Nonlinear Models With Random Effects

TAG study: Examine the effect of glucagon on reducing postpartum concentration of liver triacylglycerols (TAG) in dairy cows (fatty liver disease)

- Treatments
  - Controls :(injection with saline solution) (8 cows)
  - Low level of glucagon: (7 mg of glucagon per day, for 14 days after calving, in 60 ml of saline) (7 cows)
  - High level of glucagon: (15 mg of glucagon per day, for 14 days after calving, in 60 ml of saline) (8 cows)
- Liver biopsies taken at baseline (about 4 days prior to calving) and at days 2, 6, 9, 16, 20, 27, 34, 41

First Order Compartment Model

- Change in TAG concentration in the liver

$$\frac{\partial \mu(t)}{\partial t} = k_a \mu_a(t) - k_e \mu_(t)$$

where

$\mu(t)$ is the TAG concetration in the liver at time t

$\mu_a(t)$ is the amount of TAG to be absorbed at time (t)

k_e is the elimination rate

k_a is the accumulation rate

- The TAG concentration in the liver is

$$\mu(t) = \frac{x k_a}{V(k_a - k_e)}\left(e^{-k_e t} - e^{-k_a t}\right)$$

where x is the level of injected glucagon

When $k_e = k_a = k$ the model simplifies to

$$\mu(t) = \frac{xkte^{-kt}}{V}$$

To account for random measurement error, we will consider the model

$$Y_{ij}(t) = a_{ij} e^{b_{ij} c_{ij} t e^{-c_{ij} t}} \epsilon_{ij}(t)$$

or

$$log(Y_{ij}(t)) = log(a_{ij}) + b_{ij} c_{ij} t e^{-c_{ij} t} + log(\epsilon_{ij}(t))$$

where

$Y_{ij}(t)$ is the liver TAG concentration for the j-th cow in the i-th treatment group

$log(\epsilon_{ij}(t)) \sim NID(0, \sigma_e^2)$

$a_{ij} > 0$ is the liver tag concentration prior to calving (baseline) for the j-th cow in the i-th treatment group

$$log(a_{ij}) = \alpha + \omega_{ij}$$

where α is a fixed parameter and ω_{ij} is a random effect

$b_{ij} > 0$ is an effect of the i-th treatment that controls the maximum of the response curve for the j-th cow in the i-th treatment group

$$log(b_{ij}) = \beta_i + \eta_{ij}$$

where β is a fixed parameter and η_{ij} is a random effect
For the control group (i=1), $\beta_1 = 0$

$c_{ij} > 0$ represents the TAG accumulation and elimination rates in the liver of the j-th cow in the i-th treatment group

$$log(c_{ij}) = \gamma + \delta_{ij}$$

where γ is a fixed parameter and δ_{ij} is a random effect

The vectors of random components $(\omega_{ij},\ \eta_{ij},\ \delta_{ij})$ associated with different cows are assumed to be independent with a joint Gaussian distribution with mean vector $(0,\ 0,\ 0)$ and arbitrary covariance matrix V.

The population response curve for the i-th treatment is

$$\mu_i(t) = \alpha e^{\beta_i \gamma t exp(-\gamma t))} \text{ for } i = 1, 2, 3$$

You could consider different accumulation/elimination rates for different treatments, i.e. $(\gamma_1,\ \gamma_2,\ \gamma_3)$, but this does not significantly improve the fit of the model to the observed data.

The response curve for the i-th treatment group initally increases and achieves its maximum at $t = \gamma^{-1}$. Then the curve declines toward an asymptote at α, the "normal" pre-calving state.

The maximum height of the response curve for the i-th treatment is

$$\mu_i(1/\gamma) = \alpha e^{(\beta_i exp(-1))}$$

The goal is to find treatments that keep this maximum close to α, i.e. treatments with values of β_i close to zero.

Compare treatments by comparing the estimates of the β_i's. Test the null hypothesis $H_0 : \beta_1 = \beta_2 = \beta_3$

This nonlinear model with random effects allows different response curves to be simultaneously fit to individual cows

$$\mu_{ij}(t) = (\alpha+\omega_{ij})e^{((\beta_i+\eta_{ij})(\gamma+\delta_{ij})t\, exp(-(\gamma+\delta_{ij})t))}$$

