

DESIGN AND MIXED-MODEL ANALYSIS OF EXPERIMENTS

XII. Repeated measurements experiments

(Payne, 1996; Mead, sec. 14.5)

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XII.A Introduction to repeated measurements experiments

Definition XII.1: A **repeated measurements experiment** is one in which a sequence of measurements is made under constant treatment conditions. Generally, there will be u units (plots, animals and so) each of which is observed for s_k times. ■

Thus repeated measurement experiments are distinguished from split-plot experiments in which each unit is observed only once and from cross-over experiments in which each unit is observed more than once but the treatment conditions are not the same for all observations. Also, experiments involving the observation of different plots at different times are not repeated measurements experiments. That is, just because an experiment involves observations at different times, does not mean that it is necessarily a repeated measurements experiment.

Definition XII.2: A repeated measures experiment in which all units are observed for the same time intervals relative to the start of the experiment is called a **balanced repeated measurements experiment**. ■

Definition XII.3: The set of measurements made on a unit is called the unit's **profile**. ■

The design of repeated measurement experiments involves no special consideration. One has only to decide on the design to be applied to the units, most likely using one of the designs covered in the other chapters of this subject. For example, one might choose to run a factorial experiment laid out using an RCBD. This having been decided the experiment is laid out according to the chosen design and the repeated observations made on each unit.

There are now available several methods for the analysis of repeated measurements. These vary from an analysis of variance to complicated analyses allowing for non-linear fitting of trends and complex covariance structures between the observations from different times. We will be focussing on analysis of variance methods, although these have the limitation of often involving over-simplified models.

XII.B Repeated measurements versus split-plot experiments

In this section we consider the application of the analysis of variance to repeated measurements experiments and explore how this differs from that for split-plot experiments. In many textbooks you will see suggested that the analysis of variance for a repeated measurements experiment is a split-plot-in-time, with times being regarded as randomized to hypothetical subplots. For example, suppose that the experiment was laid out as an RCBD and then repeated measurements were made on each plot. The suggested analysis would be that for the standard split-plot experiment with the plots from the RCBD being treated as main plot and the times as being randomized to hypothetical subplots. However, the times are not randomized — they are the result of systematic observation of the plots. Further times are not nested within subplots, as is often the case with split-plot experiments, but time is crossed with plots in that each time is the same for all units. So this type of experiment is not of the split-plot kind.

First we consider experiments in which time is randomized. The first two exercises for chapter XI are examples of such experiments as they involved time factors that were randomized to subplots using a split-plot design.

Example XII.1 Celery experiment

Exercise XI.1 involved an experiment to investigate the effect on the yield of three methods of seedling propagation, two levels of nutrient and four harvest dates. The six combinations of propagation methods and nutrients were applied to main plots using a completely randomized design with three replicates of each treatment combination. The harvest dates were to be randomized to the four subplots within each main plot. The analysis of variance that was derived for this experiment was of the following form:

Source	df	E[MSq]		
MainPlots	17			
Propagation	2	σ_{MS}^2	$+4\sigma_M^2$	$+f_P(\psi)$
Nutrient	1	σ_{MS}^2	$+4\sigma_M^2$	$+f_N(\psi)$
Propagation.Nutrient	2	σ_{MS}^2	$+4\sigma_M^2$	$+f_{PN}(\psi)$
Residual	12	σ_{MS}^2	$+4\sigma_M^2$	
MainPlots.Subplots	54			
Harvests	3	σ_{MS}^2		$+f_H(\psi)$
Propagation.Harvests	6	σ_{MS}^2		$+f_{PH}(\psi)$
Nutrient.Harvests	3	σ_{MS}^2		$+f_{NH}(\psi)$
Propagation.Nutrient.Harvests	6	σ_{MS}^2		$+f_{PNH}(\psi)$
Residual	36	σ_{MS}^2		
Total	71			

Here the factor Harvests corresponds to times of harvest so that the experiment involves tracing the response variable, yield, over a period of time. However, the experiment is not a repeated measurements experiment because we did not repeatedly observe the same plot.

We next consider a repeated measurements experiment.

Example XII.2 Clones observed over several years

Consider a randomized complete block experiment in which several clones of some perennial crop are to be compared. The yield for each plot is measured in successive years without any change in the experimental layout. Generated data for such an experiment are given in the table below.

**Layout and results
for the repeated measurements experiment**

Blocks	Years	Plots					
		1		2		3	
		Clone	Yield	Clone	Yield	Clone	Yield
1	1	1	148.8	2	152.7	3	159.9
	2		142.4		142.3		150.6
	3		146.9		141.9		157.7
	4		155.4		142.9		152.2
2	1	3	160.5	1	160.5	2	156.2
	2		152.1		162.0		150.9
	3		136.7		148.1		135.0
	4		151.9		164.4		149.8
3	1	3	158.6	2	151.2	1	152.3
	2		157.5		145.1		156.6
	3		145.6		135.7		145.6
	4		163.1		154.6		165.3
4	1	1	152.5	3	159.0	2	151.4
	2		154.4		158.0		145.7
	3		162.7		166.5		151.6
	4		162.3		164.0		147.5
5	1	1	152.1	3	153.2	2	148.5
	2		158.4		149.1		144.4
	3		168.3		157.4		153.6
	4		168.2		151.7		144.7

The split-plot-in-time analysis for the generated set of data is presented in table below. In working out the expected mean squares for this analysis, Years is taken to be a fixed factor as it is unlikely that Years will display the necessary homogeneity to be regarded as a variation factor. From this analysis we conclude that there is no interaction between Clones and Years and no overall differences between the Years but that there are overall differences between the Clones.

**Split-plot-in-time analysis
for the repeated measurements experiment**

Source	df	MSq	E[MSq]	F	Prob
Blocks	4	75.38	$\sigma_{BPY}^2 + 4\sigma_{BP}^2 + 12\sigma_B^2$	1.35	0.332
Blocks.Plots	10				
Clones	2	490.52	$\sigma_{BPY}^2 + 4\sigma_{BP}^2 + f_C(\psi)$	8.77	0.010
Residual	8	55.96	$\sigma_{BPY}^2 + 4\sigma_{BP}^2$	1.50	0.192
Blocks.Plots.SubPlots	45				
Years	3	105.57	$\sigma_{BPY}^2 + f_Y(\psi)$	2.84	0.051
Clones.Years	6	48.52	$\sigma_{BPY}^2 + f_{CY}(\psi)$	1.30	0.282
Residual	36	37.22	σ_{BPY}^2		

a) Determining the analysis of variance table

We now derive the analysis for a repeated measurements experiment using the rules for determining the analysis of variance table.

Example XII.2 Clones observed over several years (continued)

What are the components of the study?

1. Observational unit – a plot in a year
2. Response variable – Yield
3. Unrandomized factors – Blocks, Plots, Years
4. Randomized factors – Clones
5. Type of study – Repeated measurements on an RCBD

The experimental structure is:

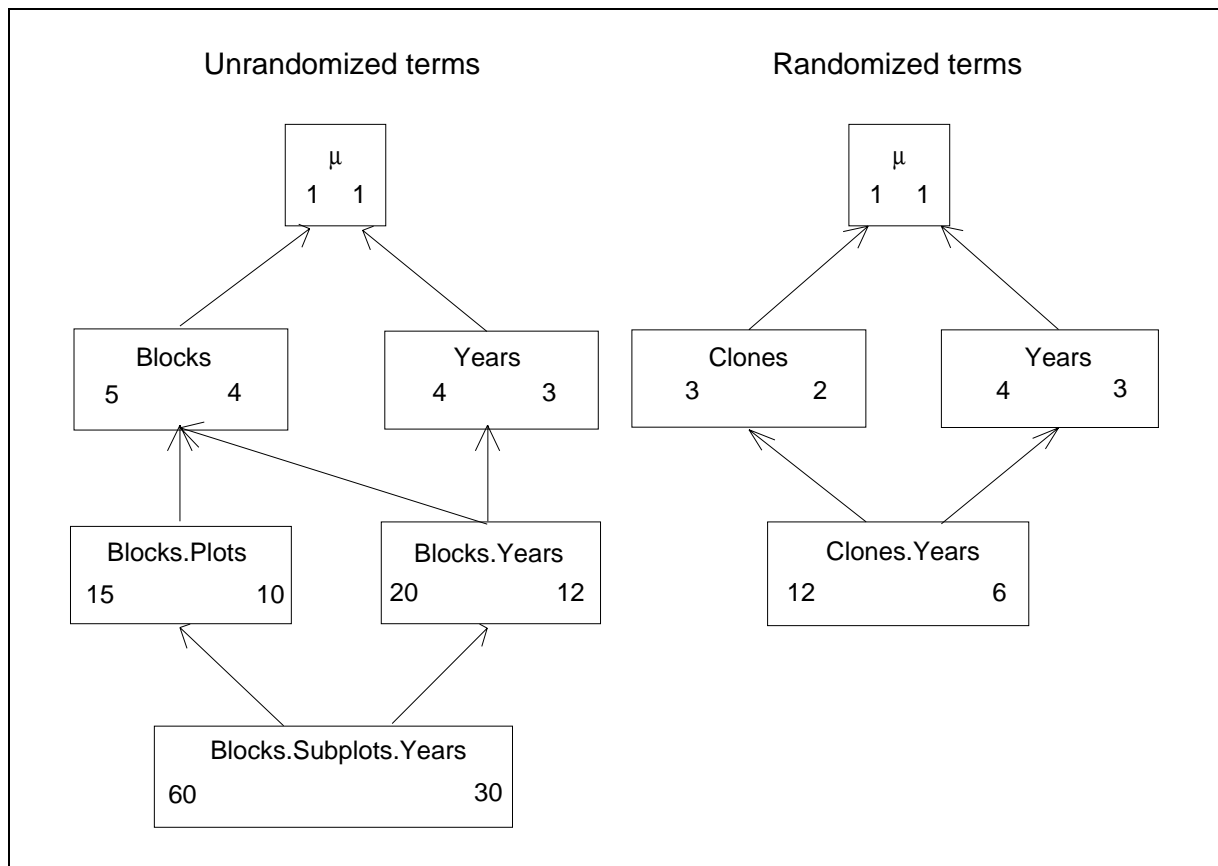
Structure	Formula
unrandomized	(5 Blocks/3 Plots)*4 Years
randomized	3 Clones*Years

Note that we have included the factor Years in the randomized structure because we are interested its interaction with Clones.

The formulae expand to give:

$$\begin{aligned}
 & (\text{Blocks/Plots}) * \text{Years} \\
 &= (\text{Blocks} + \text{Blocks.Plots}) * \text{Years} \\
 &= \text{Blocks} + \text{Blocks.Plots} + \text{Years} + \text{Blocks.Years} + \text{Blocks.Plots.Years}
 \end{aligned}$$

and $\text{Clones} \times \text{Years} = \text{Clones} + \text{Years} + \text{Clones} \cdot \text{Years}$



Note, that in working out the degrees of freedom for the terms from the randomized structure, the rule for a set of crossed factors can be used. That is, for each factor in the term, calculate the number of levels minus one and multiply these together.

The models for this experiment, based on Blocks and Plots being random factors and Clones and Years being fixed factors are:

$$E[Y] = \text{Clones} \cdot \text{Years}$$

$$\text{and } \text{var}[Y] = \text{Blocks} + \text{Blocks} \cdot \text{Plots} + \text{Blocks} \cdot \text{Years} + \text{Blocks} \cdot \text{Plots} \cdot \text{Years}.$$

The analysis of variance table for this experiment has the following form:

**Skeleton analysis of variance table
for the repeated measurements experiment**

Source	df	E[MSq]			
Blocks	4	σ_{BPY}^2	$+4\sigma_{BP}^2$	$+3\sigma_{BY}^2$	$+12\sigma_B^2$
Blocks.Plots	10				
Clones	2	σ_{BPY}^2	$+4\sigma_{BP}^2$		$+f_C(\psi)$
Residual	8	σ_{BPY}^2	$+4\sigma_{BP}^2$		
Years	3	σ_{BPY}^2		$+3\sigma_{BY}^2$	$+f_Y(\psi)$
Blocks.Years	12	σ_{BPY}^2		$+3\sigma_{BY}^2$	
Blocks.Plots.Years	30				
Clones.Years	6	σ_{BPY}^2			$+f_{CY}(\psi)$
Residual	24	σ_{BPY}^2			
Total	59				

The analysis of variance table includes a source for Blocks.Years, a term that was not included in the split-plot-in-time analysis. It should be included unless one is willing to argue that it is unlikely to occur. If this is an appreciable source of variability, one may get quite different results from an analysis that separates this term compared to one that does not. Further it is noted that a test for Years has Blocks.Years as the denominator, rather than Blocks.Plots.Years. However, this would not be the case if both Blocks and Years were designated as fixed factors.

b) Analysis of the example

The analysis in Genstat will start with plots of the profiles using the DREPMEASURES procedure. The analysis of variance will be conducted on the data and the usual diagnostic checking performed. Because time is a quantitative factor, we will investigate whether a linear trend can be used to describe the time trend for this data.

Example XII.2 Clones observed over several years (continued)

The Genstat output file for analyzing this experiment is:

Genstat 5 Release 4.1 (PC/Windows NT) 29 April 2000 11:30:52
 Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

Genstat 5 Fourth Edition - (for Windows)
 Genstat 5 Procedure Library Release PL11

```
3 "Data taken from File: D:/ANALYSES/LM/REPEATMEASURE/RMECLONE.GSH"
4 DELETE [redefine=yes] Blocks,Plots,Years,Clones,Yields
5 FACTOR [modify=yes;nvalues=60;levels=5] Blocks
6 READ Blocks; frepresentation=ordinal
```

Identifier	Values	Missing	Levels
Blocks	60	0	5

```
9 FACTOR [modify=yes;nvalues=60;levels=3] Plots
10 READ Plots; frepresentation=ordinal
```

Identifier	Values	Missing	Levels
Plots	60	0	3

```
13 FACTOR [modify=yes;nvalues=60;levels=4] Years
14 READ Years; frepresentation=ordinal
```

Identifier	Values	Missing	Levels
Years	60	0	4

```
17 FACTOR [modify=yes;nvalues=60;levels=3] Clones
18 READ Clones; frepresentation=ordinal
```

Identifier	Values	Missing	Levels
Clones	60	0	3

```
21 VARIATE [nvalues=60] Yields
22 READ Yields
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Yields	135.0	153.0	168.3	60	0

```
28
29 PRINT Blocks,Plots,Years,Clones,Yields
```

Blocks	Plots	Years	Clones	Yields
1	1	1	1	148.8
1	2	1	2	152.7
1	3	1	3	159.9
1	1	2	1	142.4
1	2	2	2	142.3
1	3	2	3	150.6
1	1	3	1	146.9
1	2	3	2	141.9
1	3	3	3	157.7
1	1	4	1	155.4
1	2	4	2	142.9
1	3	4	3	152.2
2	1	1	3	160.5
2	2	1	1	160.5
2	3	1	2	156.2
2	1	2	3	152.1
2	2	2	1	162.0
2	3	2	2	150.9
2	1	3	3	136.7
2	2	3	1	148.1
2	3	3	2	135.0
2	1	4	3	151.9
2	2	4	1	164.4
2	3	4	2	149.8
3	1	1	3	158.6
3	2	1	2	151.2
3	3	1	1	152.3


