MAXIMUM INGREDIENT LEVEL OPTIMIZATION WORKBOOK

for Estimating the Maximum Safe Levels of Feedstuffs

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Click [here] to download the MIOW.xls. All modern versions of Microsoft Excel with Visual Basic function should be enough to run the MIOW workbook. To use the workbook, the solver add-in must be activated and macros must be enabled.

MIOW QUICK REFERENCE

If you'd like to estimate MSL from existing data or to estimate coefficients for a new experiment:
Go to one of the True Parameter Estimator windows and enter your data in the input and output columns, estimate the parameters in cells H8 to I8 or J8, and click on the "Fit the Model" button.

If you'd like to simulate the effects of different numbers of reps and levels on the MSL:
Go to the "Levels & Reps" window and create a grid. Then go the "Simulations" window and choose a true model and enter its coefficients. Then enter the predicted CV and number of experiments to simulate and click "Run."

UGA extension

Why is it important to estimate the maximum safe level of a feed ingredient?

Every year, potential feedstuffs are being evaluated as new feed ingredients for livestock. The evaluation process includes feeding the test ingredients at increasing levels to groups of birds or animals, at which point the pattern of the biological response and/or the maximum safe level of this ingredient can be estimated. The biological response of feeding an ingredient varies depending on several factors, such as the age and species of the test animal and the chemical composition of the ingredient. One scenario that reflects a response to an ingredient in a feeding trial (Gamboa *et al.*, 2001) is illustrated in Figure 1. Feeding increasing levels of cottonseed meal had no impact on the growth performance of broilers (up to a certain point) as represented by the plateau segment of the curve.

Further increasing the cottonseed meal level resulted in reducing the growth performance as represented by the descending segment of the line. Underestimating the maximum safe level of cottonseed meal will not maximize the economic returns of including this ingredient in the ration, while overestimating the level of the meal will result in a significant reduction in growth performance due to the nature of the chemical composition of the ingredient (e.g. high levels of antinutritional factors). Therefore, precisely finding the maximum safe level of feed ingredients is required to maximize the performance and the profits.

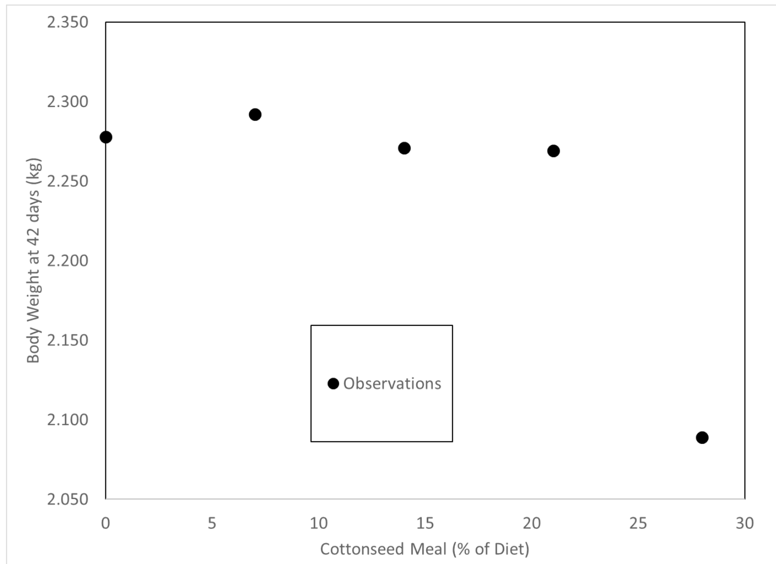

Figure 1. Growth response of broiler chickens fed increasing levels of cottonseed meal (Gamboa *et al.*, 2001).

What are the statistical methods used to estimate the maximum safe level of feedstuffs?