- The predicted response curve for a cow is smoothed toward the population response curve for it's treatment group.
- Liver TAG concentrations do not have to be measured at the same set of time points for every cow.
- A predicted response curve can be obtained for cows with only one observation


```
data fatliver;
input cow treatment d4p d2 d6 d9 d16 d20 d27 d34 d41;
datalines;
1463 1 4.287 7.341 8.506 10.099 9.362 8.960 6.226 5.821 5.081
1575 1 4.789 7.612 8.634 8.847 6.742 6.084 5.277 5.233 5.035
1581 1 5.393 7.626 6.957 12.099 11.636 11.151 9.343 7.717 5.399
1607 1 3.760 4.478 5.121 6.453 6.283 6.688 6.817 4.385 4.273
1679 1 4.108 4.765 5.916 5.190 4.292 4.227 4.627 4.365 4.084
1719 1 3.855 9.251 9.637 9.044 5.466 4.980 8.201 . .
1740 1 4.352 6.150 5.184 3.438 3.880 3.286 3.490 3.870 4.170
1764 1 . 6.518 16.159 16.721 12.290 13.705 10.063 7.890 4.165
1278 2 5.601 4.071 4.940 5.456 5.584 5.120 4.758 5.019 3.863
1572 2 4.669 8.495 12.218 5.367 7.121 5.823 4.075 3.723 3.691
1604 2 4.463 8.427 6.139 6.635 5.825 4.942 4.790 5.465 4.848
1659 2 4.287 4.598 5.521 6.146 10.399 9.585 7.499 7.091 6.425
1739 2 . 4.443 5.868 4.434 4.576 5.155 4.644 4.297 4.417
1755 2 3.703 6.122 5.129 4.895 3.667 4.001 3.681 3.714 3.583
1766 2 . 4.232 4.744 5.618 4.565 4.069 4.057 3.908 3.923
1522 3 4.036 4.200 4.746 5.220 4.318 4.052 4.090 4.448 5.022
1576 3 4.209 5.766 6.279 4.896 4.450 4.502 4.588 4.290 5.325
1611 3 3.805 3.548 5.352 7.360 5.323 3.170 4.038 2.693 3.425
1670 3 4.304 5.236 3.877 4.770 3.541 4.088 4.082 4.887 .
1703 3 4.932 4.861 7.754 5.683 5.321 4.815 4.704 5.347 5.224
1714 3 4.842 8.603 10.544 8.028 9.834 13.955 10.617 9.720 7.964
1726 3 4.662 4.276 4.641 4.428 4.635 3.934 3.613 3.896 4.123
1743 3 3.863 4.927 5.545 6.049 5.449 4.413 4.707 4.198 4.606
run;
```

```
data fatliver2 (drop=d4p d2 d6 d9 d16 d20 d27 d34 d41);
set fatliver;
tag=d4p;
time=0;
t1=0;
t2=0;
t3=0;
if treatment=1 then t1=1;
if treatment=2 then t2=1;
if treatment=3 then t3=1;
output;

tag=d2;
time=2;
t1=0;
t2=0;
t3=0;
if treatment=1 then t1=1;
if treatment=2 then t2=1;
if treatment=3 then t3=1;
output;

tag=d6;
time=6;
t1=0;
t2=0;
t3=0;
if treatment=1 then t1=1;
if treatment=2 then t2=1;
if treatment=3 then t3=1;
output;
```



```
tag=d9;
time=9;
t1=0;
t2=0;
t3=0;
if treatment=1 then t1=1;
if treatment=2 then t2=1;
if treatment=3 then t3=1;
output;

tag=d16;
time=16;
t1=0;
t2=0;
t3=0;
if treatment=1 then t1=1;
if treatment=2 then t2=1;
if treatment=3 then t3=1;
output;

tag=d20;
time=20;
t1=0;
t2=0;
t3=0;
if treatment=1 then t1=1;
if treatment=2 then t2=1;
if treatment=3 then t3=1;
output;
```



```
tag=d27;
time=27;
t1=0;
t2=0;
t3=0;
if treatment=1 then t1=1;
if treatment=2 then t2=1;
if treatment=3 then t3=1;
output;

tag=d34;
time=34;
t1=0;
t2=0;
t3=0;
if treatment=1 then t1=1;
if treatment=2 then t2=1;
if treatment=3 then t3=1;
output;

tag=d41;
time=41;
t1=0;
t2=0;
t3=0;
if treatment=1 then t1=1;
if treatment=2 then t2=1;
if treatment=3 then t3=1;
output;
run;
```



```
data fatliver2;
  set fatliver2;
   t=time;
   ltag = log(tag);
  run;

proc sort data=fatliver2;
by cow;
run;

/* First fit a model with no random coefficients */

proc nlin data=fatliver2;
parms
alp=2.0
beta1=1.5
beta2=0.7
beta3=0.7
gam=.12;

model ltag = alp+exp(log(beta1*t1+beta2*t2+beta3*t3))*
             exp(log(gam))*t*exp(-(t*exp(log(gam))));

output out=set2 predicted=logpred residual=logresid;
run;
```

The NLIN Procedure
Dependent Variable ltag
Method: Gauss-Newton

Iterative Phase

Iter	alp	beta1	beta2	beta3	gam	Sum of Squares
0	2.0000	1.5000	0.7000	0.7000	0.1200	66.2889
1	1.5004	1.6756	0.7271	0.5931	0.1173	17.6356
2	1.4990	1.6760	0.7280	0.5958	0.1161	17.6348
3	1.4990	1.6760	0.7266	0.5946	0.1160	17.6348
4	1.4990	1.6760	0.7266	0.5946	0.1160	17.6348
5	1.4990	1.6760	0.7265	0.5946	0.1160	17.6348

NOTE: Convergence criterion met.

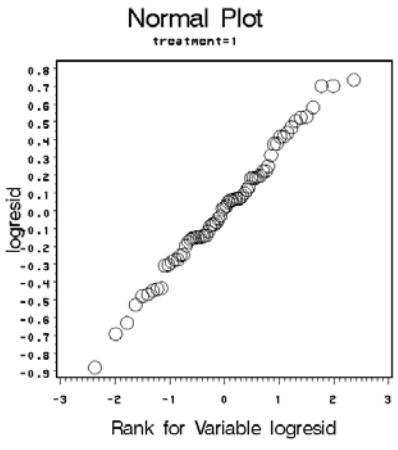
Source	DF	Sum of Squares	Mean Square	F Value	Approx Pr > F
Model	4	5.6539	1.4135	15.71	<.0001
Error	196	17.6348	0.0900		
Corrected Total	200	23.2887			