```

3      1      2      3      157.5
3      2      2      2      145.1
3      3      2      1      156.6
3      1      3      3      145.6
3      2      3      2      135.7
3      3      3      1      145.6
3      1      4      3      163.1
3      2      4      2      154.6
3      3      4      1      165.3
4      1      1      1      152.5
4      2      1      3      159.0
4      3      1      2      151.4
4      1      2      1      154.4
4      2      2      3      158.0
4      3      2      2      145.7
4      1      3      1      162.7
4      2      3      3      166.5
4      3      3      2      151.6
4      1      4      1      162.3
4      2      4      3      164.0
4      3      4      2      147.5
5      1      1      1      152.1
5      2      1      3      153.2
5      3      1      2      148.5
5      1      2      1      158.4
5      2      2      3      149.1
5      3      2      2      144.4
5      1      3      1      168.3
5      2      3      3      157.4
5      3      3      2      153.6
5      1      4      1      168.2
5      2      4      3      151.7
5      3      4      2      144.7

30  "
-31  **** separate data for years and plot profiles
-32  "
33  SUBSET [CONDITION=Years==1] OLD=Blocks,Plots,Clones; \
34      NEW=Block,Plot,Clone
35  FOR i=1...4
36      SUBSET [CONDITION=Years==i] OLD=Yields; NEW=Yield[i]
37  ENDFOR
38  PRINT Block,Plot,Clone,Yield[1...4]; FIELD=9; DEC=1

Block      Plot      Clone Yield[1] Yield[2] Yield[3] Yield[4]
1          1          1      148.8    142.4    146.9    155.4
1          2          2      152.7    142.3    141.9    142.9
1          3          3      159.9    150.6    157.7    152.2
2          1          3      160.5    152.1    136.7    151.9
2          2          1      160.5    162.0    148.1    164.4
2          3          2      156.2    150.9    135.0    149.8
3          1          3      158.6    157.5    145.6    163.1
3          2          2      151.2    145.1    135.7    154.6
3          3          1      152.3    156.6    145.6    165.3
4          1          1      152.5    154.4    162.7    162.3
4          2          3      159.0    158.0    166.5    164.0
4          3          2      151.4    145.7    151.6    147.5
5          1          1      152.1    158.4    168.3    168.2
5          2          3      153.2    149.1    157.4    151.7
5          3          2      148.5    144.4    153.6    144.7

39  DREPMEASURES [GROUPS=Clone,Block] DATA=Yield
40  DREPMEASURES [GROUPS=Clone] DATA=Yield
41  "
-42  **** perform repeated measurements ANOVA
-43  "
44  DUPLICATE OLD=Years; NEW=Year
45  BLOCK (Blocks/Plots)*Years
46  TREAT Clones*POL(Year;2)
47  ANOVA [FPROB=Y; PSE=LSD] Yields

```

47.....

***** Analysis of variance *****

Variate: Yields

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	4	302.268	75.567		
Years stratum					
Year	3	315.939	105.313		
Lin	1	6.931	6.931		
Quad	1	293.046	293.046		
Deviations	1	15.962	15.962		
Blocks.Plots stratum					
Clones	2	981.567	490.783	8.74	0.010
Residual	8	449.371	56.171	14.76	
Blocks.Years stratum	12	1247.572	103.964	27.33	
Blocks.Plots.Years stratum					
Clones.Year	6	290.200	48.367	12.71	<.001
Clones.Lin	2	266.222	133.111	34.99	<.001
Clones.Quad	2	7.267	3.633	0.96	0.399
Deviations	2	16.711	8.355	2.20	0.133
Residual	24	91.309	3.805		
Total	59	3678.226			

* MESSAGE: the following units have large residuals.

Blocks 2 Years 3 -9.66 s.e. 4.56
 Blocks 1 Plots 3 Years 3 2.98 s.e. 1.23

***** Tables of means *****

Variate: Yields

Grand mean 152.97

Clones	1	2	3		
	156.36	147.29	155.27		
Year	1	2	3	4	
	154.49	151.30	150.22	155.87	
Clones	Year	1	2	3	4
1		153.24	154.76	154.32	163.12
2		152.00	145.68	143.56	147.90
3		158.24	153.46	152.78	156.58

*** Least significant differences of means (5% level) ***

Table	Clones	Year	Clones
			Year
rep.	20	15	5
l.s.d.	5.465	*	*
d.f.	8	*	*
Except when comparing means with the same level(s) of			
Year			5.696
d.f.			11.42

```

48  CALC pBP=1-FPROB(56.171/3.805; 8; 24)
49  &    pBY=1-FPROB(103.964/3.805; 12; 24)
50  PRINT pBP,pBY

```

```

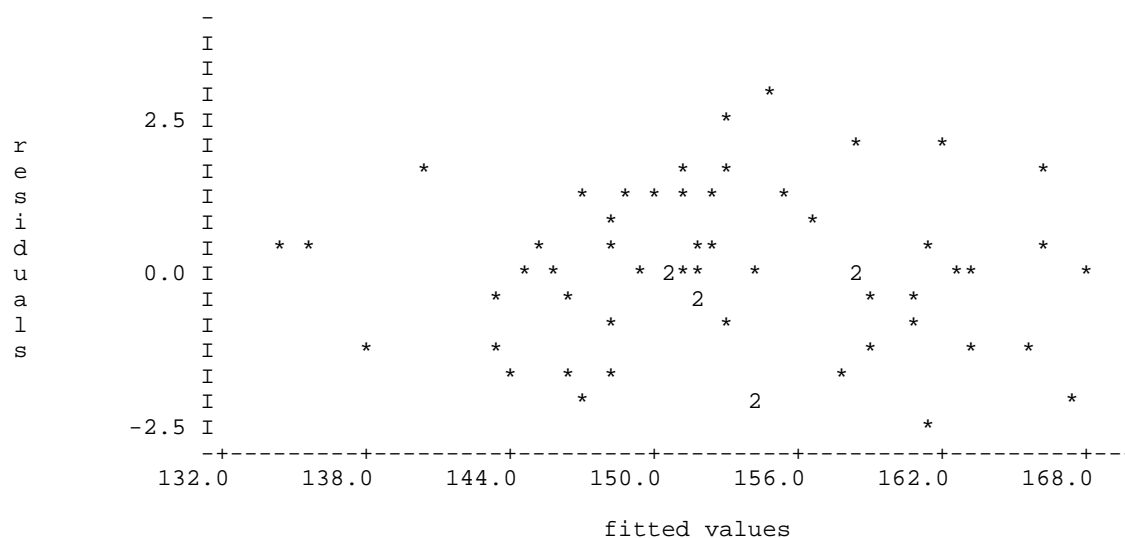
      pBP      pBY
0.000000148  0.4532E-10

```

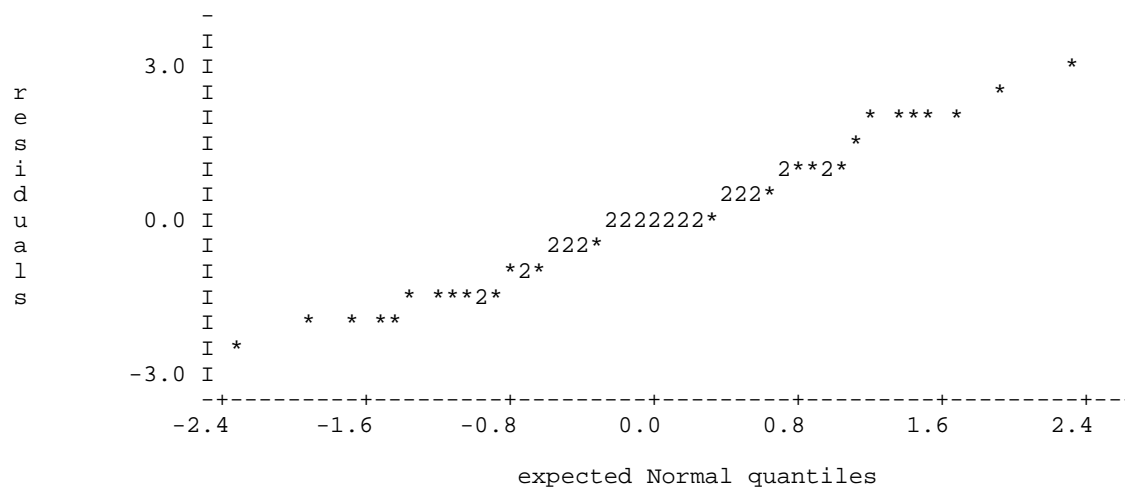
```

51  APLOTT METHOD=fit,normal

```



Normal plot



```

52  "
-53  **** Tukey's one-degree-of-freedom-for-non-additivity.
-54  **** It is the term designated covariate in the following analysis
-55  "
56  TREAT Clones*Year
57  AKEEP [FIT=Fit]
58  CALC ResSq=Fit*Fit
59  ANOVA [PRINT=*] ResSq; RES=ResSq
60  COVAR ResSq
61  ANOVA [PRINT=A; FPROB=Y] Yields

```

"A computational trick"

```

***** Warning (Code AN 40). Statement 1 on Line 61
Command: ANOVA [PRINT=A; FPROB=Y] Yields
Stratum variance cannot be estimated
Years stratum has zero residual sum of squares or degrees of freedom

```

61.....

***** Analysis of variance (adjusted for covariate) *****

Variate: Yields
Covariate: ResSq

Source of variation	d.f.	s.s.	m.s.	v.r.	cov.ef.	F pr.
Blocks stratum	4	302.268	75.567			
Years stratum						
Year	3	315.939	105.313		1.00	
Blocks.Plots stratum						
Clones	2	981.567	490.783	8.74	1.00	0.010
Residual	8	449.371	56.171	14.49	1.00	
Blocks.Years stratum	12	1247.572	103.964	26.81		
Blocks.Plots.Years stratum						
Clones.Year	6	290.200	48.367	12.47	1.00	<.001
Covariate	1	2.135	2.135	0.55		0.466
Residual	23	89.174	3.877		0.98	
Total	59	3678.226				

62 COVAR

63 "

-64 ***** obtain fitted equations

-65 "

66 VARI Yr

67 CALC Yr=Year

68 MODEL Yields

69 TERMS Clones/Yr

70 FIT Clones/Yr

70.....

***** Regression Analysis *****

Response variate: Yields
Fitted terms: Constant + Clones + Yr.Clones

*** Summary of analysis ***

	d.f.	s.s.	m.s.	v.r.
Regression	5	1255.	250.94	5.59
Residual	54	2424.	44.88	
Total	59	3678.	62.34	

Percentage variance accounted for 28.0

Standard error of observations is estimated to be 6.70

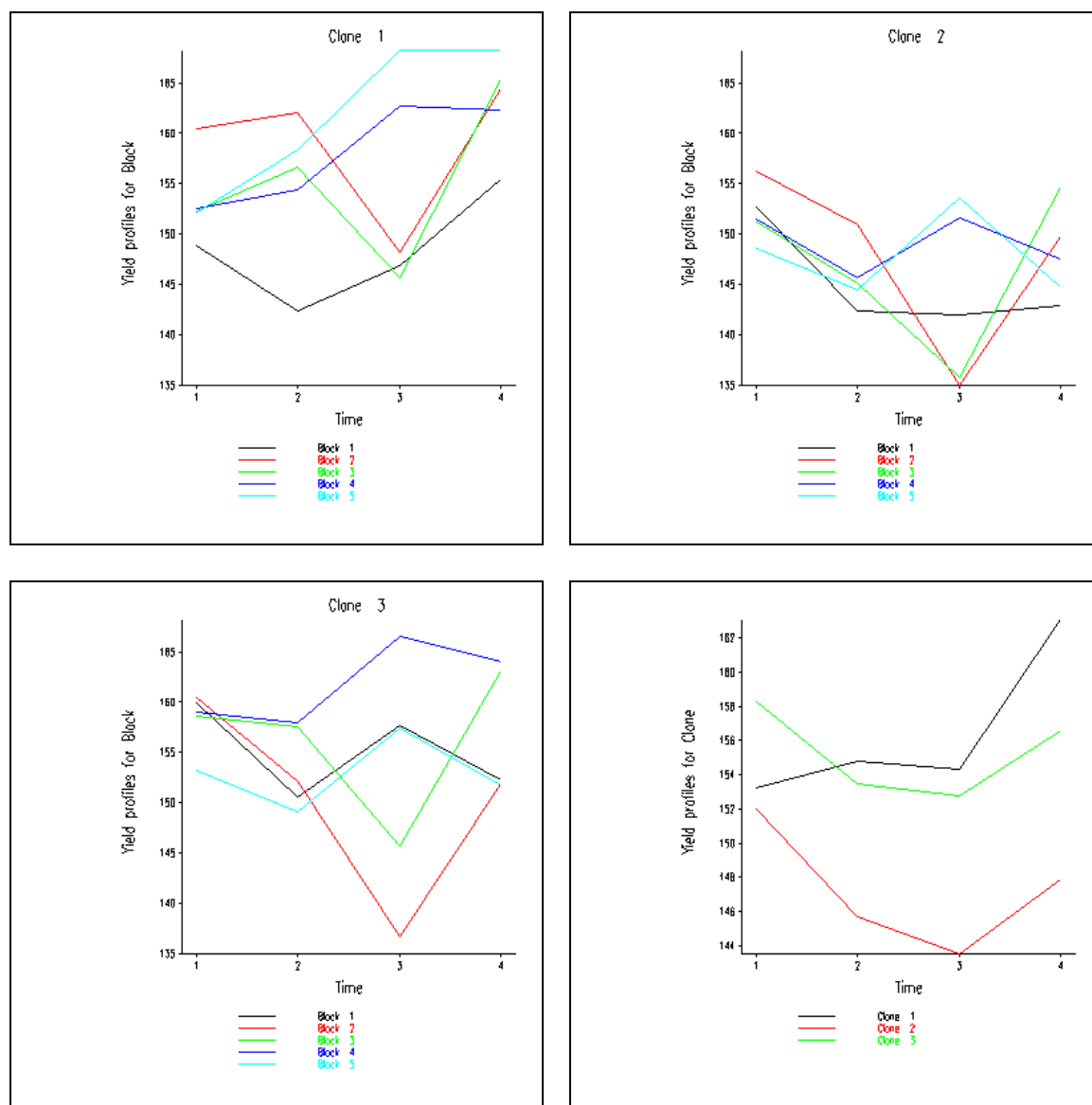
* MESSAGE: The following units have large standardized residuals:

Unit	Response	Residual
19	136.70	-2.81

*** Estimates of parameters ***

	estimate	s.e.	t(54)
Constant	149.06	3.67	40.62
Clones 2	1.83	5.19	0.35
Clones 3	7.62	5.19	1.47
Yr.Clones 1	2.92	1.34	2.18
Yr.Clones 2	-1.44	1.34	-1.08
Yr.Clones 3	-0.57	1.34	-0.42

The plots of the profiles are given in the following figures:



These plots indicate that there is considerable variability amongst the individual profiles for a particular clone. However, it also appears that the clones differ in their pattern over the years.

As far as diagnostic checking is concerned, there are no problems apparent. The residual-versus-fitted-values plot is displaying a homogenous pattern, the normal probability plot looks like a roughly linear pattern and Tukey's test for nonadditivity is nonsignificant.

The analysis of variance indicates that

1. The Deviations term for Clones.Times is not significant;
2. The Clones.Quad term is not significant;
3. The Clones.Lin term is significant.

That is, a linear trend describes the pattern over time, but that the slope of the trend differs between the clones. Regression was used to obtain the fitted equations and these are as follows:

For clone 1, $Yield = 149.06 + 2.92 Year$

For clone 2, $Yield = 150.89 - 1.44 Year$

For clone 3, $Yield = 156.68 - 0.57 Year$

We note that the Clones.Years term was not significant in the split-plot-in-time analysis, but is in this analysis. This is because the Blocks.Years term is highly significant and in the split-plot-in-time analysis it included in the Residual MSq of 37.22.

c) Computation in Genstat

The following commands were used in analyzing the example:

