

The cholesterol content of the yolks of chicken eggs can be controlled by adding certain chemicals to chicken feed. For health reasons, many people want to eat eggs with low-cholesterol yolks. A poultry scientist wishes to compare the effect of 8 chemical additives on yolk cholesterol. 160 chickens were randomly allocated to 8 treatments groups so that there was a different group of 20 chickens consuming each chemical additive. The mean cholesterol content of the eggs produced by each of the 160 chickens was recorded during the study. Are there significant differences among the 8 chemical additives? If so, describe the differences.

```
proc glm;  
  class chemical;  
  model cholesterol=chemical;  
  means chemical / tukey cldiff lines alpha=0.05;  
  lsmeans chemical / adjust=tukey pdiff cl alpha=0.05;  
run;
```

Notes on SAS program ...

#### 1<sup>st</sup> means statement

The MEANS statement above provides the value of the HSD and all possible pairwise differences with their 95% simultaneous CIs (with familywise confidence level) resulting from the TUKEY CLDIFF ALPHA=0.05 options. The HSD and CIs include the Tukey-Kramer adjustment if sample sizes are not equal across groups. After the CI output, a listing of the means and a version of a line plot is given, resulting from the LINE option.

The main advantage of this statement is that the HSD is reported and you can get a line graph. However, when we get to 2-way ANOVAs, MEANS will not provide appropriate estimates for interactions. Use LSMEANS whenever possible.

#### 2<sup>nd</sup> lsmeans statement

The LSMEANS statement above produces several items. The CL ALPHA=0.05 option provides estimated treatment means (not differences) and their individual 95% CIs – this is not a family-wise confidence level.

The ADJUST=TUKEY PDIFF ALPHA=0.05 option provides a p-value for the test of  $H_0: \mu_i = \mu_k$  (vs. a difference) for each pair of means ( $i, k$ ) and an estimated difference and the family-wise confidence intervals using the Tukey-Kramer procedure.

## The GLM Procedure

Dependent Variable: cholesterol

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	13122.78184	1874.68312	56.20	<.0001
Error	152	5070.00758	33.35531		
Corrected Total	159	18192.78942			

Tukey's Studentized Range (HSD) Test for cholesterol

NOTE: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	152
Error Mean Square	33.35531
Critical Value of Studentized Range	4.34676
Minimum Significant Difference	5.6135

Comparisons significant at the 0.05 level are indicated by \*\*\*.

chemical Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
1 - 8	0.152	-5.461	5.766	
1 - 6	5.834	0.221	11.448	***
1 - 5	7.693	2.079	13.306	***
1 - 4	10.808	5.195	16.422	***
1 - 7	12.029	6.416	17.643	***
1 - 2	22.896	17.283	28.510	***
1 - 3	26.421	20.808	32.035	***
8 - 1	-0.152	-5.766	5.461	
8 - 6	5.682	0.069	11.295	***
8 - 5	7.540	1.927	13.154	***
8 - 4	10.656	5.042	16.269	***
8 - 7	11.877	6.263	17.490	***
8 - 2	22.744	17.131	28.358	***
8 - 3	26.269	20.655	31.882	***
6 - 1	-5.834	-11.448	-0.221	***
6 - 8	-5.682	-11.295	-0.069	***
6 - 5	1.858	-3.755	7.472	
6 - 4	4.974	-0.640	10.587	
6 - 7	6.195	0.581	11.808	***
6 - 2	17.062	11.449	22.676	***
6 - 3	20.587	14.973	26.200	***
5 - 1	-7.693	-13.306	-2.079	***
5 - 8	-7.540	-13.154	-1.927	***
5 - 6	-1.858	-7.472	3.755	
5 - 4	3.115	-2.498	8.729	
5 - 7	4.336	-1.277	9.950	
5 - 2	15.204	9.590	20.817	***