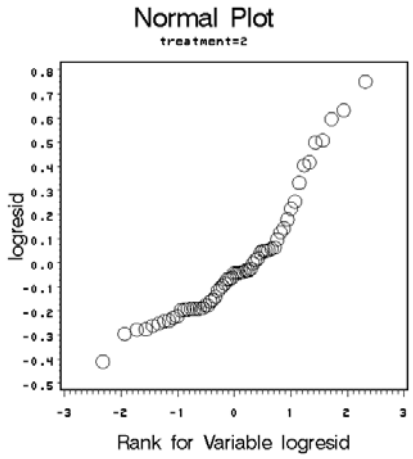
Parameter	Estimate	Approx Std Error	Approximate 95% Confidence Limits	
alp	1.4990	0.0431	1.4140	1.5840
beta1	1.6760	0.2128	1.2564	2.0956
beta2	0.7265	0.2254	0.2820	1.1710
beta3	0.5946	0.2181	0.1643	1.0248
gam	0.1160	0.0134	0.0896	0.1424

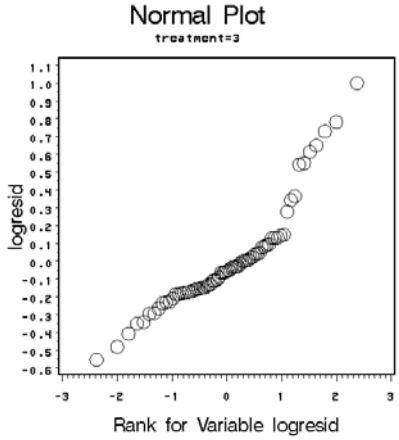
Approximate Correlation Matrix

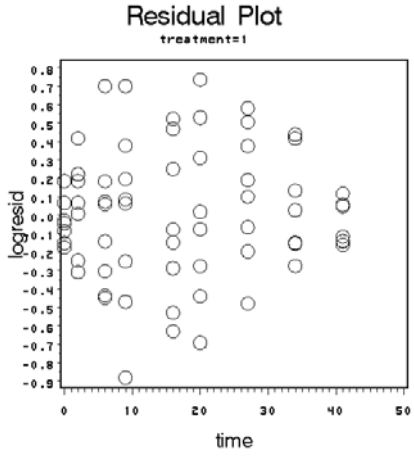
	alp	beta1	beta2	beta3	gam
alp	1.0000	-0.6166	-0.6541	-0.6841	0.4379969
beta1	-0.6166	1.0000	0.4318	0.4458	-0.0349298
beta2	-0.6541	0.4318	1.0000	0.4574	-0.1885094
beta3	-0.6841	0.4458	0.4574	1.0000	-0.2171932
gam	0.4379	-0.0349	-0.1885	-0.2171	1.0000000

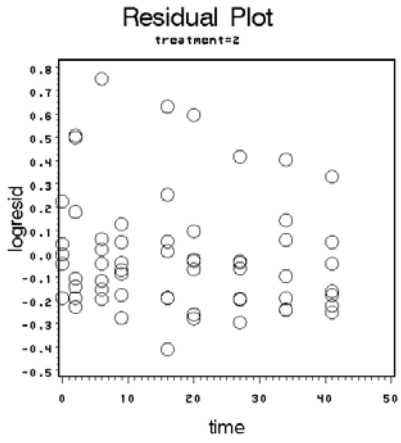

```
proc sort data=set2; by treatment; run;

proc rank data=set2 out=set2 normal=blom; by treatment;
  var logresid; ranks q; run;

proc univariate normal; by treatment;
  var logresid;
  run;

goptions  device=WIN target=WINPRTC;

  axis1 length = 5 in
        label = (h=1.5 f=swiss r=0 a=90)
        color = black width=8.0 style=1;

  axis2 label = (h=1.5 f=swiss )
        length = 5.5 in
        color=black width=8.0 style=1;

  symbol1 v=circle i=none h=2 c=black width=2;
  symbol2 v=none i=spline c=black width=4;

proc gplot data=set2; by treatment;
  plot logresid*q / vaxis=axis1 haxis=axis2;
  title h=3 f=swiss  'Normal Plot';
  run;

proc gplot data=set2; by treatment;
  plot logresid*time / vaxis=axis1 haxis=axis2;
  title h=3 f=swiss  'Residual Plot';
  run;
```

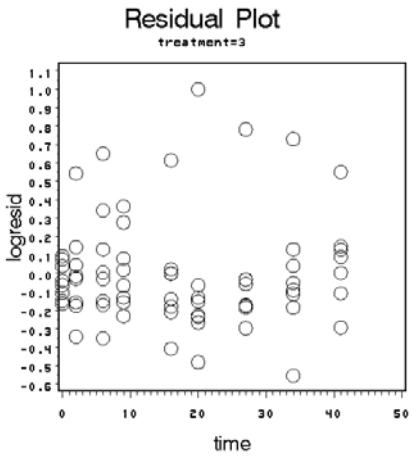




Normal Plot
treatment=2
logresid
Rank for Variable logresid


Normal Plot
treatment=3
logresid
Rank for Variable logresid


Residual Plot
treatment=1
logresid
time


Residual Plot
treatment=2
logresid
time




```
/* Now include random coefficients */

proc nlmixed data=fatliver2 cov method=firo tech=newrap;
parms
alp=1.5
beta1=1.2
beta2=0.80
beta3=0.60
gam=.12

g11=.10
g21= 0.0
g22=0.3
g31 = 0.0
g32= 0.0
g33=1.0
s2=0.05;

lform=exp(log(alp)+w)+exp(log(beta1*t1+beta2*t2+beta3*t3)
             +eta)*exp(log(gam)+del)*
            t*exp(-(t*exp(log(gam)+del)));

res=ltag-lform;

model ltag~normal(lform,s2);
random w eta del~normal([0,0,0],[g11, g21, g22,g31, g32,g33])
       subject=cow;
predict exp(lform)  out=pred;
predict lform out=pred2;
predict res out=res;
run;
```


