there is a biological connection between the pancreas and the regulation of glucose, which leads to a prior expectation of a negative correlation between pancreas weight and serum glucose. It may then be sensible to perform difference and equivalence testing for an additional variable such as the ratio or log-ratio of these variables. Such ratios have not been defined in the current study.

Another tool to study variables together is pairwise plotting of results per experimental unit. In section 4.6 (Figure 32 - Figure 39) this was done for three variables related to liver damage and three variables related to kidney damage. The rationale was that correlations between variables would show up in these plots, but this was hardly observed in these cases. To assist in the interpretation, the effects were plotted together with proposed target effect sizes. Most effects were below these limits also at the cage level (as was already observed for the means in section 4.2). We may conclude that correlations between these variables related to the same organ are nevertheless not prominent as long as the effects are within the targeted range. Of course, correlations could be (and are expected to be) more evident for effect sizes that would exceed the limits by large amounts.

A more detailed approach to testing than reported here would also be possible based on a more detailed consultation with toxicologists. For example, nephrotoxic effects can lead to decreased or increased kidney weights. However, in both of these cases, the toxicologists would expect to see increased urea (Urea) and/or creatinine (Crea) levels. In addition, there might be a decreased level of glucose in the urine (Glu) or an increased level of amino acids, but these effects are less predictable. Increases in Urea or Crea may indicate nephrotoxic effects that are not yet discernible as deviating kidney weights. It is concluded that increased Urea and/or Crea levels are the primary indicators of kidney damage, and only increased levels represent a toxicological concern. Considering observed normal ranges, an increase by 50% in at least one of the two key variables could be seen as potentially concerning, and provide a level to be used as equivalence limit. Specific hypotheses to be tested for the differences Δ (on the log scale) between the treatment groups (GM vs. comparator) would then be as follows.

Difference tests:

$$H_0: \ E(\Delta_{Urea}) = 0 \qquad \text{vs.} \qquad H_1: \ E(\Delta_{Urea}) > 0$$

$$H_0: \ E(\Delta_{Crea}) = 0 \qquad \text{vs.} \qquad H_1: \ E(\Delta_{Crea}) > 0$$

Equivalence tests:

$$H_0: \ E(\Delta_{Urea}) = EL_{Urea} \qquad \text{vs} \qquad H_1: \ E(\Delta_{Urea}) < EL_{Urea}$$

$$H_0: \ E(\Delta_{Crea}) = EL_{Crea} \qquad \text{vs.} \qquad H_1: \ E(\Delta_{Crea}) < EL_{Crea}$$

where $EL_{Urea} = \ EL_{Crea} = \log(1.5)$.

In cases when a difference is found or an equivalence cannot be shown, the other variables (kidney weight, Glu, amino acids) may provide further interpretation to the toxicologist. These variables are therefore considered as secondary: the results can be summarised in terms of absolute values and confidence intervals for Δ (also shown graphically), but they would not be part of the testing framework based on primary variables. However, fine-tuning of statistical analyses as suggested here demands a large investment of time from both toxicologists and statisticians, and it will be very difficult to perform such exercises across the whole spectrum of endpoints.

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List of Appendices

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Table 14   95% Confidence interval plus estimate for the ratio Δ of the feeds with 50% maize inclusion rate versus the corresponding 33% inclusion rate for females, and for the GM 33% feeds versus the non-GM 33% feed. Ratios with corresponding 95/99% intervals that do not encompass the value 1 are coloured <mark style="background-color:#f4b8b8">red</mark>/<mark style="background-color:#e5b84b">gold</mark>; this is equivalent to a significant difference according to a 5/1% t-test.

Table 15   Targeted effect sizes from Table 1 in Hong *et al* (2017) along with their implied lower and upper limits on the ratio scale and on the log-ratio scale.

Table 16   P-values of equivalence tests for the ratio of two means using targeted effect sizes of Hong et al (2017), see Table 15, as equivalence limits. P-values smaller than 0.01/0.05 have a gold/yellow background.

Table 17   Means per feeding group and results of statistical tests for nine comparisons for male rats. Significant difference are marked, with red background colouring, as follows: *D*: P<0.01 by Dunnett-test, *d*: P<0.05 by Dunnett-test, *T*: P<0.01 by t-test but not by Dunnett-test, *t*: P<0.05 by t-test but not by Dunnett-test, *W*: P<0.01 by Wilcoxon signed rank test, *w*: P<0.05 by Wilcoxon signed rank test. See text.

Table 18   Means per feeding group and results of statistical tests for nine comparisons for female rats. Significant difference are marked, with red background colouring, as follows: *D*: P<0.01 by Dunnett-test, *d*: P<0.05 by Dunnett-test, *T*: P<0.01 by t-test but not by Dunnett-test, *t*: P<0.05 by t-test but not by Dunnett-test, *W*: P<0.01 by Wilcoxon signed rank test, *w*: P<0.05 by Wilcoxon signed rank test. See text.

Table 19   Diets organized as a 2 × 3 factorial with treatments MaizeRate with 2 levels (33% and 50%) and MaizeType with three levels (Control, NK- and NK+), with additional treatments NK11-/50 and NK11+/50.

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Table 21   P values for significance of contrasts obtained for the factorial model. Type1 / Type2 denote contrasts MaizeType.GM / MaizeType.RoundUp, while Int1 / Int2 denote the interaction contrasts MaizeRate.MaizeType.GM / MaizeRate.MaizeType.RoundUp. P-values smaller than 0.01/0.05 have a <mark style="background-color:#e5b84b">gold</mark>/<mark style="background-color:#ffff00">yellow</mark> background.

Table 22   Ratios for significant contrasts for males and females according to the testing sequence for the factorial model (see text).

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