Several statistical methods have been used in animal feeding trials to estimate the maximum safe level of feed ingredients. The most common and easiest method is by separating the means of the response variable using a multiple range test. The multiple range tests are based on one-way analysis of variance and were designed for categorical data to distinguish between feeding cottonseed meal versus soybean meal, not different levels of an independent variable like cottonseed meal. In terms of multiple-range tests, the working definition of “maximum safe level” is the maximum level of the feed ingredient that results in a response not significantly different from the maximum or minimum response at a chosen level of significance. These tests are not valid to analyze data obtained from feeding trials where the factor is continuous because the actual safe level can only be on or between two levels; more conservative tests (e.g. Scheffe’s test, 1953, vs. Duncan’s test, 1955) will result in detecting fewer significant differences, and extrapolation and constructing a confidence interval for a mean maximum safe level is not possible.

Another method used in feeding trials is the orthogonal contrast, which compares levels against the control group. Since fewer numbers of comparisons are made, this method is more precise than the multiple range tests, but they are not really orthogonal and distinguish only between levels as with the multiple range tests.

Employing polynomial regression analysis helps to understand the pattern of the data (e.g. linear, quadratic, or second-order polynomial). With second-order polynomial (2OP) regression, the independent variable is treated as a continuous variable and the maximum safe level is determined by finding the first derivative (level of input at the maximum response). However, second-order polynomial regression models provide no feature to fit a plateau segment of the response function and the estimated maximum safe levels may be considerably less than levels actually resulting in maximum performance (Figure 2).

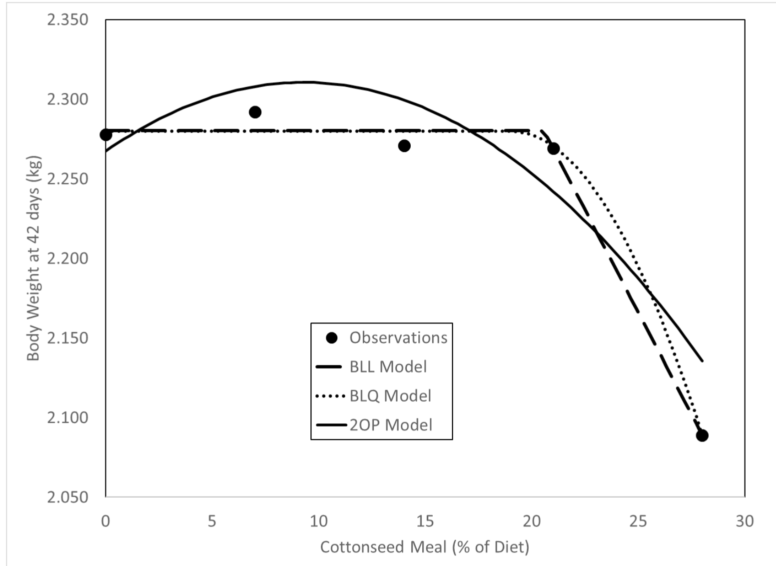

Figure 2. Growth response of broiler chickens fed increasing levels of cottonseed meal (Gamboa *et al.*, 2001) fitted to three different models for estimating the maximum safe level to feed.

There are two spline functions that may be fit to ingredient response data to find the MSL and its confidence interval. The broken-line linear (BLL) model depicts a constant response to increasing levels of the ingredient (plateau with a slope of zero) followed by a linear response (descending line). The broken-line quadratic (BLQ) model depicts a constant response to increasing levels of the ingredient (plateau with a slope of zero) then a non-linear diminishing returns response (descending curve). For both of these spline models, the break or transition point between the two segments represents the MSL and its confidence interval (standard error or SE) can be calculated.

It's difficult to identify which of these models will best represent the actual shape of the response to the ingredient levels. Particular models may fit one set of data best, but there does not appear to be a best model for all response data sets. Nutritionists usually like to include a margin of safety with such determinations to be sure that there are no detrimental effects from including the ingredient in question. Since the BLQ model gives lower estimates than the BLL model, a smaller margin of safety will probably be required when the BLQ model is used. The important consideration when determining margins of safety is the batch-to-batch variation that is found in deliveries to the feed mill.

What does the Maximum Ingredient Level Optimization Workbook do?