The NLMIXED Procedure

Specifications

Data Set	WORK.FATLIVER2
Dependent Variable	ltag
Distribution for Dependent Variable	Normal
Random Effects	w eta del
Distribution for Random Effects	Normal
Subject Variable	cow
Optimization Technique	Newton-Raphson
Integration Method	First Order

Dimensions

Observations Used	201
Observations Not Used	6
Total Observations	207
Subjects	23
Max Obs Per Subject	9
Parameters	12

Parameters

alp	beta1	beta2	beta3	gam	g11	g21
1.5	1.2	0.8	0.6	0.12	0.1	0

g22	g31	g32	g33	s2	NegLogLike
0.3	0	0	1	0.05	-4.8588037

Iteration History

Iter	Calls	NegLogLike	Diff	MaxGrad	Slope
1*	247	-23.681871	18.82307	458.5758	-3.636E7
2*	375	-24.353729	0.671858	386.6239	-4.17544
3*	490	-26.031314	1.677585	109.0627	-3.08626
4*	646	-39.986904	13.95559	333.7329	-2.8298
5*	762	-42.287742	2.300838	217.5092	-5.93616
6*	877	-43.054497	0.766755	39.2621	-1.1668
7*	992	-43.445451	0.390954	4.387036	-0.45157
8*	1107	-44.048677	0.603225	4.782687	-0.7302
9*	1222	-44.781015	0.732338	17.07981	-0.98178
10*	1337	-44.962976	0.181961	6.573504	-0.31911
11*	1452	-44.97251	0.009535	0.245998	-0.01781
12*	1567	-44.972599	0.000088	0.002818	-0.00017
13*	1682	-44.972599	3.623E-7	0.000208	-6.97E-7
14*	1797	-44.972599	5.41E-10	0.000011	-1E-9

NOTE: GCONV convergence criterion satisfied.

Fit Statistics

-2 Log Likelihood	-89.9
AIC (smaller is better)	-65.9
AICC (smaller is better)	-64.3
BIC (smaller is better)	-52.3

Parameter	Estimate	Standard Error	DF	t Value	Pr > |t|	Lower
alp	1.5062	0.03083	20	48.86	<.0001	1.4419
beta1	1.3963	0.2695	20	5.18	<.0001	0.8342
beta2	0.9737	0.2183	20	4.46	0.0002	0.5183
beta3	0.6093	0.1476	20	4.13	0.0005	0.3014
gam	0.1273	0.01100	20	11.57	<.0001	0.1043
g11	0.0057	0.003046	20	1.86	0.0775	-0.00069
g21	0.0136	0.01823	20	0.75	0.4631	-0.02439
g22	0.3762	0.2101	20	1.79	0.0885	-0.06202
g31	-0.0272	0.02003	20	-1.36	0.1887	-0.06903
g32	-0.1502	0.1433	20	-1.05	0.3073	-0.4492
g33	0.4686	0.2332	20	2.01	0.0582	-0.01785
s2	0.0213	0.002596	20	8.22	<.0001	0.01593

Parameter Estimates

Parameter	Upper	Gradient
alp	1.5705	1.083E-7
beta1	1.9584	-9.56E-7
beta2	1.4292	-5.27E-7
beta3	0.9171	-3.37E-7
gam	0.1502	-9.36E-8
g11	0.01202	0.000011
g21	0.05166	-7.59E-7
g22	0.8144	7.341E-7
g31	0.01452	-4.45E-6
g32	0.1488	-5.11E-7
g33	0.9550	1.078E-6
s2	0.02676	-1.28E-6

Covariance Matrix of Parameter Estimates

Row	Parameter	alp	beta1	beta2	beta3	gam	g11
1	alp	0.000950	-0.00109	-0.00073	-0.00073	2.183E-6	-9.06E-6
2	beta1	-0.00109	0.07261	0.01276	0.01067	-0.00019	0.000018
3	beta2	-0.00073	0.01276	0.04766	0.007512	-0.00018	-7.11E-6
4	beta3	-0.00073	0.01067	0.007512	0.02178	-0.00044	-0.00002
5	gam	2.183E-6	-0.00019	-0.00018	-0.00044	0.000121	8.593E-6
6	g11	-9.06E-6	0.000018	-7.11E-6	-0.00002	8.593E-6	9.278E-6
7	g21	0.000028	-0.00058	0.000096	-0.00052	7.742E-6	-0.00001
8	g22	0.000594	-0.03018	-0.00974	-0.00761	-0.00042	-0.00002
9	g31	-0.00003	0.000539	0.000587	0.001145	-0.00010	-0.00002
10	g32	-0.00030	0.01439	0.001230	0.002839	0.000375	0.000058
11	g33	0.000910	-0.02555	-0.02346	-0.01644	0.000626	0.000036
12	s2	7.878E-7	9.303E-6	-0.00001	-0.00001	7.86E-7	-1.15E-6