```
PRINT Blocks,Plots,Years,Clones,Yields
"
**** separate data for years and plot profiles
"
SUBSET [CONDITION=Years==1] OLD=Blocks,Plots,Clones; \
      NEW=Block,Plot,Clone
FOR i=1...4
  SUBSET [CONDITION=Years==i] OLD=Yields; NEW=Yield[i]
ENDFOR
PRINT Block,Plot,Clone,Yield[1...4]; FIELD=9; DEC=1
DREPMEASURES [GROUPS=Clone,Block] DATA=Yield
DREPMEASURES [GROUPS=Clone] DATA=Yield
"
**** perform repeated measurements ANOVA
"
DUPLICATE OLD=Years; NEW=Year
BLOCK (Blocks/Plots)*Years
TREAT Clones*POL(Year;2)
ANOVA [FPROB=Y; PSE=LSD] Yields
CALC pBP=1-FPROB(56.171/3.805; 8; 24)
&    pBY=1-FPROB(103.964/3.805; 12; 24)
PRINT pBP,pBY
APLOT METHOD=fit,normal
"
**** Tukey's one-degree-of-freedom-for-non-additivity.
**** It is the term designated covariate in the following analysis
"
TREAT Clones*Year
AKEEP [FIT=Fit]
CALC ResSq=Fit*Fit
ANOVA [PRINT=*] ResSq; RES=ResSq
COVAR ResSq
ANOVA [PRINT=A; FPROB=Y] Yields
COVAR
"
**** obtain fitted equations
"
VARI Yr
CALC Yr=Year
MODEL Yields
TERMS Clones/Yr
FIT Clones/Yr
```

"A computational trick"

XII.C Problems with ANOVA on individual measurements for all timepoints

The major problem with using an ANOVA on the individual measurements for all timepoints is that it is very likely that the assumptions are not met. In practice it is assumed that variances at the different timepoints are equal and the correlations between pairs of timepoints are the same for all timepoints. That is the variance matrix for times should have the following form:

$$\sigma^2 \begin{bmatrix} 1 & \rho & \rho & \dots & \rho \\ \rho & 1 & \rho & \dots & \rho \\ \rho & \rho & 1 & \dots & \rho \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \rho & \rho & \rho & \dots & 1 \end{bmatrix}$$

This pattern is known as **uniform covariance structure** and the matrix is said to show **compound symmetry**. Further the values of σ^2 and ρ in this matrix must be the same for each of the terms not involving times in the analysis. Tests are available for compound symmetry and equality of variance matrices.

There will be situations in which these assumptions are met and the single ANOVA on the individual measurements for all timepoints is appropriate. However, when the observations are made over a period of time during which there is a considerable change in the organisms being observed, it is very likely that these assumptions will not be met. If they are not met, alternative analyses must be used instead. Some of the possible alternative analyses are:

- a) separate analyses of each timepoint
- b) ad hoc summary statistics over timepoints
- c) summary statistics from fitted curves
- d) Greenhouse-Geisser and Huynh-Feldt adjustments to the degrees of freedom of ANOVA on individual measurements for all timepoints
- e) multivariate ANOVA
- f) analysis assuming ante-dependence structure
- g) nonlinear-mixed models
- h) more general covariance models and nonparametric smoothing of time trends

Of these, only the first three will be discussed in this course.

XII.D Separate analyses of each timepoint

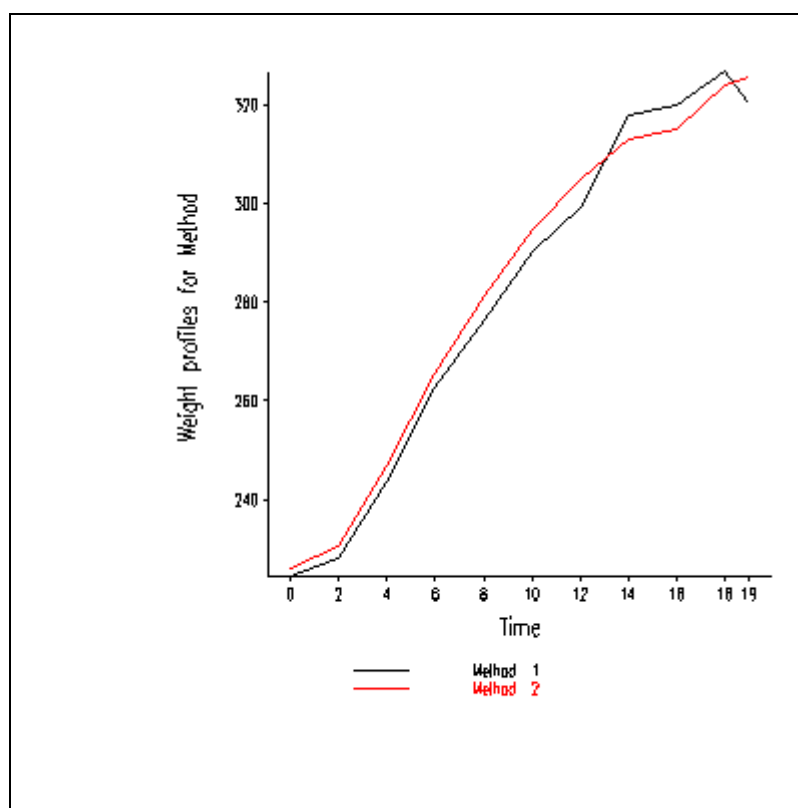
Often the data from repeated measurements experiments is analysed using separate analyses of the data from each timepoint. This is not invalid but separate tests must be interpreted correctly, and this means in a very limited sense. The major errors that occur in the use of this procedure are:

- The tests are incorrectly used to assess changes over time. These tests ignore the successive nature of the observations. They are based on *between-unit* comparisons. The test examines whether the group means over different units at a particular time are different using variability in the response variable between units within a group. On the other hand assessment of change requires *within-unit* comparisons. That is, a comparison of the means of the time differences computed for each unit using the variability of the within-unit time differences. The variability of within-unit time differences is likely to be quite different to the variability between units at a particular time. *Further, a change in statistical significance is **not** equivalent to a statistically significant change.*
- The tests may not detect differences because they are very inefficient. They ignore the within-unit correlations and treat each timepoint as independent.
- It is inappropriate to simply divide timepoints into those that are “significant” and those that are “not significant” for a process that is essentially continuous in time.

Example XII.3 Internal parasites in calves

An experiment to compare two methods for the control of intestinal parasites in calves involved 60 calves. At the start of the grazing season the calves were randomly assigned to the two methods so that 30 calves received each method. The weights of each calf were measured at weeks 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 19.

The plot of the mean profile for the two methods is shown in the following diagram.



There are only two treatments and so it is a simple matter to perform t-tests on the data for each timepoint (very easy to do in Excel). The results of the t-tests are shown in the following output:

	test	d.f.	prob
ttest[0]	-0.60	58.00	0.55
ttest[2]	-0.82	58.00	0.42
ttest[4]	-1.03	58.00	0.31
ttest[6]	-0.88	58.00	0.38
ttest[8]	-1.21	58.00	0.23
ttest[10]	-1.12	58.00	0.27
ttest[12]	-1.28	58.00	0.21
ttest[14]	1.10	58.00	0.28
ttest[16]	0.95	58.00	0.34
ttest[18]	0.53	58.00	0.60
ttest[19]	-0.85	58.00	0.40

These tests indicate that there are no differences between the methods over time. We will see how alternative analyses can give more useful information about the effects of treatments in this experiment.

XII.E Analyses of summary statistics

The summary statistic approach to the analysis of repeated measurements experiments is, in principle, very simple. A small number of statistics is computed for each unit from the observations over time for that unit.

The analysis of these summary statistics is generally straightforward. Each statistics is treated a single response and analyzed using conventional techniques, for example analysis of variance, regression or even a nonparametric test. If there is more than one-statistic from each subject then these will usually not be independent and multivariate analyses can be used, for example multivariate analysis of variance. So this method moves the problem from the realm of repeated measurements into that of more conventional methodology.

The advantage of this approach is that the unlikely-to-be-met assumptions about the behaviour through time are avoided. The choice of summary statistic depends very much on the context and there exist many possible alternatives. It is important that the chosen statistics have some meaning or interpretation in terms of the experiment. However, it is not critical that all information in a unit's profile is extracted, provided that features believed to be relevant are examined.

It is likely that in choosing the statistics there will be some trade-off between exploratory and confirmatory analysis. It is preferable that the statistics be chosen in the planning stage of the experiment. However, this may require knowledge in advance about the form of the profile and this may well not be the case. If there is insufficient knowledge available in the planning stage, one may be forced into a more exploratory analysis where an examination of the observed profiles is used to choose a suitable summary statistic.

The summary statistic approach is particularly valuable when

- individual profiles vary considerably;
- the repeated measurements are unbalanced because not all units are measured at the same set of timepoints, provided the differences between units are not too great.

We distinguish between two forms of summary statistics: a) ad hoc summary statistics and b) summary statistics from fitted curves.

a) Ad hoc summary statistics

Often a profile will not follow a simple functional form and many types of ad hoc statistics can be considered. Some commonly used ones are:

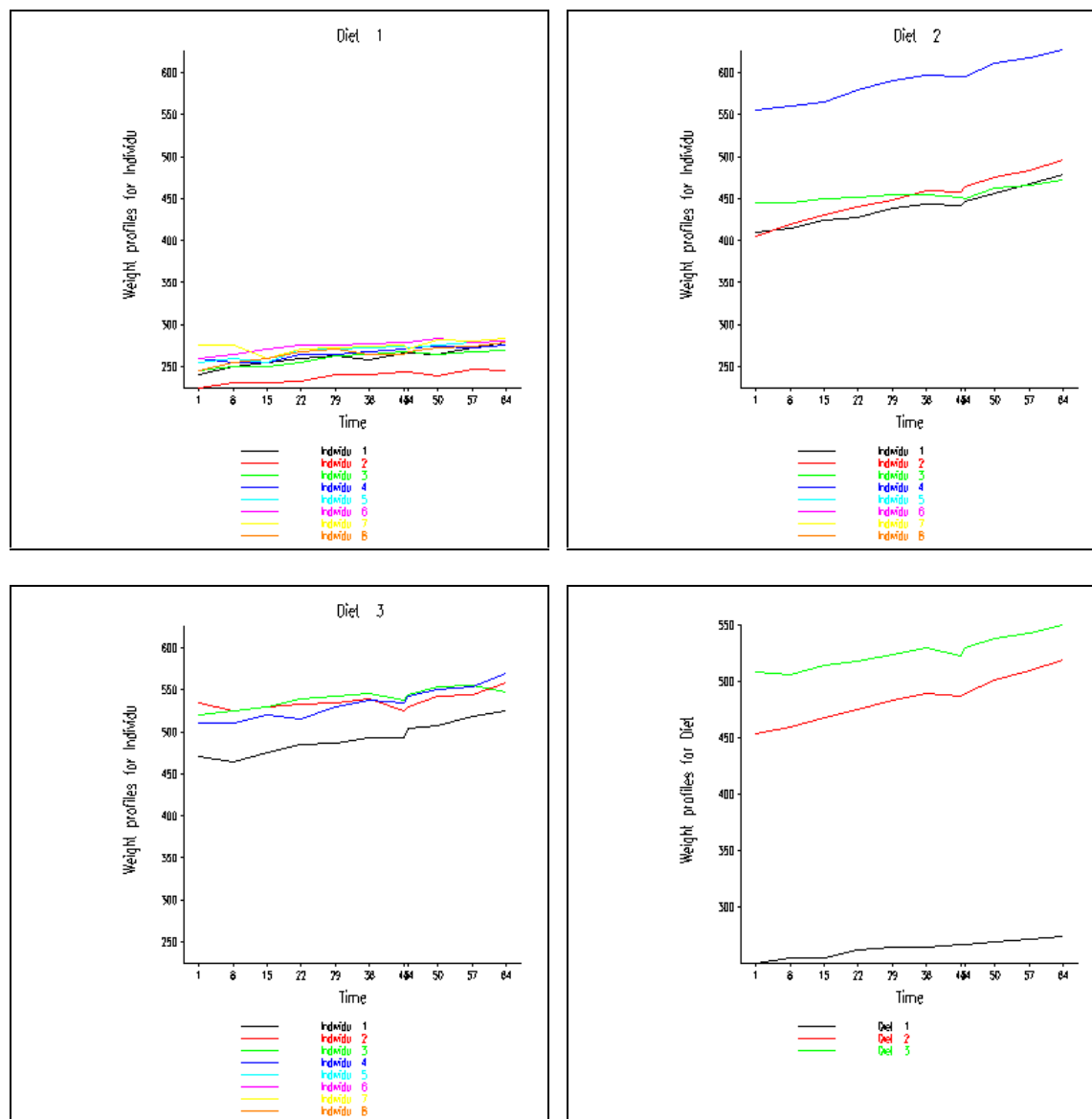
- particular end point(s)
- mean over the whole or part of the profile
- area under the profile
- maximum or minimum during the whole, or part, of the period of measurement
- change between two times
- time to a pre-defined threshold, to return to a baseline or to the maximum/minimum
- the number of events or changes
- proportion of successes

The great advantage of summary statistics is that they provide a way of handling categorical (particularly binary) repeated measurements without the need for sophisticated modelling. However, some summary statistics may be far from normally distributed whatever the distribution of the original measurements. Examples are extrema or times to particular events when there are only a few timepoints.

One danger with this approach is to create a very large number of statistics and to analyse them all. It is unlikely that all the statistics will be independent. Consequently, the analysis and interpretation of the results would not be independent and the statistical properties of the analyses would be compromised.

Example XII.4 Rat weight

An experiment to investigate the effects of 3 diets is described in Crowder and Hand (1996). It involved 16 rats, eight randomly assigned to diet 1 and four to each of diets 2 and 3. The body weights, in grams, were measured over 64 days. The profiles are plotted in the following diagrams.



The profiles show a roughly linear pattern with all rats having parallel patterns — rather well-behaved profiles.

In this case, the weight gain over the 64 days is likely to be of interest to the experiment and so we choose it as our summary statistic for analysis. Below is the Genstat output that contains the commands to produce the profiles and analyses and the output from these commands.

Genstat 5 Release 4.1 (PC/Windows NT) 30 April 2000 12:14:48
Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

Genstat 5 Fourth Edition - (for Windows)
Genstat 5 Procedure Library Release PL11

```

3 "Data taken from File: D:/ANALYSES/LM/REPEATMEASURE/RMERAT.GSH"
4 DELETE [redefine=yes] Days,Rats,Diets,Weights
5 FACTOR [modify=yes;nvalues=176;levels=!(1,8,15,22,29,36,43,44,50,57,64)\
6 ] Days
7 READ Days; frepresentation=ordinal

```

```

Identifier    Values    Missing    Levels
  Days         176         0         11

14 FACTOR [modify=yes;nvalues=176;levels=16] Rats
15 READ Rats; frepresentation=ordinal

Identifier    Values    Missing    Levels
  Rats         176         0         16