The MIOW (Microsoft Corp., Redmond, WA) provides two basic functions for estimating MSLs. First, it has spreadsheets for estimating MSLs from experimental data. With the "True Parameter Estimator" (BLL) & "True Parameter Estimator" (BLQ) spreadsheets, experimental results can be entered and the various parameters can be solved for. Second, the "Levels & Reps" and "Simulations" spreadsheets can be used for planning new experiments. They can be used to find the combination of ingredient levels and replications per level that maximize experimental efficiency.

If you already have experimental data, or need to know what the parameters might be to start investigating experimental possibilities, the true parameter estimator spreadsheets determine the maximum safe level of feed ingredients and the related descriptive statistics: confidence interval (CI), standard deviation (SD), standard error (SE) and the R_2 for the fitted relationship estimates.

If you want to plan an experiment and need to know the best combination of levels and reps for your experiment, you need to first find coefficients for the model you think should best represent the response. The important things to know are the mean and standard deviation of the response expected (body weight, FCR, bone ash, etc.). Coefficient estimates can be made for the two-spline functions, and one of those must be chosen to represent the expected response on the "Simulations" spreadsheet. The mean and standard error of the MSL are then estimated from simulated experiments. MSLs are estimated from the broken-line linear model (BLL), broken-line quadratic model (BLQ), and second-order polynomial (2OP) model.

What does the Maximum Ingredient Level Optimization Workbook consist of?

The workbook contains several individual spreadsheets for experimental planning (Figure 3): "Home page," "Instructions," "Levels & Reps," "Simulations" and "Calculations" spreadsheets. The "Levels & Reps" spreadsheet is designed to generate an experimental grid (combinations of level and replications) for the experiment being simulated. The experimental grid contains the levels of the ingredient as well as the number of replications of the experiment. The "Simulations" spreadsheet contains sections for the entry of the true parameters of the response function, initial guesses for the parameters of fitted functions, simulation parameters, results, and a graph of the results.