Covariance Matrix of Parameter Estimates

Row	g21	g22	g31	g32	g33	s2
1	0.000028	0.000594	-0.00003	-0.00030	0.000910	7.878E-7
2	-0.00058	-0.03018	0.000539	0.01439	-0.02555	9.303E-6
3	0.000096	-0.00974	0.000587	0.001230	-0.02346	-0.00001
4	-0.00052	-0.00761	0.001145	0.002839	-0.01644	-0.00001
5	7.742E-6	-0.00042	-0.00010	0.000375	0.000626	7.86E-7
6	-0.00001	-0.00002	-0.00002	0.000058	0.000036	-1.15E-6
7	0.000332	-0.00028	-0.00011	-0.00063	0.000609	8.719E-6
8	-0.00028	0.04413	0.000178	-0.01699	0.01564	-0.00006
9	-0.00011	0.000178	0.000401	-0.00022	-0.00221	-4.53E-6
10	-0.00063	-0.01699	-0.00022	0.02055	-0.01311	8.604E-6
11	0.000609	0.01564	-0.00221	-0.01311	0.05438	-0.00002
12	8.719E-6	-0.00006	-4.53E-6	8.604E-6	-0.00002	6.738E-6


```
proc sort data=res; by treatment; run;

proc rank data=res out=res normal=blom; by treatment;
  var pred; ranks q;
    run;

proc univariate data=res normal; by treatment;
  var pred;
  run;
```


---------- treatment=1 ----------

The UNIVARIATE Procedure
Variable: Pred (Predicted Value)

Moments

N	69	Sum Weights	69
Mean	-0.0976224	Sum Observations	-6.735947
Std Deviation	0.2551544	Variance	0.06510377
Skewness	-1.7614104	Kurtosis	4.78923546
Uncorrected SS	5.08463576	Corrected SS	4.42705631
Coeff Variation	-261.36865	Std Error Mean	0.03071698

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.82869	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.197925	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.688562	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	3.935943	Pr > A-Sq	<0.0050

---------- treatment=2 ----------

The UNIVARIATE Procedure
Variable: Pred (Predicted Value)

Moments

N	61	Sum Weights	61
Mean	-0.00341	Sum Observations	-0.2080099
Std Deviation	0.14982385	Variance	0.02244719
Skewness	1.16006476	Kurtosis	1.64426801
Uncorrected SS	1.34754043	Corrected SS	1.34683112
Coeff Variation	-4393.6641	Std Error Mean	0.01918298

Tests for Normality

Test		--Statistic---		-----p Value------
Shapiro-Wilk	W	0.914699	Pr < W	0.0004
Kolmogorov-Smirnov	D	0.139323	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.276215	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.708408	Pr > A-Sq	<0.0050

---------- treatment=3 ----------

The UNIVARIATE Procedure
Variable: Pred (Predicted Value)

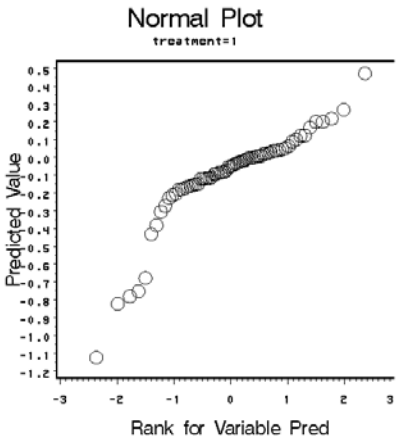
Moments

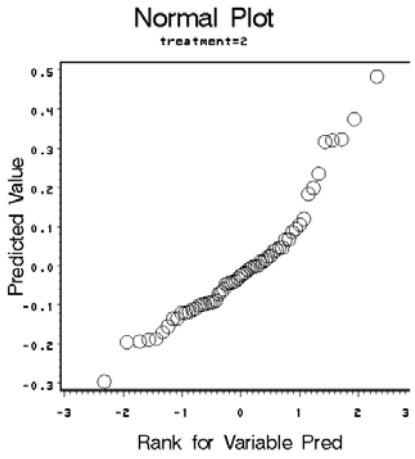
N	71	Sum Weights	71
Mean	-0.0227645	Sum Observations	-1.6162774
Std Deviation	0.14752248	Variance	0.02176288
Skewness	-0.024921	Kurtosis	1.12898985
Uncorrected SS	1.56019543	Corrected SS	1.52340173
Coeff Variation	-648.03826	Std Error Mean	0.0175077

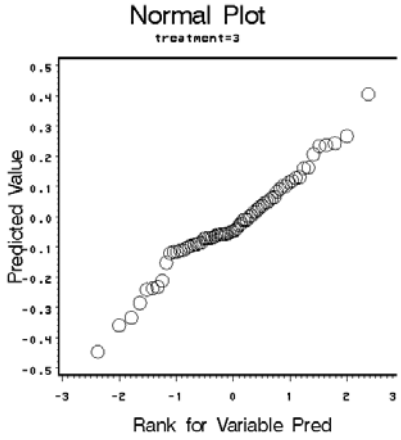
Tests for Normality

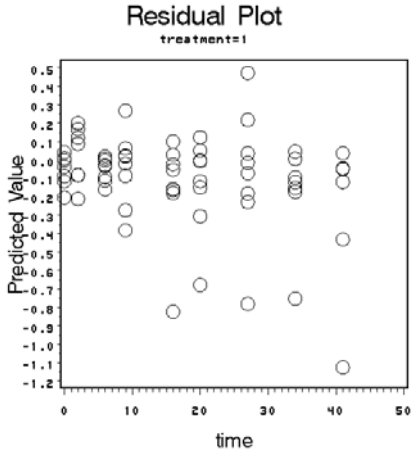
Test		--Statistic---		-----p Value------
Shapiro-Wilk	W	0.970777	Pr < W	0.0963
Kolmogorov-Smirnov	D	0.126726	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.174405	Pr > W-Sq	0.0112
Anderson-Darling	A-Sq	0.936665	Pr > A-Sq	0.0182


```
  axis1 length = 5 in
        label = (h=1.5 f=swiss r=0 a=90)
        color = black width=8.0 style=1;

  axis2 label = (h=1.5 f=swiss )
        length = 5.5 in
        color=black width=8.0 style=1;

  symbol1 v=circle i=none h=2 c=black width=2;
  symbol2 v=none i=spline c=black width=4;

proc gplot data=res; by treatment;
  plot pred*q / vaxis=axis1 haxis=axis2;
  title h=3 f=swiss  'Normal Plot';
  run;

proc gplot data=res; by treatment;
  plot pred*time / vaxis=axis1 haxis=axis2;
  title h=3 f=swiss  'Residual Plot';
  run;
```