22 FACTOR [modify=yes;nvalues=176;levels=3] Diets
23 READ Diets; frepresentation=ordinal

Identifier    Values    Missing    Levels
  Diets         176         0         3

29 VARIATE [nvalues=176] Weights
30 READ Weights

Identifier    Minimum    Mean    Maximum    Values    Missing
  Weights      225.0    384.5    628.0     176         0

41
42 "
-43 **** separate data for Days and Rat profiles
-44 "
45 SUBSET [CONDITION=Days==1] OLD=Rats,Diets; \
46                                NEW=Rat,Diet
47 FOR i=1,8...43,44,50,57,64
48     SUBSET [CONDITION=Days==i] OLD=Weights; NEW=Weight[i]
49 ENDFOR
50 PRINT Rat,Diet,Weight[1,8...43,44,50,57,64]; FIELD=9; DEC=0

Rat    Diet  Weight[1]  Weight[8]  Weight[15]  Weight[22]  Weight[29]  Weight[36]
  1      1      240      250      255      260      262      258
  2      1      225      230      230      232      240      240
  3      1      245      250      250      255      262      265
  4      1      260      255      255      265      265      268
  5      1      255      260      255      270      270      273
  6      1      260      265      270      275      275      277
  7      1      275      275      260      270      273      274
  8      1      245      255      260      268      270      265
  9      2      410      415      425      428      438      443
 10      2      405      420      430      440      448      460
 11      2      445      445      450      452      455      455
 12      2      555      560      565      580      590      597
 13      3      470      465      475      485      487      493
 14      3      535      525      530      533      535      540
 15      3      520      525      530      540      543      546
 16      3      510      510      520      515      530      538

Weight[43]  Weight[44]  Weight[50]  Weight[57]  Weight[64]
  266      266      265      272      278
  243      244      238      247      245
  267      267      264      268      269
  270      272      274      273      275
  274      273      276      278      280
  278      278      284      279      281
  276      271      282      281      284
  265      267      273      274      278
  442      446      456      468      478
  458      464      475      484      496
  451      450      462      466      472
  595      595      612      618      628
  493      504      507      518      525
  525      530      543      544      559
  538      544      553      555      548
  535      542      550      553      569

51 FACTOR [LEV=30] Individuals
52 CALC Individuals=NEWLEVELS(Rat; !v(1...8,(1...4)2))

```

```

53 DREPMEASURES [GROUPS=Diet,Individuals] DATA=Weight
* MESSAGE: There are missing values in the plot

```

```

***** Warning (Code HG 20). Statement 130 in Procedure DREPMEAS
Command: DGRAPH [WINDOW= window; KEYWINDOW= keywindow; TITLE= Title_] GroupMns
Key window full

```

```

* MESSAGE: There are missing values in the plot

```

```

***** Warning (Code HG 20). Statement 130 in Procedure DREPMEAS
Command: DGRAPH [WINDOW= window; KEYWINDOW= keywindow; TITLE= Title_] GroupMns
Key window full

```

```

* MESSAGE: There are missing values in the plot

```

```

***** Warning (Code HG 20). Statement 130 in Procedure DREPMEAS
Command: DGRAPH [WINDOW= window; KEYWINDOW= keywindow; TITLE= Title_] GroupMns
Key window full

```

```

54 DREPMEASURES [GROUPS=Diet] DATA=Weight
55 "
-56 **** analyse weight gain
-57 "
58 CALC WtGain=Weight[64]-Weight[1]
59 BLOCK Rat
60 TREAT Diet
61 ANOVA [FPROB=Y; PSE=LSD] WtGain

```

```

61.....

```

```

***** Analysis of variance *****

```

```

Variate: WtGain

```

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rat stratum					
Diet	2	4681.1	2340.6	8.07	0.005
Residual	13	3772.6	290.2		
Total	15	8453.7			

```

* MESSAGE: the following units have large residuals.

```

```

Rat 11          -37.7   s.e. 15.4

```

```

***** Tables of means *****

```

```

Variate: WtGain

```

```

Grand mean  38.1

```

Diet	1	2	3
	23.1	64.7	41.5
rep.	8	4	4

```

*** Least significant differences of means (5% level) ***

```

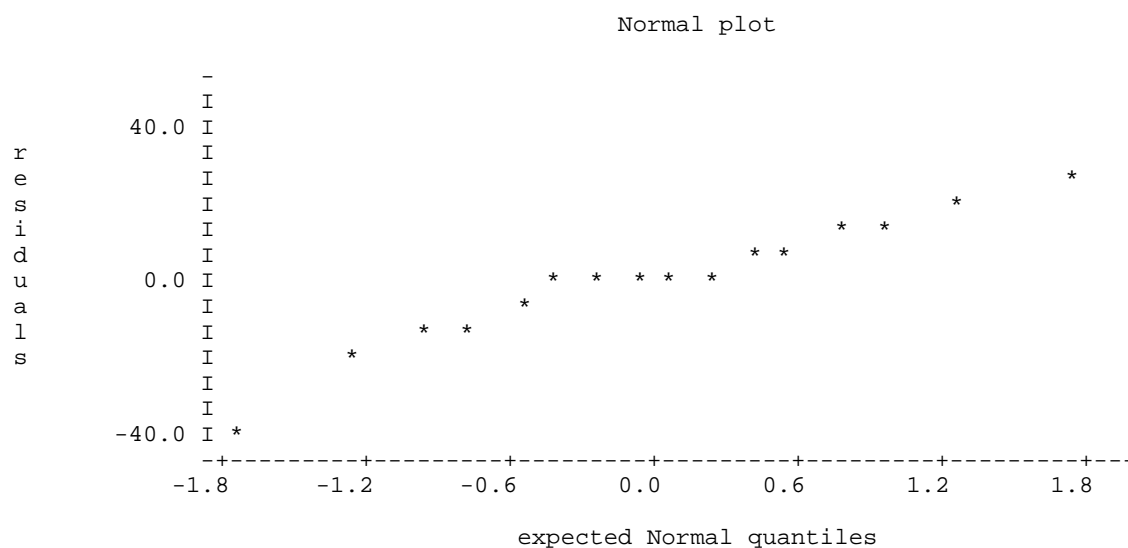
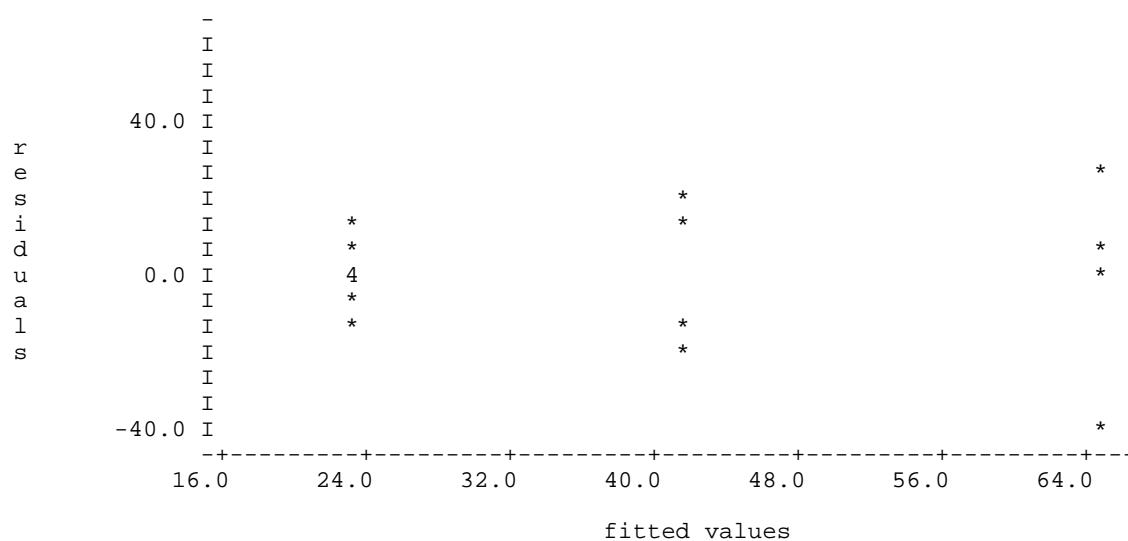
Table	Diet	
rep.	unequal	
d.f.	13	
l.s.d.	26.02	min.rep
	22.54	max-min
	18.40X	max.rep

```

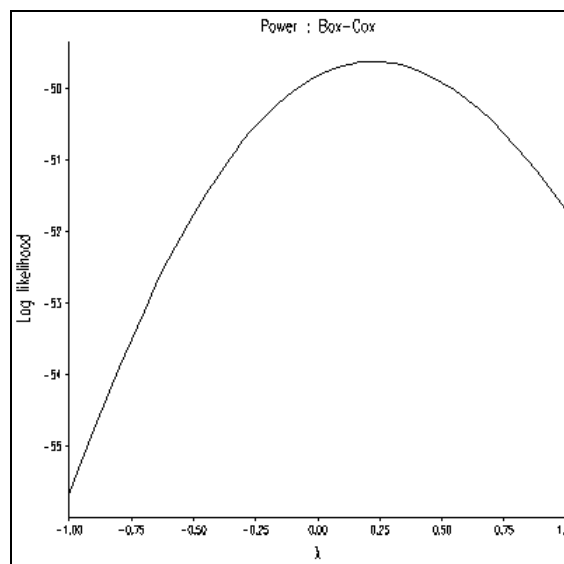
(No comparisons in categories where s.e.d. marked with an X)

```

62 APLOT METHOD=fit,normal



The assumptions underlying the analysis of weight gain appear not to be met in that the variance appears to be increasing with the weight gain. So we need to find modify the analysis to take this into account. The following diagram contains the plot produced by YTRANSFORM to identify a power transformation. The log transformation was chosen because it is close to the maximum and is interpretable. The output below shows the analysis of the natural logarithm of the weight gain.



```

63  YTRANSFORM [TERMS=Diet; LOWER=-1; UPPER=1] WtGain; SAVE=s
64  CALC TWtGain=LOG(WtGain)
65  ANOVA [FPROB=Y; PSE=LSD] TWtGain

```

65.....

***** Analysis of variance *****

Variate: TWtGain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rat stratum					
Diet	2	2.9587	1.4794	6.55	0.011
Residual	13	2.9344	0.2257		
Total	15	5.8932			

* MESSAGE: the following units have large residuals.

Rat 7 -0.86 s.e. 0.43

***** Tables of means *****

Variate: TWtGain

Grand mean 3.46

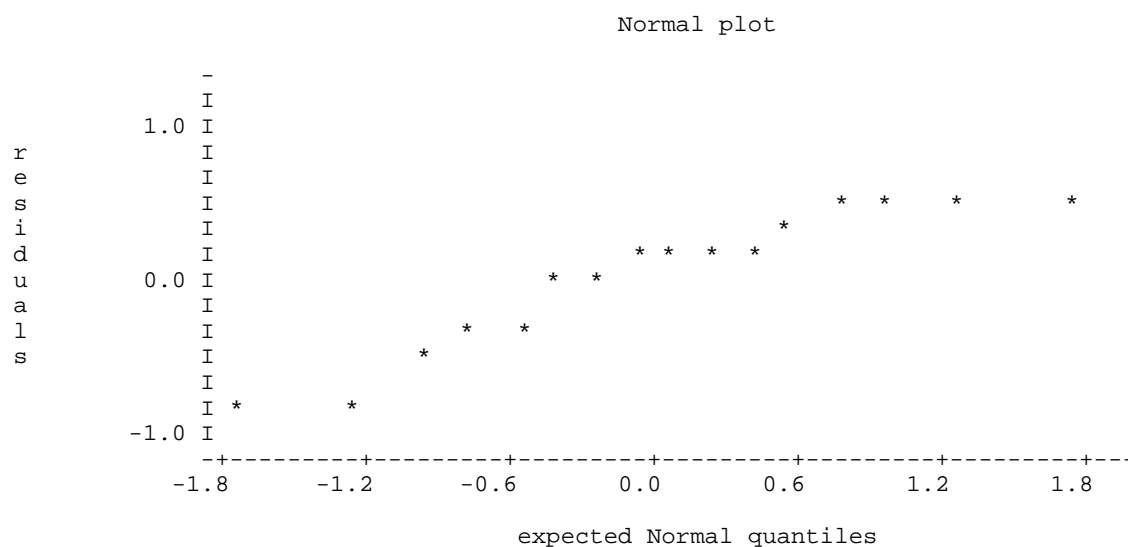
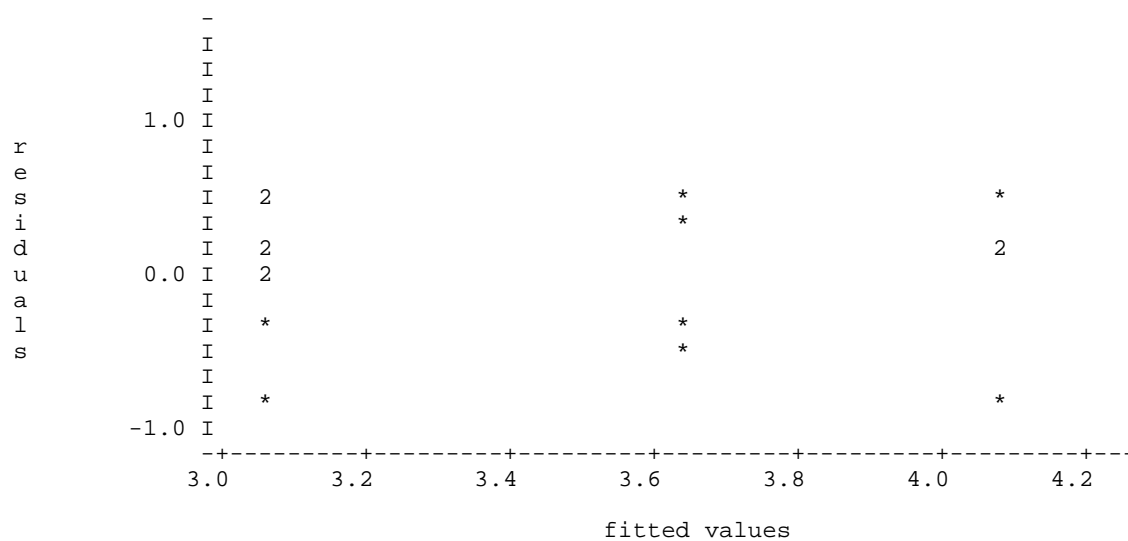
Diet	1	2	3
	3.06	4.08	3.65
rep.	8	4	4

*** Least significant differences of means (5% level) ***

Table	Diet	
rep.	unequal	
d.f.	13	
l.s.d.	0.726	min.rep
	0.629	max-min
	0.513X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