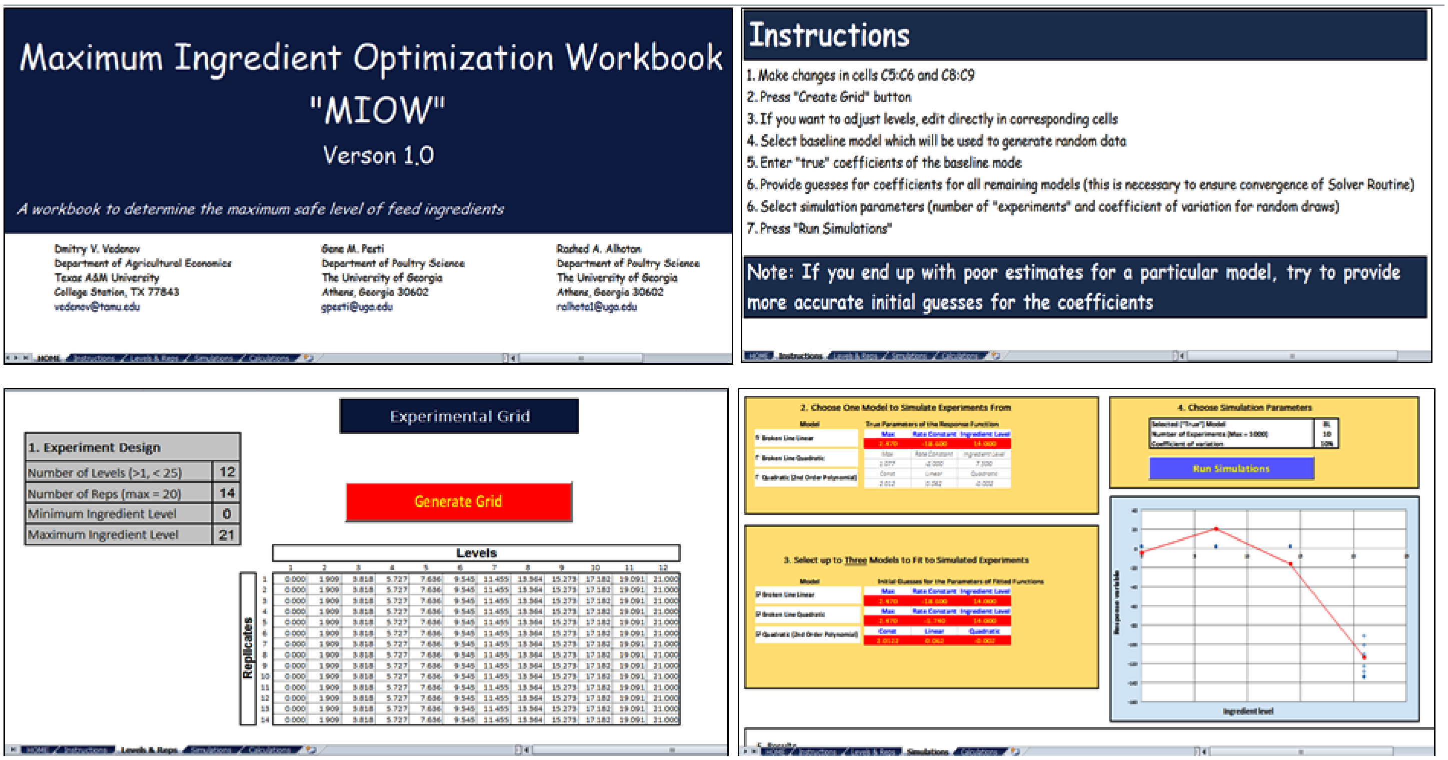

Figure 3. Overview of the Maximum Ingredient Level Optimization Workbook spreadsheets.

How is the Maximum Ingredient Level Optimization Workbook used?

The MIOW can be used by following the next steps:

1. Design the experiment being simulated by making changes in cells C5:C6 and C8:C9.
   The number of ingredient levels and the number of replications of the experiment being simulated can be entered in cells C5 and C6, respectively. It should be noted that the maximum number of levels that can be used here is limited to 24 while the maximum number of replicates is 20. The minimum and maximum ingredient levels should be entered in cells C8 and C9, respectively.

2. Click "Generate Grid" to create the experimental grid.
   By clicking on the "Generate Grid" button, a table containing the experimental replicates and the associated ingredient levels will be created. The levels of the ingredient being used will be evenly spaced. In a feeding trial (Moghaddam *et al*, 2012), four levels of sunflower meal (0-21 percent) were used, and each dietary treatment was replicated 4 times. Figure 4 shows the experimental grid after updating the experimental design section with the experimental design information from this research. The data contained in the table will be used in the "Calculations" spreadsheet in model fitting.