Normal Plot
treatment=2
Predicted Value
Rank for Variable Pred


Normal Plot
treatment=3
Predicted Value
Rank for Variable Pred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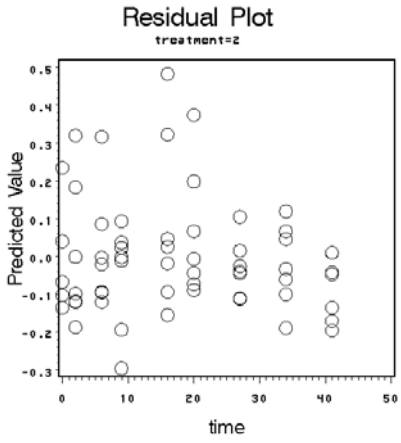
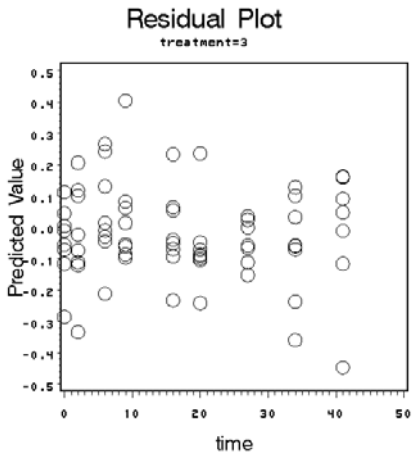
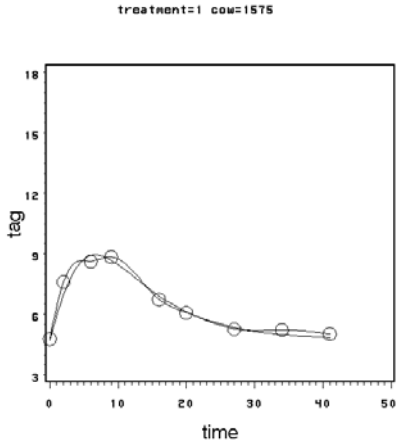
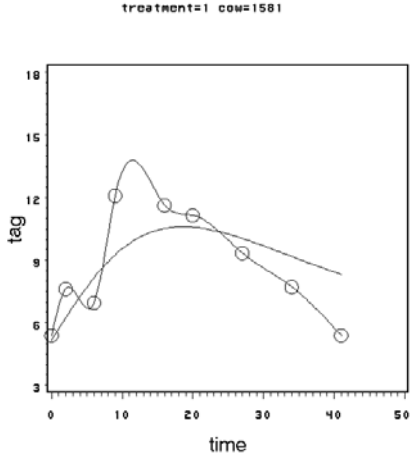
treatment=1
Predicted Value
time


Residual Plot
treatment=2
Predicted Value
time

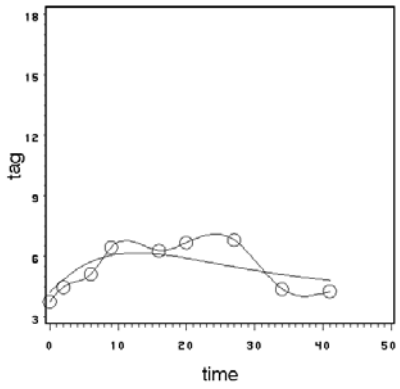



```
proc sort data=pred; by treatment cow; run;


axis1 length = 5 in order=3 to 18 by 3
      label = (h=1.5 f=swiss r=0 a=90)
      color = black width=8.0 style=1;

axis2 label = (h=1.5 f=swiss )
      length = 5.5 in
      color=black width=8.0 style=1;

symbol1 v=circle i=spline h=2 c=black width=2;
symbol2 v=none i=spline c=black width=4;


proc gplot data=pred; by treatment cow;
  plot (tag pred)*time / overlay vaxis=axis1 haxis=axis2;
  title h=3 f=swiss  ' ';
  run;
```



treatment=1 cow=1607



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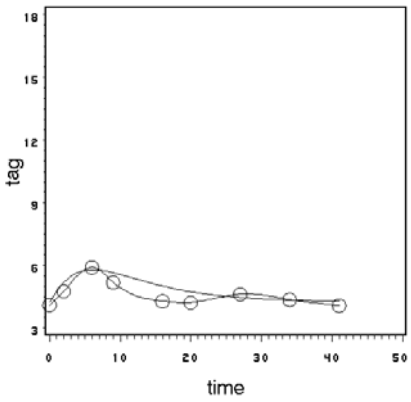