```
66  APLLOT METHOD=fit,normal
```



```
67  AKEEP Diet; MEANS=DietMn
68  CALC DietMn=EXP(DietMn)
69  PRINT DietMn
```

Diet	DietMn
1	21.32
2	59.10
3	38.43

This analysis indicates a significant difference between the weight gains under the different diets ($p = 0.011$). To determine the differences between the diet we examine the table of log weight gain means and use the LSD; the relevant part of the output is given below.

***** Tables of means *****

Variate: TWtGain

Grand mean 3.46

Diet	1	2	3
	3.06	4.08	3.65
rep.	8	4	4

*** Least significant differences of means (5% level) ***

Table	Diet	
rep.	unequal	
d.f.	13	
l.s.d.	0.726	min.rep
	0.629	max-min
	0.513X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

Note to compare diet 1 with the other diets, use the max-min LSD as these comparisons involve a mean of 8 with a mean of 4; for diet 2 versus 3, use the min-min LSD as these two means are both based on 4 observations. Using the max-min LSD we see that diet 1 results in less weight gain than diet 3 but is not significantly different to diet 2. Nor is there a significant difference between the mean weight gains of diets 2 and 3.

The back-transformed means have also been computed and are given at the end of the output — they are the geometric means of the original data. We can interpret the results from using the LSD as indicating which ratios of geometric means are significantly different. In this case, the only significant difference is between diets 1 and 2 and the ratio of diet 2 to diet 1 is $59.1/21.32 = 2.77$.

b) Summary statistics from fitted curves

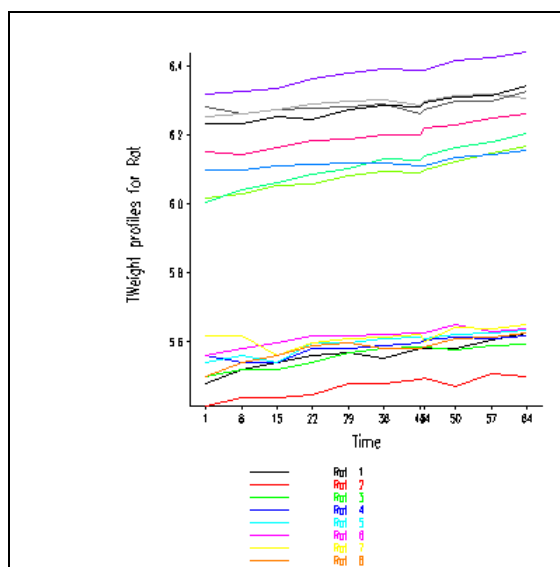
If the individual profiles are smooth, it may be possible to summarize an individual profile with a response function. In general, this response function can be any suitable function and the parameters of the fitted equation provide the summary statistics for analysis. We will examine the use of low-degree polynomials for this purpose.

Although the measurements at different timepoints for a subject are not independent, it is still suggested that ordinary, unweighted, least-squares estimates be used, possibly after transformation of the response. Provided that the variability of the repeated measurements does not change much over time (a transformation may be needed to ensure this), the ordinary, unweighted, least-squares estimates will be as efficient, or nearly as efficient, as more sophisticated methods that take into account the correlations between the timepoints. The ordinary, unweighted, least-squares estimates will always be valid, in the sense that they will be consistent, and they have the great advantage of simplicity. However, standard errors, R^2 and other inferential, regression procedures, in particular the use of differences in the residual mean squares to compare models, are **not** valid and can be very misleading. Here it is to be emphasized that we merely computed fitted parameters as summary statistics describing particular aspects of the behaviour of the data. The analysis that

we then perform on these statistics involves assumptions about a model for the distribution of the values of the fitted parameters over the sample of units, and not the distribution of the residuals from the fit of a curve to an individual's profile. One consequence of this is that it is not even required that the curves are particularly good fit to the data, although clearly this may be a desirable aid to interpretation.

Example XII.4 Rat weight (continued)

In the previous section we described an experiment involving 16 rats to investigate the effects of 3 diets. The weight gain over the period of observation was used as an ad hoc summary statistic to analyse this data. However, it was found that a log transformation was needed to stabilise the variance. A plot of the profiles is the natural logarithms of the weights is shown in the diagram below.



From this plot it is concluded that the trend remains linear, as would be expected if the range of the weight gains for each rat covers a narrow range ($\max/\min < 3$).

Given this approximately linear trend, another possible summary statistic is the slope of a fitted straight line. Genstat's VORTHOPOL procedure can be used to compute the coefficients of orthogonal polynomials. The degree of the polynomials to be fitted is specified by the option MAXDEGREE and the coefficients are saved in variates specified by the CONTRAST parameter. For example CONTRAST=pol saves the mean for each unit in pol[0], the slope for each in pol[1], the quadratic coefficient for each in pol[2] and so on. The following Genstat output contains the commands to compute the mean and linear and quadratic coefficients and to analyse them. The output of these commands is also given.

Genstat 5 Release 4.1 (PC/Windows NT) 30 April 2000 13:11:19
Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

Genstat 5 Fourth Edition - (for Windows)
Genstat 5 Procedure Library Release PL11

```
3 "Data taken from File: D:/ANALYSES/LM/REPEATMEASURE/RMERAT.GSH"
4 DELETE [redefine=yes] Days,Rats,Diets,Weights
```

```

5  FACTOR [modify=yes;nvalues=176;levels=!(1,8,15,22,29,36,43,44,50,57,64)\
6  ] Days
7  READ Days; frepresentation=ordinal

```

Identifier	Values	Missing	Levels
Days	176	0	11

```

14 FACTOR [modify=yes;nvalues=176;levels=16] Rats
15 READ Rats; frepresentation=ordinal

```

Identifier	Values	Missing	Levels
Rats	176	0	16

```

22 FACTOR [modify=yes;nvalues=176;levels=3] Diets
23 READ Diets; frepresentation=ordinal

```

Identifier	Values	Missing	Levels
Diets	176	0	3

```

29 VARIATE [nvalues=176] Weights
30 READ Weights

```

Identifier	Minimum	Mean	Maximum	Values	Missing
Weights	225.0	384.5	628.0	176	0

```

41
42 "
-43 **** separate data for Days and Rat profiles
-44 "
45 SUBSET [CONDITION=Days==1] OLD=Rats,Diets; \
46                               NEW=Rat,Diet
47 FOR i=1,8...43,44,50,57,64
48     SUBSET [CONDITION=Days==i] OLD=Weights; NEW=Weight[i]
49 ENDFOR
50 PRINT Rat,Diet,Weight[1,8...43,44,50,57,64]; FIELD=9; DEC=0

```

	Rat	Diet	Weight[1]	Weight[8]	Weight[15]	Weight[22]	Weight[29]	Weight[36]
1	1	1	240	250	255	260	262	258
2	1	1	225	230	230	232	240	240
3	1	1	245	250	250	255	262	265
4	1	1	260	255	255	265	265	268
5	1	1	255	260	255	270	270	273
6	1	1	260	265	270	275	275	277
7	1	1	275	275	260	270	273	274
8	1	1	245	255	260	268	270	265
9	2	2	410	415	425	428	438	443
10	2	2	405	420	430	440	448	460
11	2	2	445	445	450	452	455	455
12	2	2	555	560	565	580	590	597
13	3	3	470	465	475	485	487	493
14	3	3	535	525	530	533	535	540
15	3	3	520	525	530	540	543	546
16	3	3	510	510	520	515	530	538

Weight[43]	Weight[44]	Weight[50]	Weight[57]	Weight[64]
266	266	265	272	278
243	244	238	247	245
267	267	264	268	269
270	272	274	273	275
274	273	276	278	280
278	278	284	279	281
276	271	282	281	284
265	267	273	274	278
442	446	456	468	478
458	464	475	484	496
451	450	462	466	472
595	595	612	618	628
493	504	507	518	525
525	530	543	544	559
538	544	553	555	548

```

535      542      550      553      569

51  "
-52  **** analyse means, linear and quadratic coefficients
-53  "
54  DUPLICATE OLD=Weight; NEW=TWeight
55  CALC #TWeight=LOG(#Weight)
56  DREPMEASURES [GROUP=Rat] DATA=TWeight

***** Warning (Code HG 20). Statement 96 in Procedure DREPMEAS
Command:  DGRAPH [WINDOW= window; KEYWINDOW= keywindow; TITLE= Title_]  GroupMns
Key window full

57  VORTHPOL [MAXDEGREE=2] TWeight; CONTRAST=pol
58  BLOCK Rat
59  TREAT Diet
60  ANOVA [FPROB=Y; PSE=LSD] pol[0]

60.....

**** Analysis of variance ****

Variate: pol[0]

Source of variation      d.f.      s.s.      m.s.      v.r.  F pr.

Rat stratum
Diet                    2    1.685232    0.842616  141.88  <.001
Residual                13    0.077208    0.005939
Total                   15    1.762440

* MESSAGE: the following units have large residuals.

Rat 12          0.205   s.e. 0.069

**** Tables of means ****

Variate: pol[0]

Grand mean  5.897

      Diet      1      2      3
      5.573    6.175    6.264
rep.      8      4      4

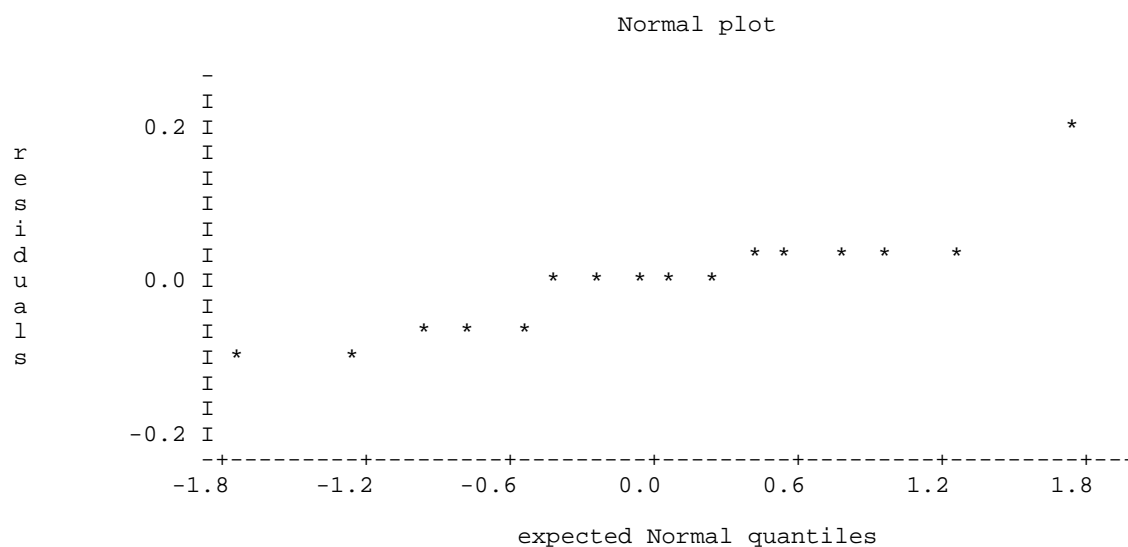
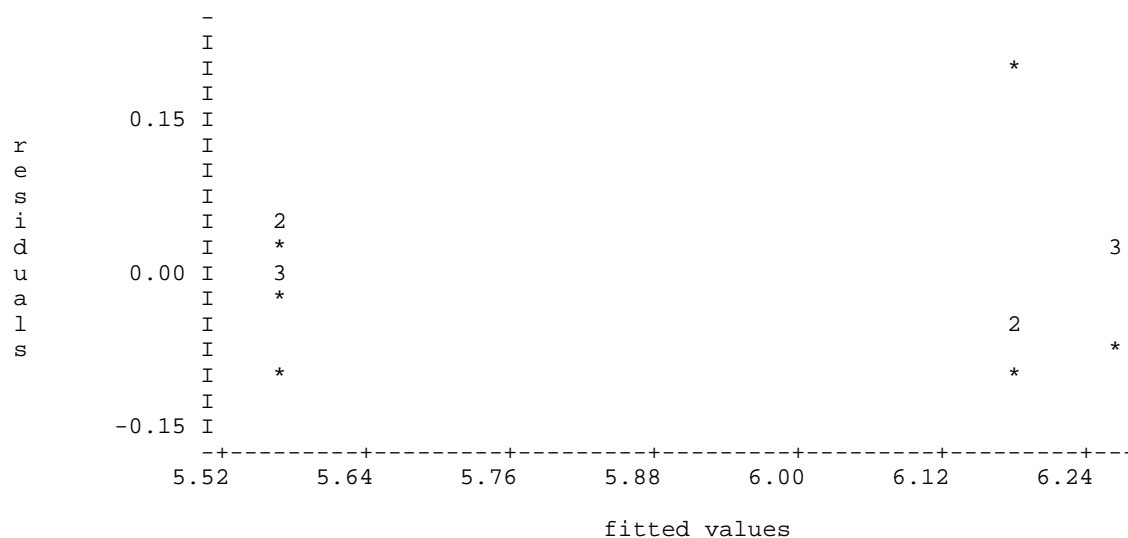
*** Least significant differences of means (5% level) ***

Table      Diet
rep.      unequal
d.f.      13
l.s.d.      0.1177 min.rep
            0.1020 max-min
            0.0832X max.rep

(No comparisons in categories where s.e.d. marked with an X)