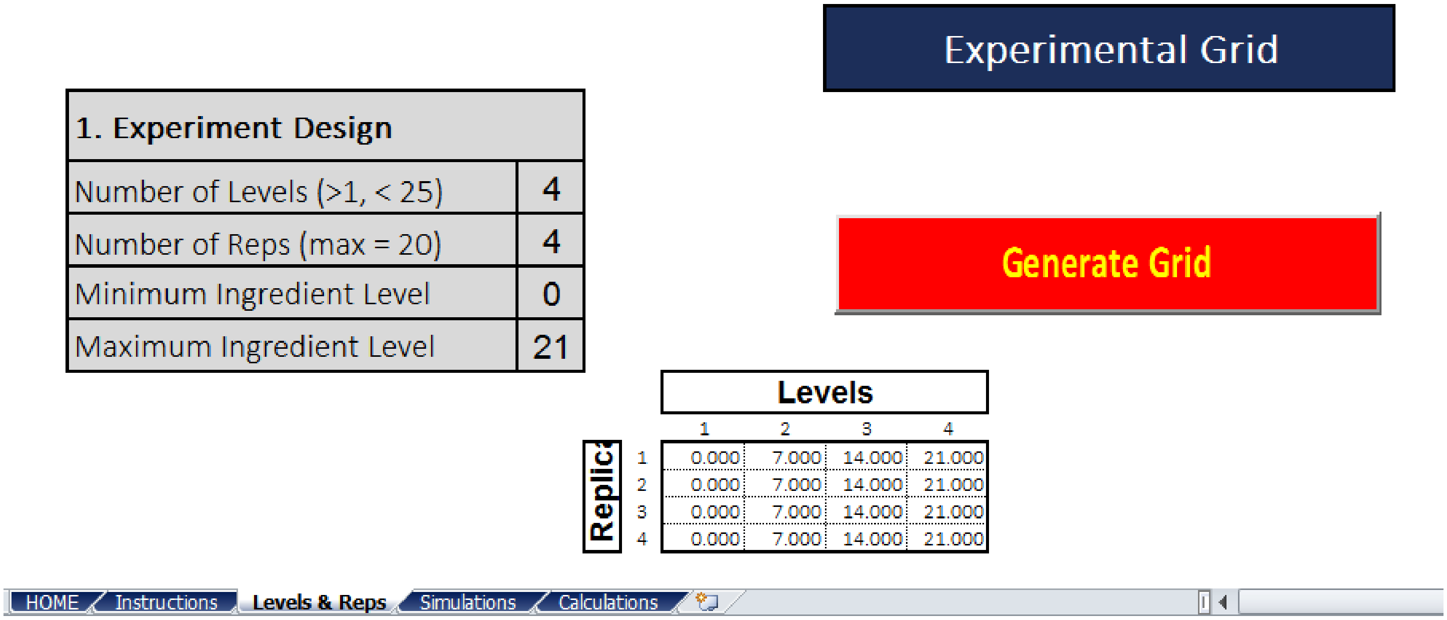

Figure 4. Experimental grid generation, part of the "Levels & Reps" spreadsheet.

3. Select the baseline model that will be used to generate random data.

Three mathematical models are available in section two of the "Simulations" spreadsheet (Figure 5). The models are broken-line linear (BLL), broken-line quadratic (BLQ), and second-order polynomial (2OP). Only one model can be selected at a time to simulate experiments.

Enter "true" coefficients of the baseline mode

The maximum value of the response variable (e.g. weight gain), the rate constant of the fitted function, and the level of the ingredient producing the maximum response should be entered in the corresponding cells for each of the broken line models. The true parameters of the second-order polynomial of the form of $y=\beta_o+\beta_1 x+\beta_2 x^2+\epsilon$ include constant term (β_o), linear term (β_1) and quadratic term (β_2) and should be entered in the specified cells. In the example (Moghaddam *et al*, 2012), the maximum weight gain at 49 days was 2.472 kg for the group of chickens fed 14 percent sunflower meal. Section two of the "Simulations" spreadsheet was updated with these values as true coefficients. The true coefficients will be used to generate random data using simulation.