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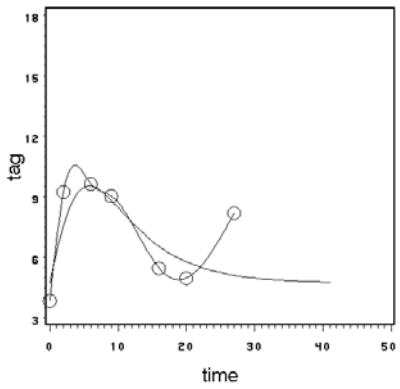

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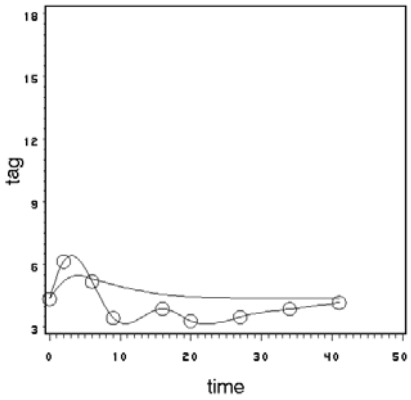

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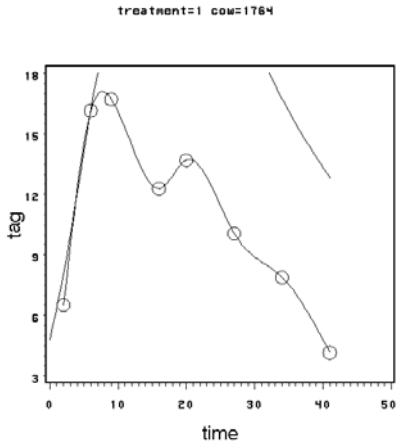

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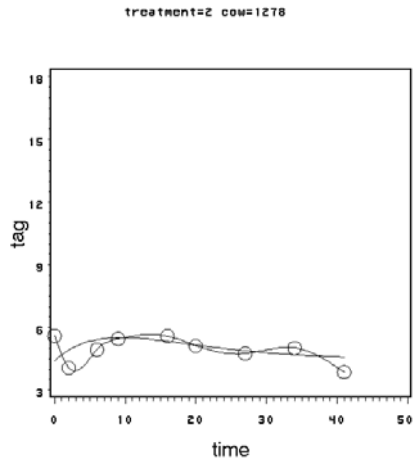

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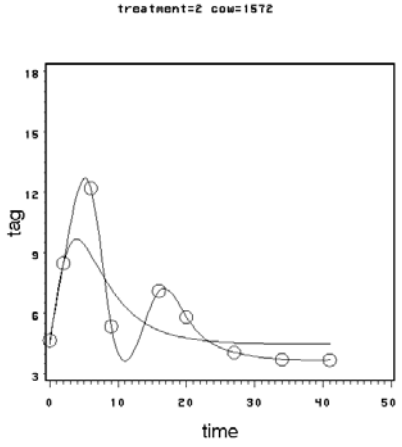

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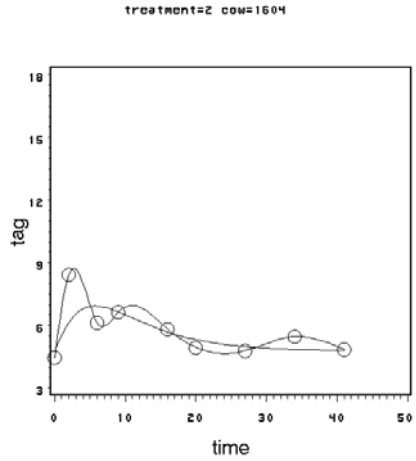

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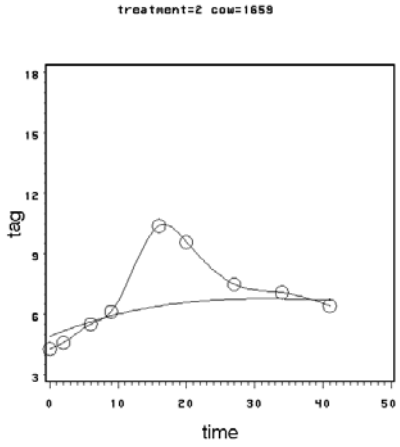

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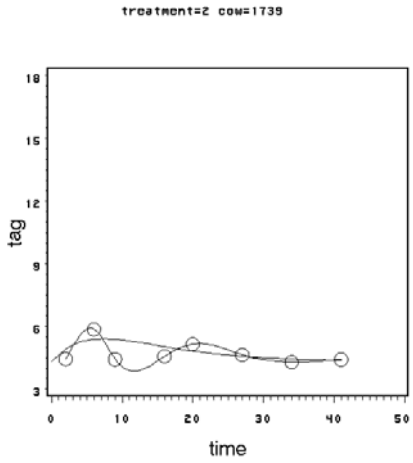

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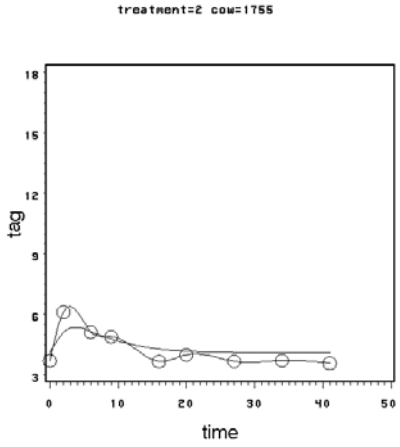