61  APLOT METHOD=fit,normal

```



62 ANOVA [FPROB=Y; PSE=LSD] pol[1]

62.....

***** Analysis of variance *****

Variate: pol[1]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rat stratum					
Diet	2	0.136E-05	0.678E-06	1.99	0.177
Residual	13	0.443E-05	0.341E-06		
Total	15	0.579E-05			

* MESSAGE: the following units have large residuals.

Rat 11 -0.00121 s.e. 0.00053

***** Tables of means *****

Variate: pol[1]

Grand mean 0.00151

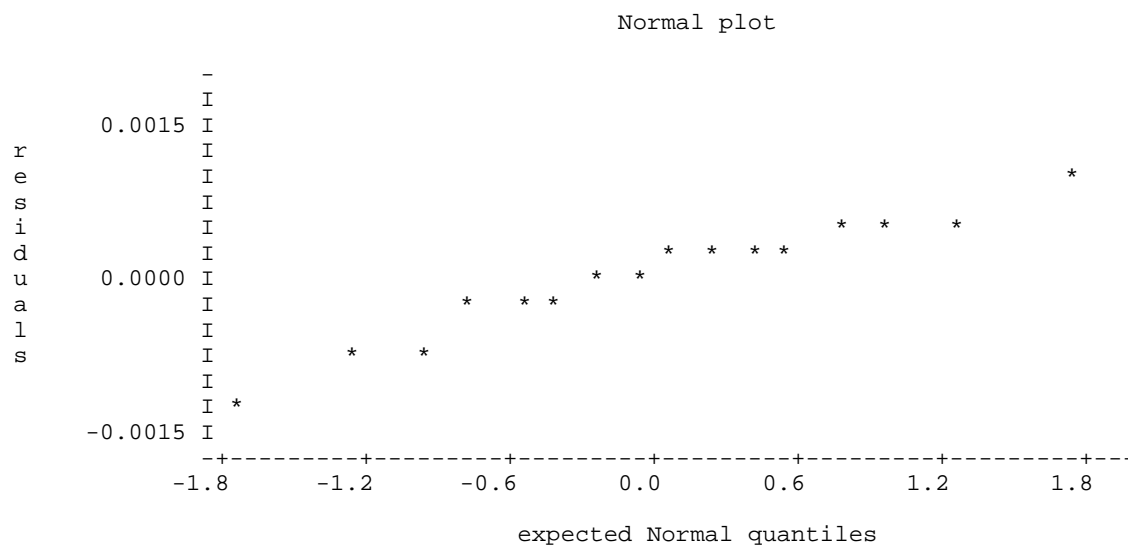
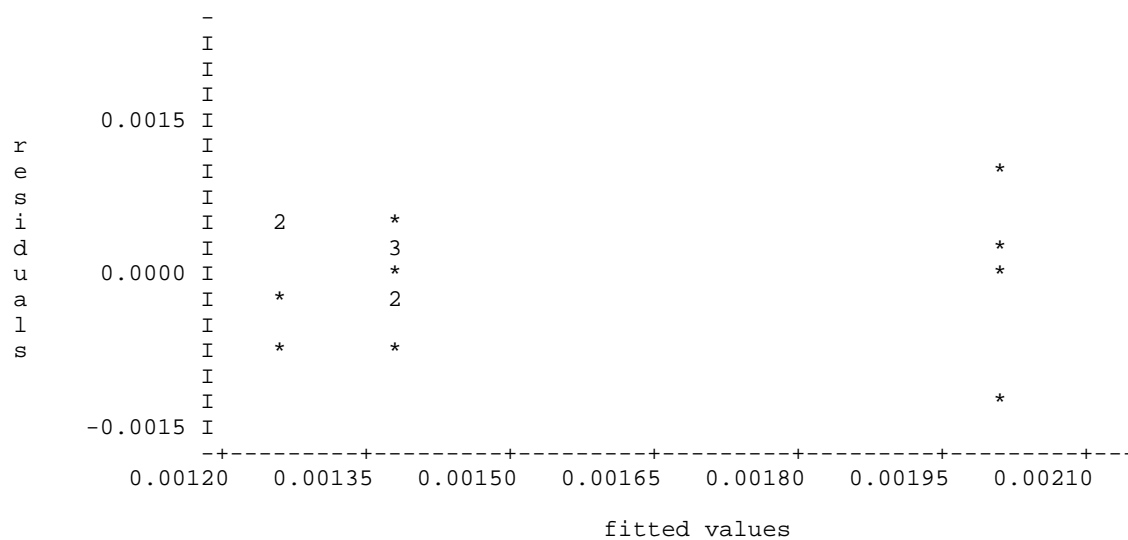
Diet	1	2	3
	0.00138	0.00200	0.00126
rep.	8	4	4

*** Least significant differences of means (5% level) ***

Table	Diet	
rep.	unequal	
d.f.	13	
l.s.d.	0.000892	min.rep
	0.000773	max-min
	0.000631X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

63 APLOT METHOD=fit,normal



64 ANOVA [FPROB=Y; PSE=LSD] pol[2]

64.....

***** Analysis of variance *****

Variate: pol[2]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rat stratum					
Diet	2	0.117E-08	0.587E-09	2.31	0.138
Residual	13	0.330E-08	0.254E-09		
Total	15	0.448E-08			

* MESSAGE: the following units have large residuals.

Rat 7 0.0000401 s.e. 0.0000144

***** Tables of means *****

Variate: pol[2]

Grand mean -0.0000013

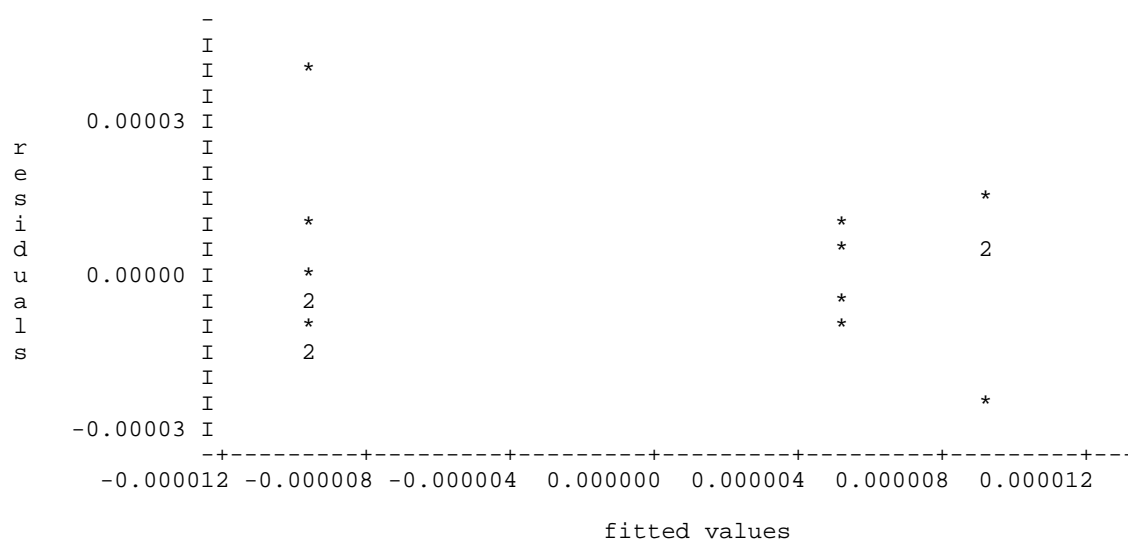
Diet	1	2	3
	-0.975903E-05	0.511111E-05	0.916205E-05
rep.	8	4	4

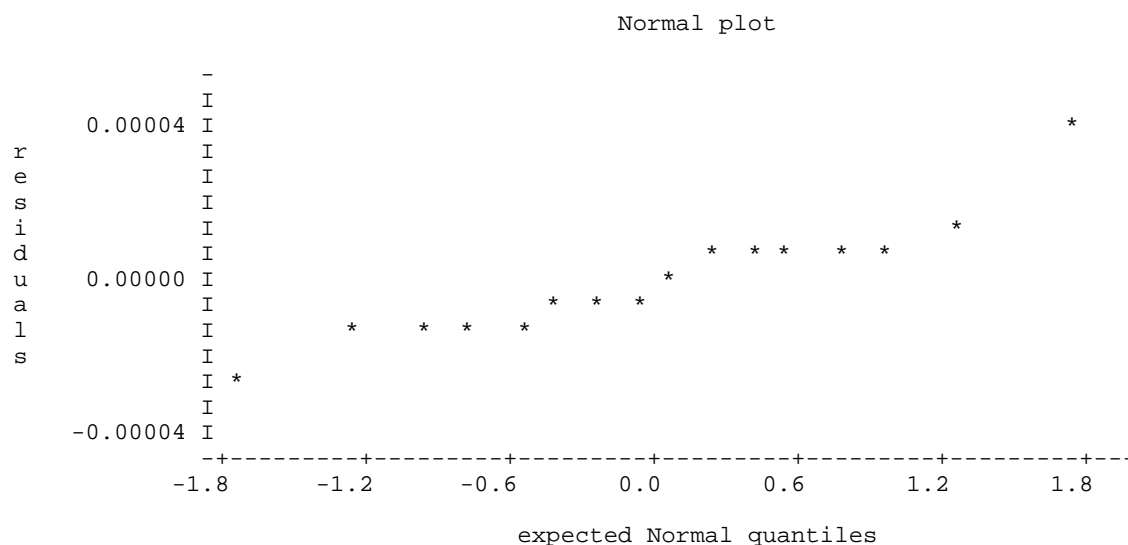
*** Least significant differences of means (5% level) ***

Table	Diet	
rep.	unequal	
d.f.	13	
l.s.d.	0.00002435	min.rep
	0.00002109	max-min
	0.00001722X	max.rep

(No comparisons in categories where s.e.d. marked with an X)

65 APLOT METHOD=fit,normal





The assumptions underlying these analyses appear to be met, although there is an outlier in the mean values that would require investigation to see if a reason could be established for it. The analyses indicate that there are significant differences between the diets in the overall rat means ($p < 0.001$) and that there are not significant differences between the diets in the slopes ($p = 0.177$) nor in the quadratic coefficients ($p = 0.138$). That is all the information about the differences between the diets appears to be contained in the overall rat means. The advantage of the overall rat means, compared to the weight gains, is that the overall rat means are based on all the observations for a rat. However, the final choice must be based on what is most important to the experimenter.

The diet means computed from the overall rat means are given in the following table. Again the exponential of these means is the geometric mean of the observations for that diet and the ratios of these could be reported.

***** Tables of means *****

Variate: pol[0]

Grand mean 5.897

Diet	1	2	3
	5.573	6.175	6.264
rep.	8	4	4

*** Least significant differences of means (5% level) ***

Table	Diet	
rep.	unequal	
d.f.	13	
l.s.d.	0.1177	min.rep
	0.1020	max-min
	0.0832X	max.rep

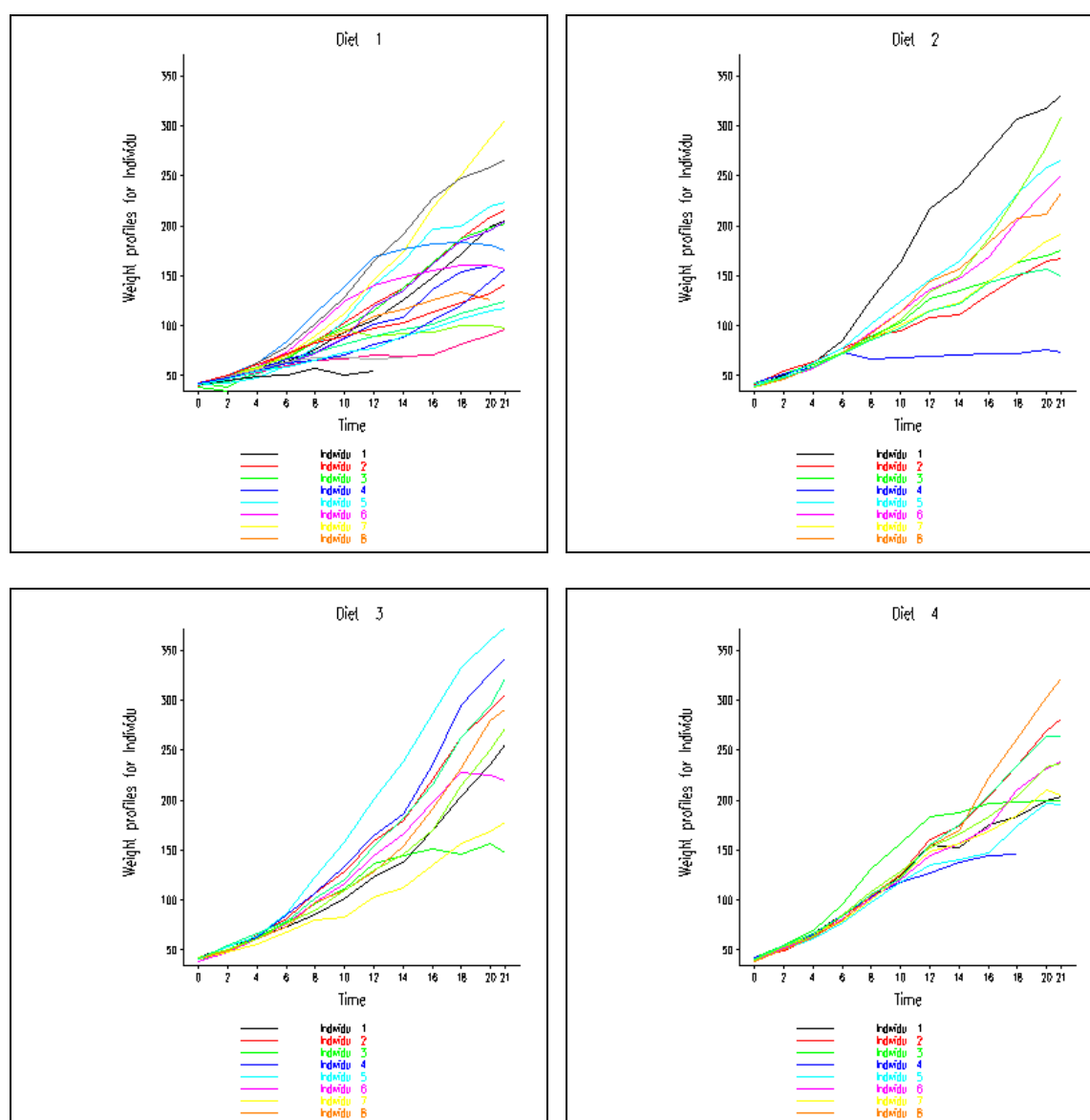
(No comparisons in categories where s.e.d. marked with an X)

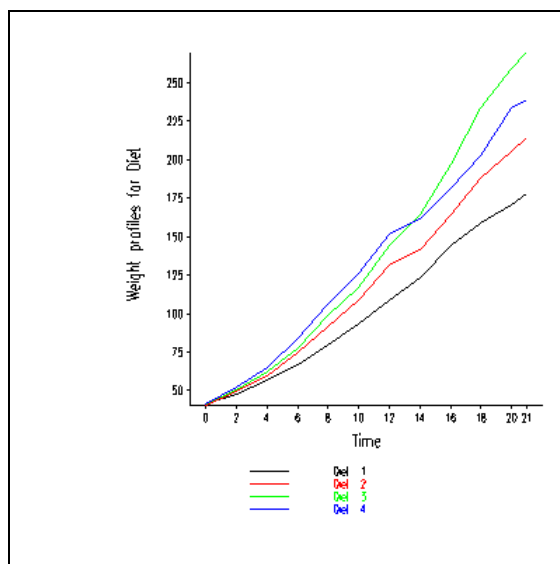
In this case diet 1 is significantly different to diets 2 and 3, the latter two diets not being significantly different. The back-transformed diet means and ratios are given in the following table.

Diet	Geometric mean	Ratio to diet 1
1	263.4	
2	480.7	1.82
3	525.2	1.99

Example XII.5 Chick body weights under 4 diets

Fifty chicks were involved in the study of the effect of four diets on the body weight of chicks. The chicks were observed on alternative days over a 3-week period. The plots of the individual profiles are shown in the following diagrams.





To analyse this data polynomials of order 6 are fitted. The results are shown in the following Genstat output.

Genstat 5 Release 4.1 (PC/Windows NT) 30 April 2000 21:36:45
 Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

Genstat 5 Fourth Edition - (for Windows)
 Genstat 5 Procedure Library Release PL11

```

3  "Data taken from File: D:/ANALYSES/LM/REPEATMEASURE/RMECHICKDIET.GSH"
4  DELETE [redefine=yes] Days,Chicks,Diets,Weights
5  FACTOR [modify=yes;nvalues=600;levels=!(0,2,4,6,8,10,12,14,16,18,20,21)\
6  ] Days
7  READ Days; frepresentation=ordinal

```

Identifier	Values	Missing	Levels
Days	600	0	12

```

26 FACTOR [modify=yes;nvalues=600;levels=50] Chicks
27 READ Chicks; frepresentation=ordinal

```

Identifier	Values	Missing	Levels
Chicks	600	0	50

```

51 FACTOR [modify=yes;nvalues=600;levels=4] Diets
52 READ Diets; frepresentation=ordinal

```

Identifier	Values	Missing	Levels
Diets	600	0	4

```

69 VARIATE [nvalues=600] Weights
70 READ Weights

```

Identifier	Minimum	Mean	Maximum	Values	Missing
Weights	35.0	121.8	373.0	600	22

```

99
100 "
-101 **** separate data for Days and Chick profiles
-102 "
103 SUBSET [CONDITION=Days==0] OLD=Chicks,Diets; \
104      NEW=Chick,Diet
105 FOR i=0,2...20,21
106     SUBSET [CONDITION=Days==i] OLD=Weights; NEW=Weight[i]

```