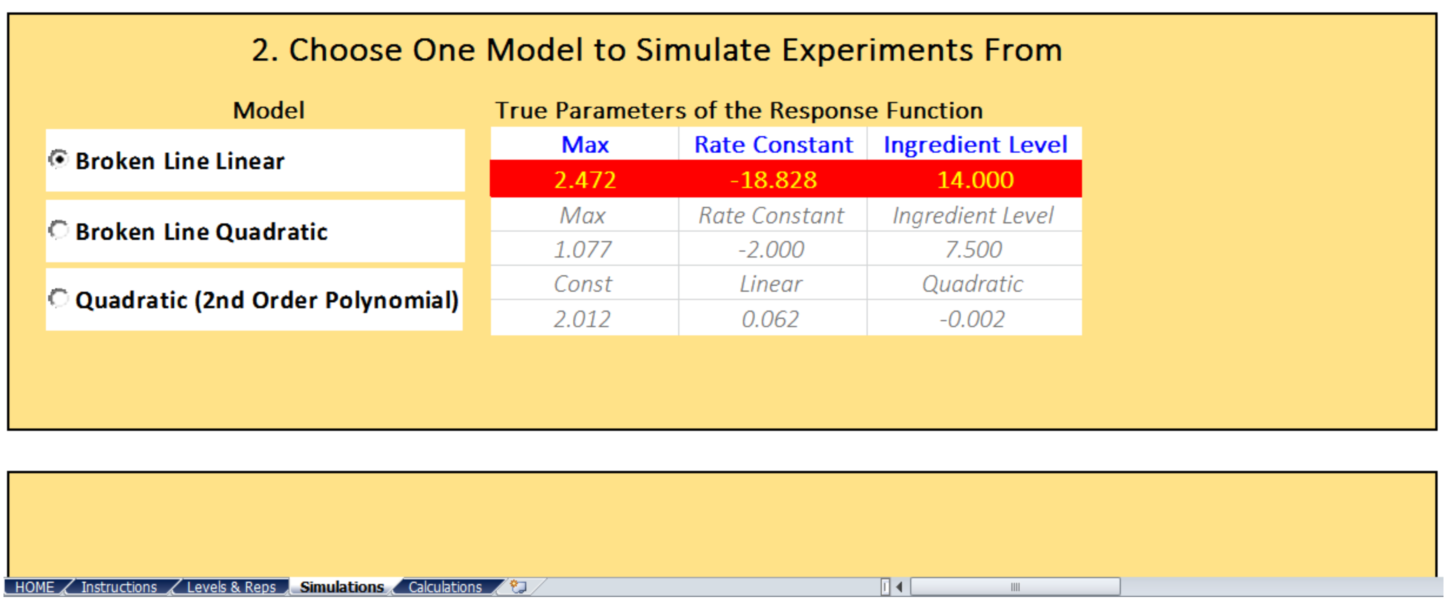

Figure 5. Baseline model selection, part of the "Simulations" spreadsheet.

4. Provide guesses for coefficients for all remaining models.

As in Figure 6, initial guesses for the regression coefficients should be entered to ensure convergence of solver routine. The rate constant should be a negative value if the second part of the curve is descending (e.g. weight gain).