5	- 3	18.728	13.115	24.342	***
4	- 1	-10.808	-16.422	-5.195	***
4	- 8	-10.656	-16.269	-5.042	***
4	- 6	-4.974	-10.587	0.640	
4	- 5	-3.115	-8.729	2.498	
4	- 7	1.221	-4.392	6.834	
4	- 2	12.088	6.475	17.702	***
4	- 3	15.613	10.000	21.226	***
7	- 1	-12.029	-17.643	-6.416	***
7	- 8	-11.877	-17.490	-6.263	***
7	- 6	-6.195	-11.808	-0.581	***
7	- 5	-4.336	-9.950	1.277	
7	- 4	-1.221	-6.834	4.392	
7	- 2	10.867	5.254	16.481	***
7	- 3	14.392	8.779	20.005	***
2	- 1	-22.896	-28.510	-17.283	***
2	- 8	-22.744	-28.358	-17.131	***
2	- 6	-17.062	-22.676	-11.449	***
2	- 5	-15.204	-20.817	-9.590	***
2	- 4	-12.088	-17.702	-6.475	***
2	- 7	-10.867	-16.481	-5.254	***
2	- 3	3.525	-2.089	9.138	
3	- 1	-26.421	-32.035	-20.808	***
3	- 8	-26.269	-31.882	-20.655	***
3	- 6	-20.587	-26.200	-14.973	***
3	- 5	-18.728	-24.342	-13.115	***
3	- 4	-15.613	-21.226	-10.000	***
3	- 7	-14.392	-20.005	-8.779	***
3	- 2	-3.525	-9.138	2.089	

Tukey's Studentized Range (HSD) Test for cholesterol

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	152
Error Mean Square	33.35531
Critical Value of Studentized Range	4.34676
Minimum Significant Difference	5.6135

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	chemical
A	219.828	20	1
A			
A	219.676	20	8
B	213.994	20	6
B			
C B	212.135	20	5
C B			
C B	209.020	20	4
C			
C	207.799	20	7
D	196.932	20	2
D			
D	193.407	20	3

Least Squares Means  
Adjustment for Multiple Comparisons: Tukey

chemical	cholesterol LSMEAN	LSMEAN Number
1	219.828050	1
2	196.931750	2
3	193.406950	3
4	209.019950	4
5	212.135450	5
6	213.993800	6
7	207.798950	7
8	219.675800	8

Least Squares Means for effect chemical  
Pr > |t| for H0: LSmean(i)=LSmean(j)

Dependent Variable: cholesterol

i/j	1	2	3	4	5	6	7	8
1		<.0001	<.0001	<.0001	0.0011	0.0354	<.0001	1.0000
2	<.0001		0.5327	<.0001	<.0001	<.0001	<.0001	<.0001
3	<.0001	0.5327		<.0001	<.0001	<.0001	<.0001	<.0001
4	<.0001	<.0001	<.0001		0.6835	0.1236	0.9977	<.0001
5	0.0011	<.0001	<.0001	0.6835		0.9712	0.2616	0.0015
6	0.0354	<.0001	<.0001	0.1236	0.9712		0.0195	0.0450
7	<.0001	<.0001	<.0001	0.9977	0.2616	0.0195		<.0001
8	1.0000	<.0001	<.0001	<.0001	0.0015	0.0450	<.0001	

chemical	cholesterol LSMEAN	95% Confidence Limits	
1	219.828050	217.276599	222.379501
2	196.931750	194.380299	199.483201
3	193.406950	190.855499	195.958401
4	209.019950	206.468499	211.571401
5	212.135450	209.583999	214.686901
6	213.993800	211.442349	216.545251
7	207.798950	205.247499	210.350401
8	219.675800	217.124349	222.227251

Least Squares Means  
Adjustment for Multiple Comparisons: Tukey

Least Squares Means for Effect chemical

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	22.896300	17.282812	28.509788
1	3	26.421100	20.807612	32.034588
1	4	10.808100	5.194612	16.421588
1	5	7.692600	2.079112	13.306088
1	6	5.834250	0.220762	11.447738
1	7	12.029100	6.415612	17.642588
1	8	0.152250	-5.461238	5.765738
2	3	3.524800	-2.088688	9.138288
2	4	-12.088200	-17.701688	-6.474712
2	5	-15.203700	-20.817188	-9.590212
2	6	-17.062050	-22.675538	-11.448562
2	7	-10.867200	-16.480688	-5.253712
2	8	-22.744050	-28.357538	-17.130562
3	4	-15.613000	-21.226488	-9.999512
3	5	-18.728500	-24.341988	-13.115012
3	6	-20.586850	-26.200338	-14.973362
3	7	-14.392000	-20.005488	-8.778512
3	8	-26.268850	-31.882338	-20.655362
4	5	-3.115500	-8.728988	2.497988
4	6	-4.973850	-10.587338	0.639638
4	7	1.221000	-4.392488	6.834488
4	8	-10.655850	-16.269338	-5.042362
5	6	-1.858350	-7.471838	3.755138
5	7	4.336500	-1.276988	9.949988
5	8	-7.540350	-13.153838	-1.926862
6	7	6.194850	0.581362	11.808338
6	8	-5.682000	-11.295488	-0.068512
7	8	-11.876850	-17.490338	-6.263362