```

107  ENDFOR
108  PRINT Chick,Diet,Weight[0,2...20,21]; FIELD=9; DEC=0

```

Chick	Diet	Weight[0]	Weight[2]	Weight[4]	Weight[6]	Weight[8]	Weight[10]
1	1	42	51	59	64	76	93
2	1	40	49	58	72	84	103
3	1	43	39	55	67	84	99
4	1	42	49	56	67	74	87
5	1	41	42	48	60	79	106
6	1	41	49	59	74	97	124
7	1	41	49	57	71	89	112
8	1	42	50	61	71	84	93
9	1	42	51	59	68	85	96
10	1	41	44	52	63	74	81
11	1	43	51	63	84	112	139
12	1	41	49	56	62	72	88
13	1	41	48	53	60	65	67
14	1	41	49	62	79	101	128
15	1	41	49	56	64	68	68
16	1	41	45	49	51	57	51
17	1	42	51	61	72	83	89
18	1	39	35	*	*	*	*
19	1	43	48	55	62	65	71
20	1	41	47	54	58	65	73
21	2	40	50	62	86	125	163
22	2	41	55	64	77	90	95
23	2	43	52	61	73	90	103
24	2	42	52	58	74	66	68
25	2	40	49	62	78	102	124
26	2	42	48	57	74	93	114
27	2	39	46	58	73	87	100
28	2	39	46	58	73	92	114
29	2	39	48	59	74	87	106
30	2	42	48	59	72	85	98
31	3	42	53	62	73	85	102
32	3	41	49	65	82	107	129
33	3	39	50	63	77	96	111
34	3	41	49	63	85	107	134
35	3	41	53	64	87	123	158
36	3	39	48	61	76	98	116
37	3	41	48	56	68	80	83
38	3	41	49	61	74	98	109
39	3	42	50	61	78	89	109
40	3	41	55	66	79	101	120
41	4	42	51	66	85	103	124
42	4	42	49	63	84	103	126
43	4	42	55	69	96	131	157
44	4	42	51	65	86	103	118
45	4	41	50	61	78	98	117
46	4	40	52	62	82	101	120
47	4	41	53	66	79	100	123
48	4	39	50	62	80	104	125
49	4	40	53	64	85	108	128
50	4	41	54	67	84	105	122

Weight[12]	Weight[14]	Weight[16]	Weight[18]	Weight[20]	Weight[21]
106	125	149	171	199	205
122	138	162	187	209	215
115	138	163	187	198	202
102	108	136	154	160	157
141	164	197	199	220	223
141	148	155	160	160	157
146	174	218	250	288	305
110	116	126	134	125	*
90	92	93	100	100	98
89	96	101	112	120	124
168	177	182	184	181	175
119	135	162	185	195	205
71	70	71	81	91	96
164	192	227	248	259	266
67	68	*	*	*	*

54	*	*	*	*	*
98	103	113	123	133	142
*	*	*	*	*	*
82	88	106	120	144	157
77	89	98	107	115	117
217	240	275	307	318	331
108	111	131	148	164	167
127	135	145	163	170	175
70	71	72	72	76	74
146	164	197	231	259	265
136	147	169	205	236	251
115	123	144	163	185	192
145	156	184	207	212	233
134	150	187	230	279	309
115	122	143	151	157	150
123	138	170	204	235	256
159	179	221	263	291	305
137	144	151	146	156	147
164	186	235	294	327	341
201	238	287	332	361	373
145	166	198	227	225	220
103	112	135	157	169	178
128	154	192	232	280	290
130	146	170	214	250	272
154	182	215	262	295	321
155	153	175	184	199	204
160	174	204	234	269	281
184	188	197	198	199	200
127	138	145	146	*	*
135	141	147	174	197	196
144	156	173	210	231	238
148	157	168	185	210	205
154	170	222	261	303	322
152	166	184	203	233	237
155	175	205	234	264	264

```

109 FACTOR [LEV=30] Individuals
110 CALC Individuals=NEWLEVELS(Chick; !v(1...20,(1...10)3))
111 DREPMEASURES [GROUPS=Diet,Individuals] DATA=Weight

```

```

***** WARNING message from procedure DREPMEAS :
There are missing values in the DATA pointer
Plots of means can be misleading

```

```
* MESSAGE: There are missing values in the plot
```

```

***** Warning (Code HG 20). Statement 130 in Procedure DREPMEAS
Command: DGRAPH [WINDOW= window; KEYWINDOW= keywindow; TITLE= Title_] GroupMns
Key window full

```

```
* MESSAGE: There are missing values in the plot
```

```

***** Warning (Code HG 20). Statement 130 in Procedure DREPMEAS
Command: DGRAPH [WINDOW= window; KEYWINDOW= keywindow; TITLE= Title_] GroupMns
Key window full

```

```
* MESSAGE: There are missing values in the plot
```

```

***** Warning (Code HG 20). Statement 130 in Procedure DREPMEAS
Command: DGRAPH [WINDOW= window; KEYWINDOW= keywindow; TITLE= Title_] GroupMns
Key window full

```

```
* MESSAGE: There are missing values in the plot
```

```

***** Warning (Code HG 20). Statement 130 in Procedure DREPMEAS
Command: DGRAPH [WINDOW= window; KEYWINDOW= keywindow; TITLE= Title_] GroupMns
Key window full

```

```
112 DREPMEASURES [GROUPS=Diet] DATA=Weight
```

```
***** WARNING message from procedure DREPMEAS :
There are missing values in the DATA pointer
Plots of means can be misleading
```

```

113  "
-114  **** analyse polynomial coefficients to degree 6
-115  "
116  VORTHPOL [MAXDEGREE=6] Weight; CONTRAST=pol
117  BLOCK Chick
118  TREAT Diet
119  FOR k=0...6
120      ANOVA [PRINT=aov; FPROB=Y; PSE=LSD] pol[k]
121      APLOT METHOD=fit,normal
122  ENDFOR