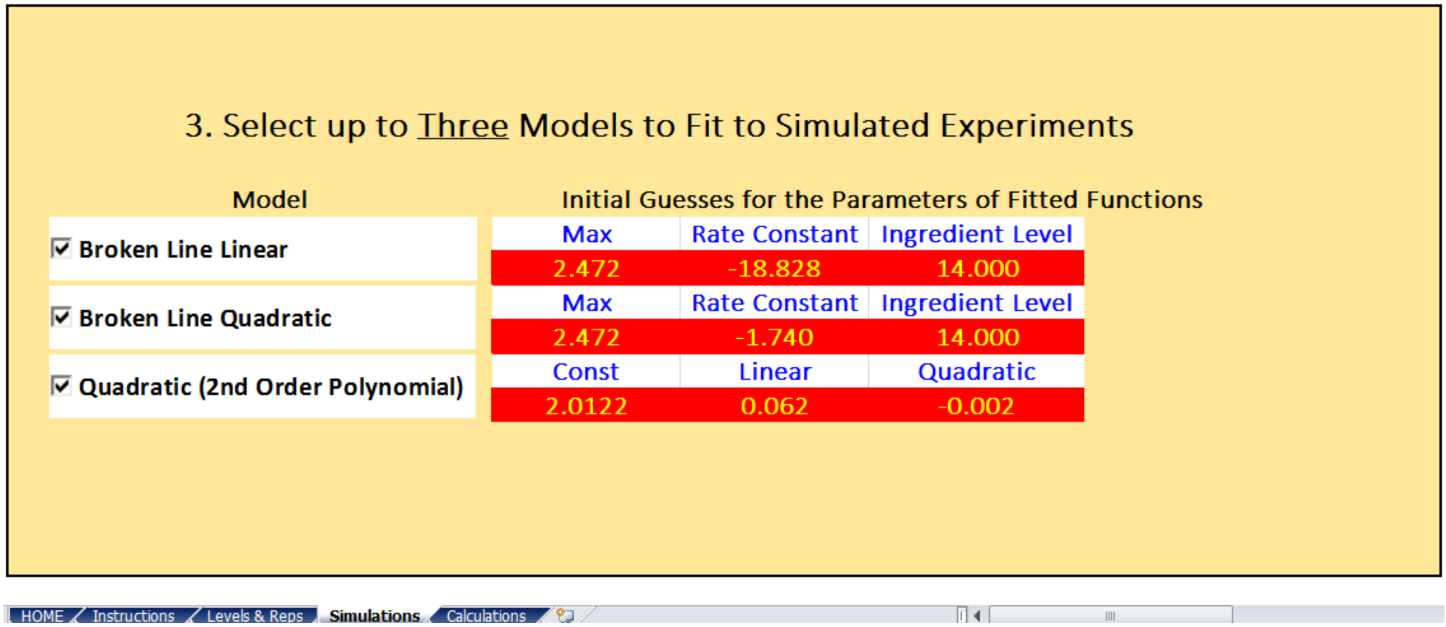

Figure 6. Initial guesses and model selection, part of the "Simulations" spreadsheet.

5. Select simulation parameters.

The number of experiments to be simulated and coefficient of variation (CV) for the simulated experiments should be provided in cells O6 and O7, respectively. Table 1 suggests that a minimum of 50 simulated experiments is enough (based on minimizing SD and SE) when estimating the MSL with broken-line models. To reflect the real-life situations, a certain amount of variability must exist in the simulated experiments. Table 2 shows that as the coefficient of variability (CV) increases the SD and SE increase accordingly and the goodness of fit (R2) decreases. A number of 100 simulated experiments and a CV value of 10 percent were chosen in the current simulation example to ensure minimum SD and SE and high R2.

Table 1. Effect of increasing the number of simulated experiments on estimating the maximum safe level of sunflower meal by broken-line models at a 10 percent fixed CV, five ingredient levels, and four replications.

	Broken-Line Linear						Broken-Line Quadratic					
	95%						95%					
N[1]	MSL (%)[2]	± SD	± SE	Confidence Lower	Confidence Upper	R^2	MSL (%)[2]	± SD	± SE	Confidence Lower	Confidence Upper	R^2
2	13.91	0.06	0.29	13.83	13.99	0.98	10.30	0.13	0.65	10.12	10.48	0.98
10	13.96	0.22	0.21	13.83	14.09	0.99	10.42	0.48	0.48	10.12	10.72	0.99
50	13.97	0.17	0.21	13.93	14.02	0.99	10.44	0.39	0.48	10.34	10.55	0.99
100	13.99	0.16	0.22	13.96	14.02	0.99	10.48	0.35	0.49	10.41	10.55	0.99
500	14.01	0.17	0.20	14.00	14.03	0.99	10.53	0.37	0.45	10.50	10.56	0.99
1000	13.99	0.16	0.20	13.98	14.00	0.99	10.48	0.36	0.46	10.46	10.50	0.99

[1] Number of simulated experiments
[2] Maximum safe level of the test ingredient

Table 2. Effect of increasing variation of the simulated experiments on estimating the maximum safe level of sunflower meal by broken-line models with 100 simulated experiments, five ingredient levels, and four replications.

	Broken-Line Linear						Broken-Line Quadratic					
	95%						95%					
CV (%)[1]	MSL (%)[2]	± SD	± SE	Confidence Lower	Confidence Upper	R^2	MSL (%)[2]	± SD	± SE	Confidence Lower	Confidence Upper	R^2
0	14.00	0.00	0.00	NA[3]	NA	1.00	10.50	0.00	0.00	10.50	10.50	1.00
5	14.00	0.08	0.11	13.99	14.02	1.00	10.50	0.19	0.24	10.47	10.54	1.00
10	13.98	0.14	0.21	13.96	14.01	0.99	10.47	0.31	0.48	10.40	10.53	0.99
20	13.97	0.34	0.41	13.91	14.04	0.96	10.46	0.73	0.93	10.31	10.60	0.96
50	13.82	1.13	NA	13.60	14.05	0.79	9.90	1.73	2.72	9.56	10.24	0.80
100	14.62	2.43	NA	14.15	15.10	0.51	9.18	6.04	NA	7.99	10.36	0.51