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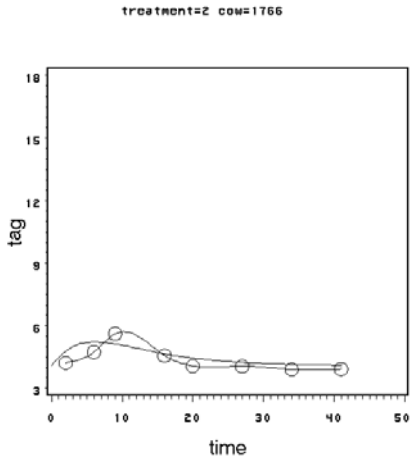

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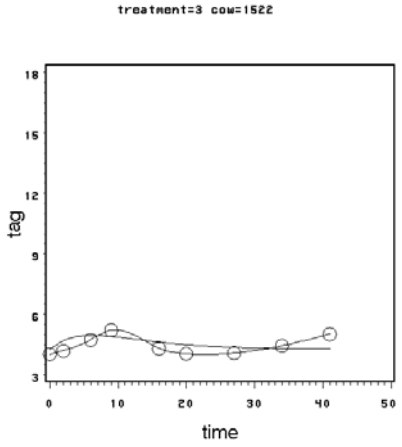

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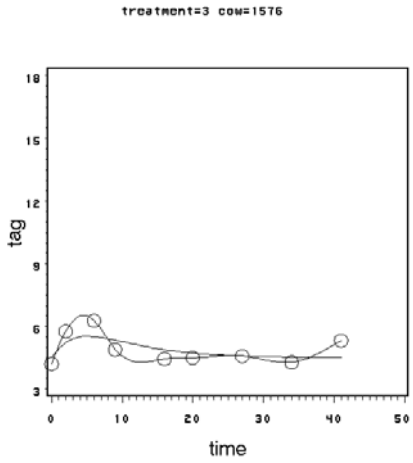

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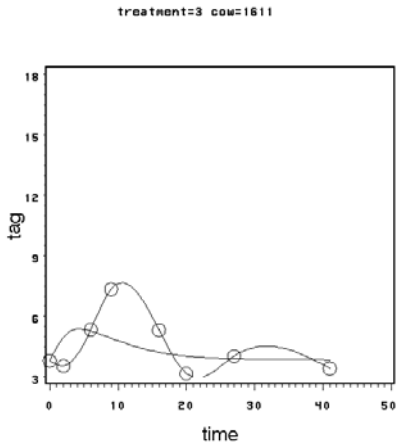

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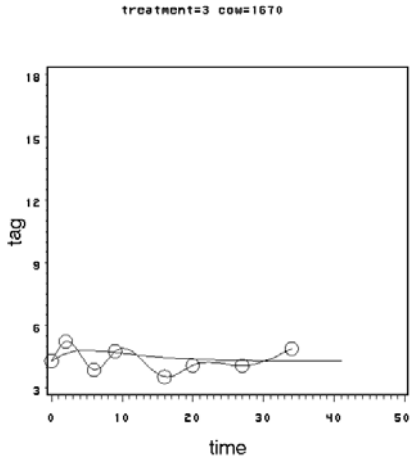

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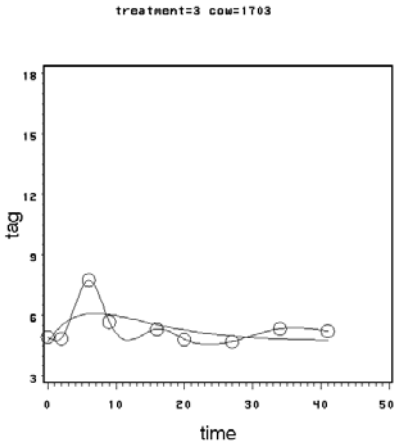
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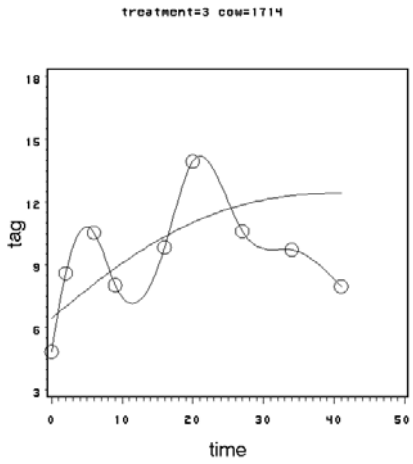
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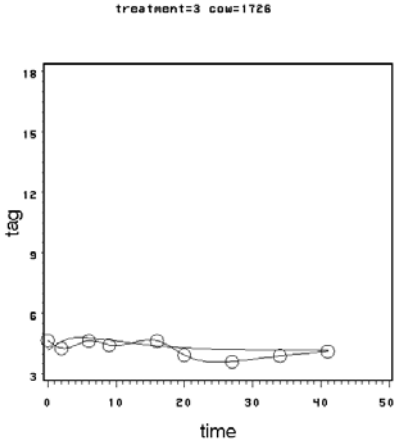
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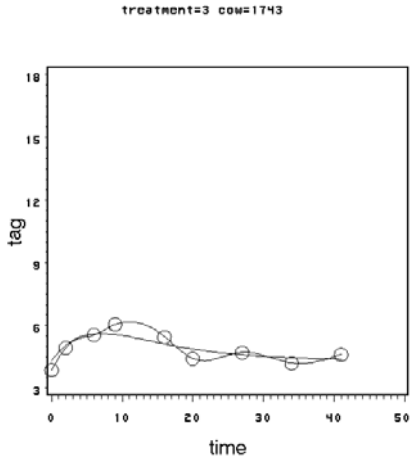
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References

Lindsey, J. K. (2001) Nonlinear Models in Medical Statistics, Oxford University Press, New York. (ISBN 0 19 850812 3)