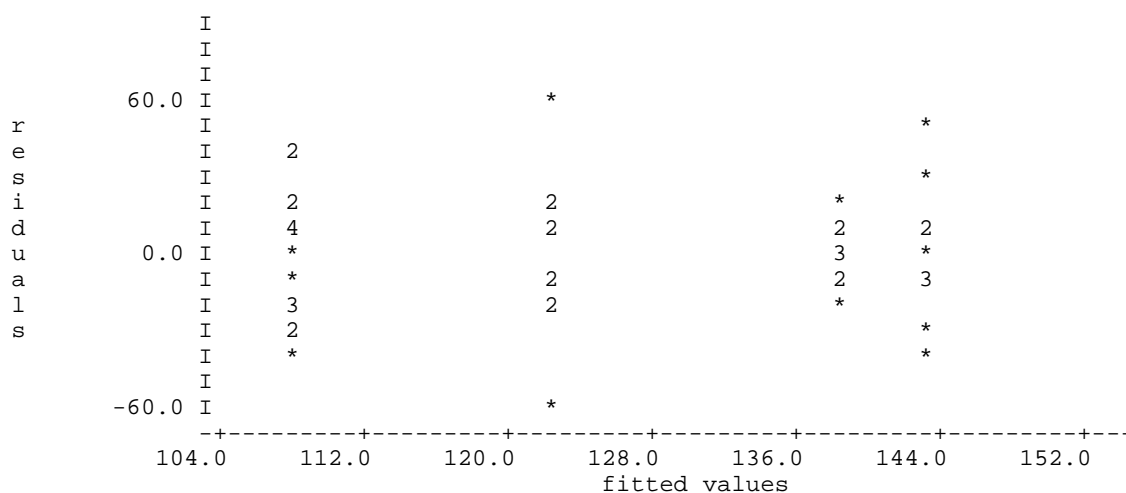
```

```
***** Analysis of variance *****
```

```
Variate: pol[0]
```

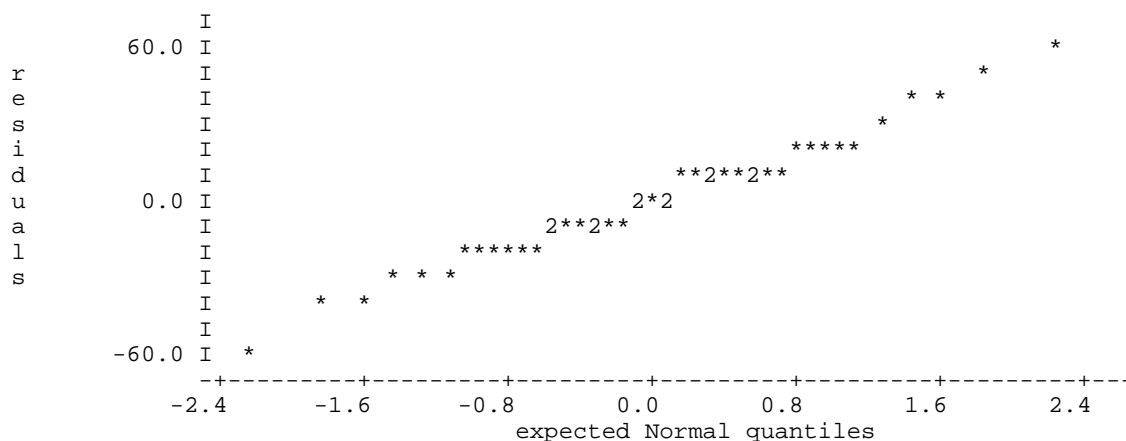
Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Chick stratum					
Diet	3	11015.2	3671.7	5.76	0.002
Residual	41(5)	26124.6	637.2		
Total	44(5)	35824.9			

5



Normal plot

5

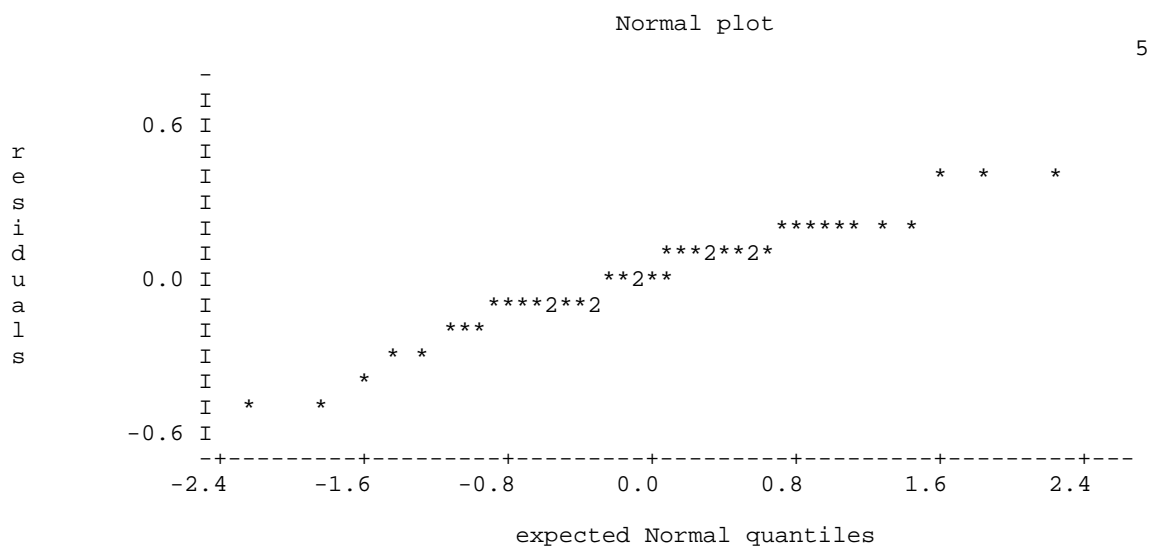
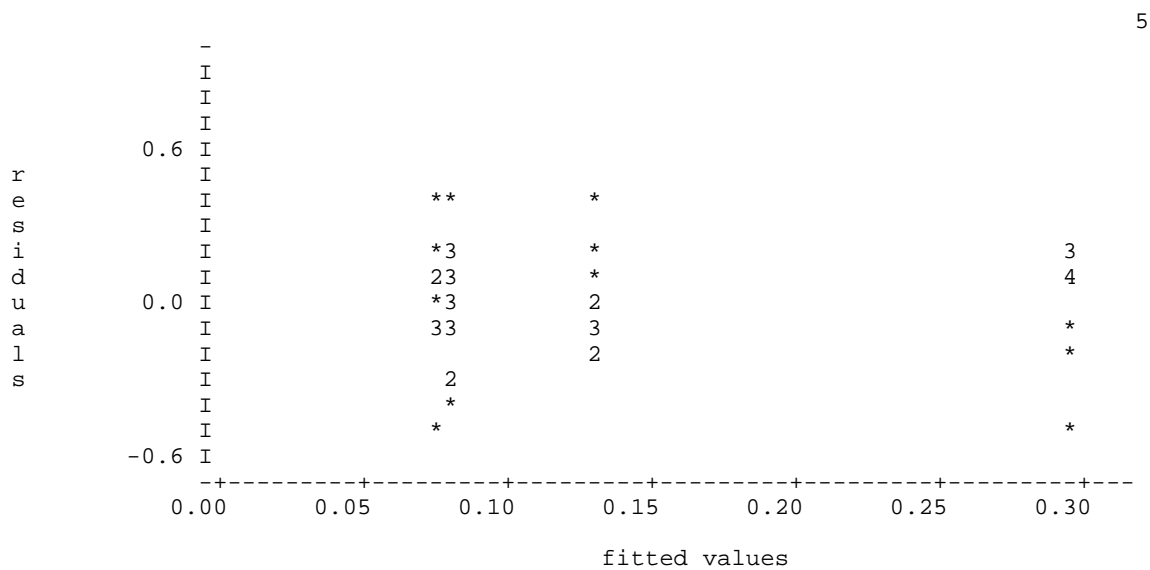


122.....

***** Analysis of variance *****

Variate: pol[2]

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Chick stratum					
Diet	3	0.35795	0.11932	2.54	0.069
Residual	41(5)	1.92227	0.04688		
Total	44(5)	2.26415			

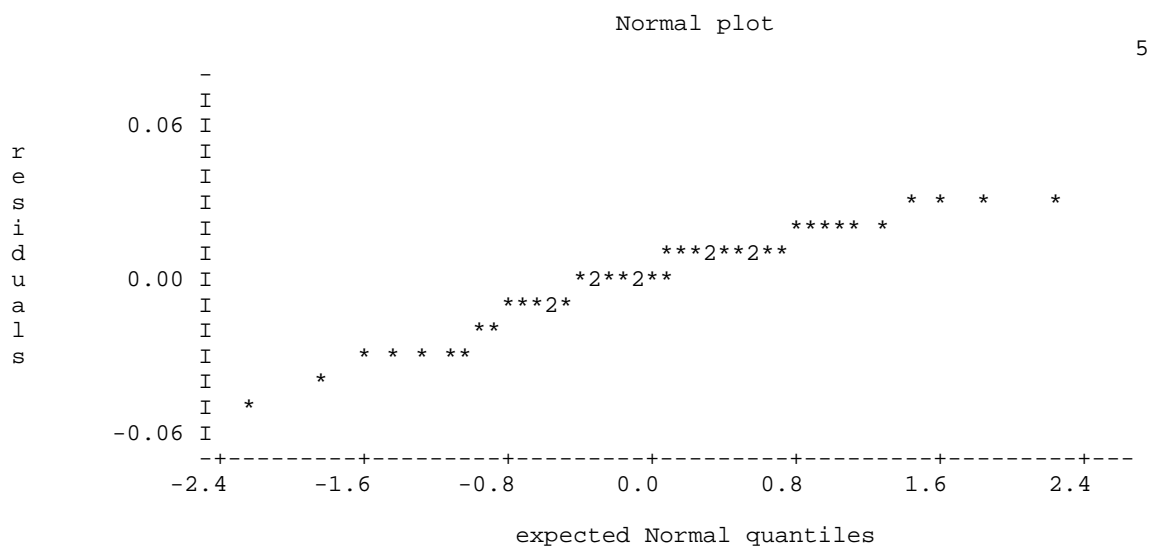
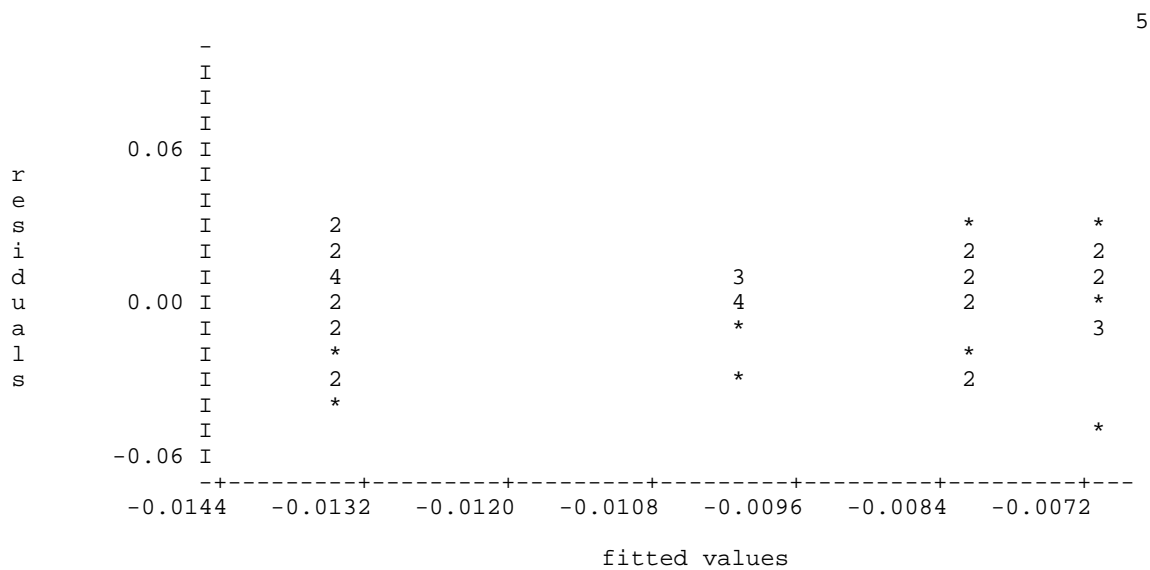


122.....

***** Analysis of variance *****

Variate: pol[3]

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Chick stratum					
Diet	3	0.0003445	0.0001148	0.28	0.841
Residual	41(5)	0.0169446	0.0004133		
Total	44(5)	0.0172501			

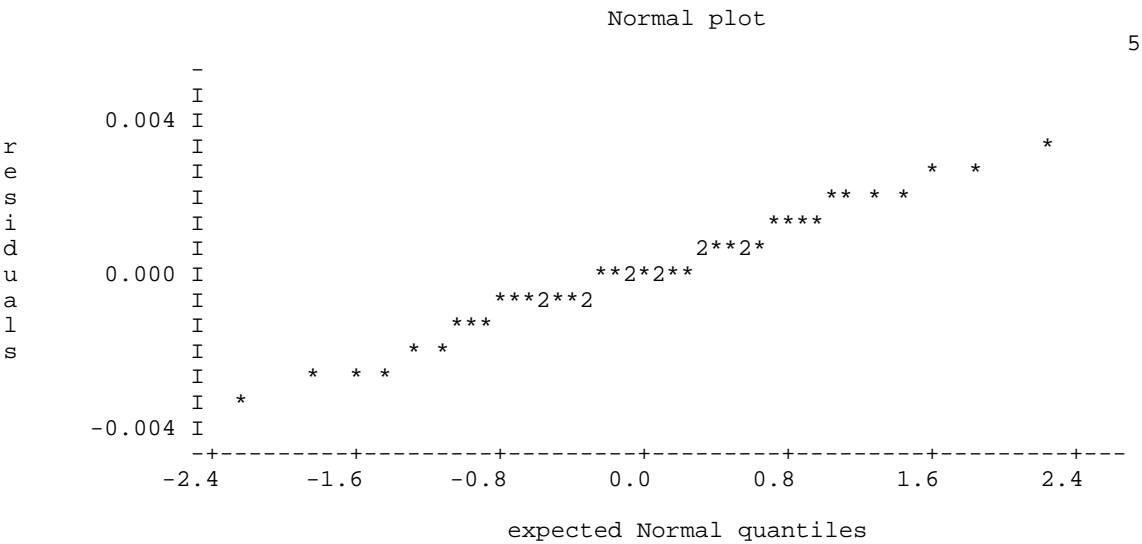
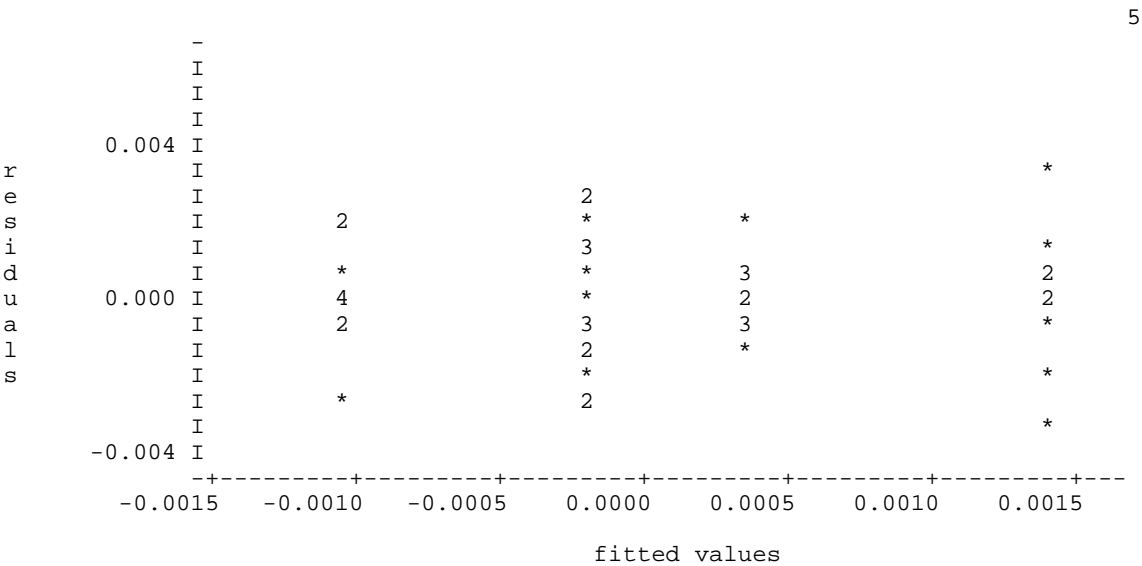


122.....

***** Analysis of variance *****

Variate: pol[4]

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Chick stratum					
Diet	3	0.323E-04	0.108E-04	4.44	0.009
Residual	41(5)	0.994E-04	0.242E-05		
Total	44(5)	0.130E-03			



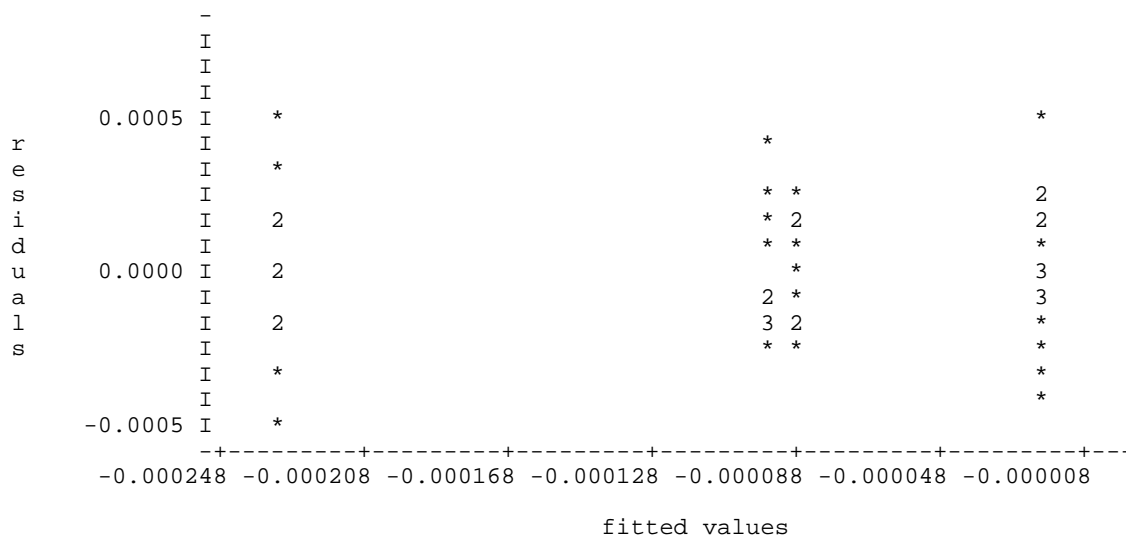
122.....

***** Analysis of variance *****

Variate: pol[5]

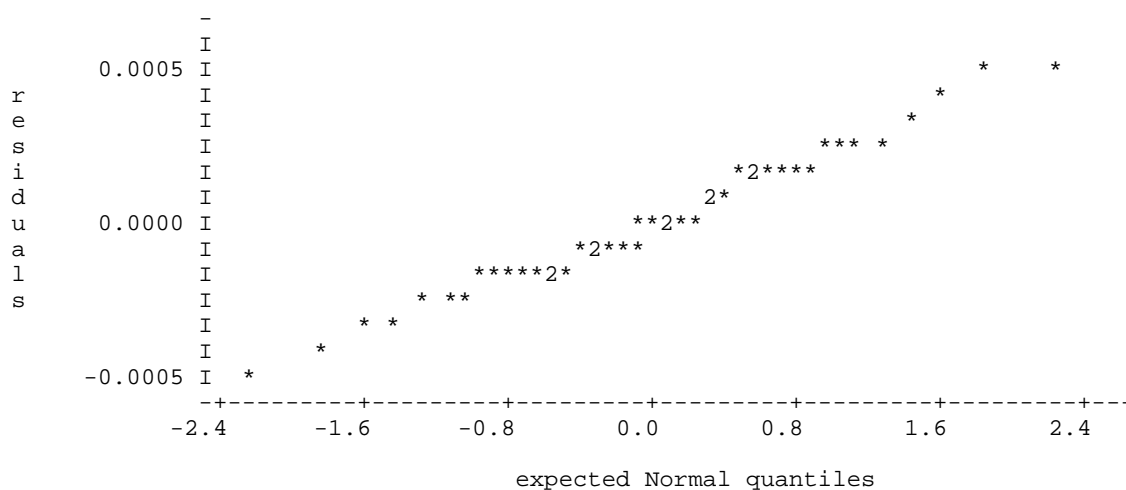
Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Chick stratum					
Diet	3	0.292E-06	0.974E-07	1.65	0.194
Residual	41(5)	0.243E-05	0.592E-07		
Total	44(5)	0.270E-05			

5



Normal plot

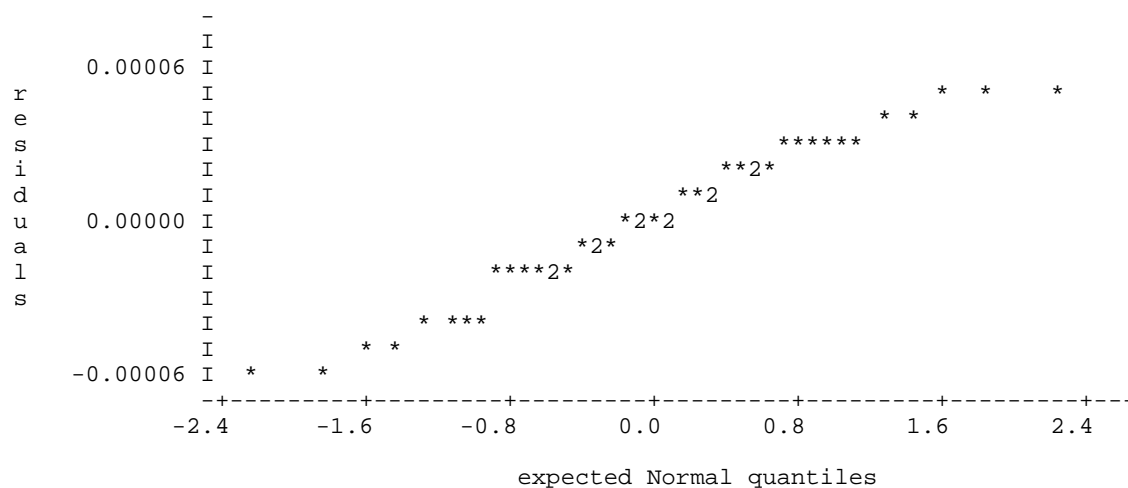
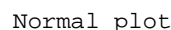
5



122.....

```
Variate: pol[6]
```

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Chick stratum					
Diet	3	0.189E-07	0.630E-08	6.69	<.001
Residual	41(5)	0.386E-07	0.942E-09		
Total	44(5)	0.551E-07			



While the assumptions for the analysis appear to be met, the fourth- and sixth-degree polynomial coefficients are significant. The analysis of polynomial coefficients does not appear to be appropriate for this example. Perhaps non-linear models would provide a more satisfactory basis for analysis of this example. In fitting nonlinear models one has to be particularly careful about the normality of the coefficients and very high correlations between the estimates of different parameters.

c) Computation in Genstat

Example XII.4 Rat weight (continued)

The commands used in the analysis of summary statistics for this example are as follows:

```
"
**** separate data for Days and Rat profiles
"
SUBSET [CONDITION=Days==1] OLD=Rats,Diets; \
      NEW=Rat,Diet
FOR i=1,8...43,44,50,57,64
  SUBSET [CONDITION=Days==i] OLD=Weights; NEW=Weight[i]
ENDFOR
PRINT Rat,Diet,Weight[1,8...43,44,50,57,64]; FIELD=9; DEC=0
FACTOR [LEV=30] Individuals
CALC Individuals=NEWLEVELS(Rat; !v(1...8,(1...4)2))
DREPMEASURES [GROUPS=Diet,Individuals] DATA=Weight
DREPMEASURES [GROUPS=Diet] DATA=Weight
"
**** analyse weight gain
"
CALC WtGain=Weight[64]-Weight[1]
BLOCK Rat
TREAT Diet
ANOVA [FPROB=Y; PSE=LSD] WtGain
APLOT METHOD=fit,normal
YTRANSFORM [TERMS=Diet; LOWER=-1; UPPER=1] WtGain; SAVE=s
CALC TWtGain=LOG(WtGain)
ANOVA [FPROB=Y; PSE=LSD] TWtGain
APLOT METHOD=fit,normal
AKEEP Diet; MEANS=DietMn
CALC DietMn=EXP(DietMn)
PRINT DietMn
"
**** analyse means, linear and quadratic coefficients
"
DUPLICATE OLD=Weight; NEW=TWeight
CALC #TWeight=LOG(#Weight)
DREPMEASURES [GROUP=Rat] DATA=TWeight
VORTHPOL [MAXDEGREE=2] TWeight; CONTRAST=pol
BLOCK Rat
TREAT Diet
FOR k=0...2
  ANOVA [FPROB=Y; PSE=LSD] pol[k]
  APLOT METHOD=fit,normal
ENDFOR
```