[1] Coefficient of Variation
[2] Maximum safe level of the test ingredient
[3] Not estimated

6. Press "Run Simulations."

   The "Run Simulations" function (Figure 7) will optimize the simulation problem, producing a graph (Figure 8) and the results of the simulation (Figure 9).

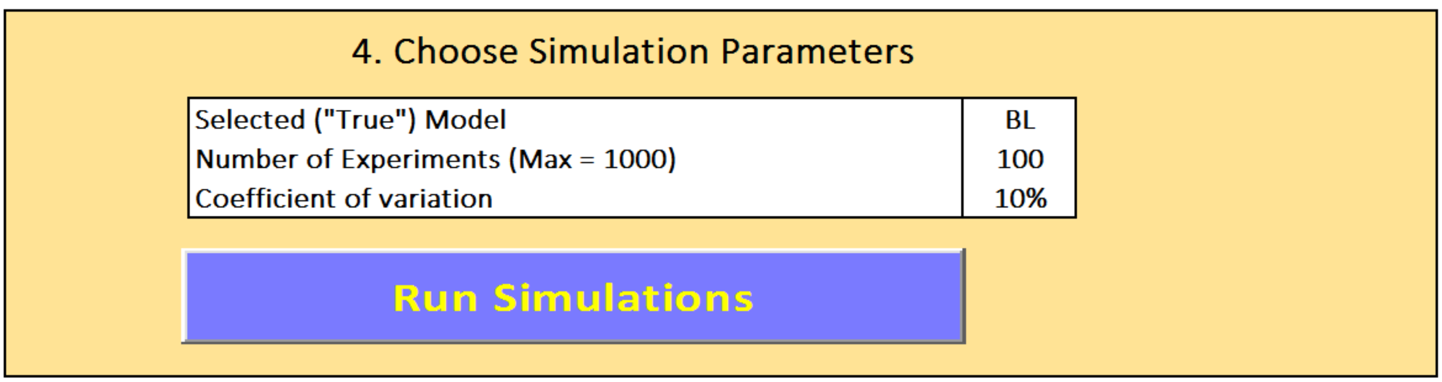

Figure 7. Simulation parameters selection and the "Run Simulations" button, part of the "Simulations" spreadsheet.

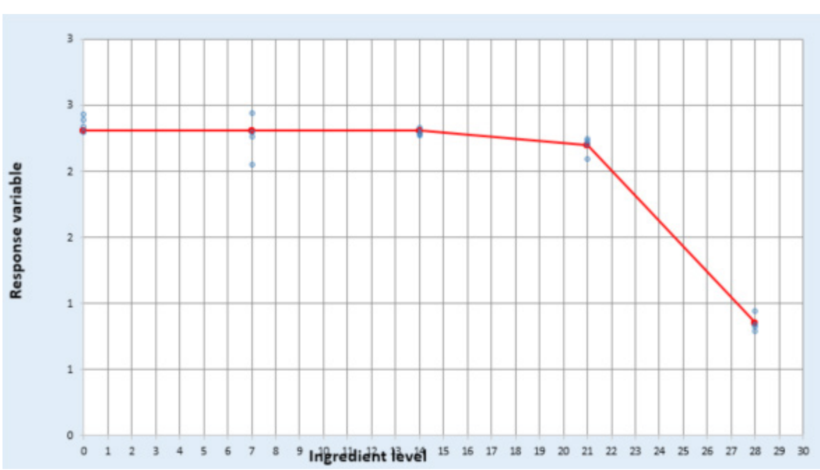

Figure 8. Graph of the results, part of the "Simulations" spreadsheet.

Experiment #	Model Fits														
	Broken Line Model					Broken Line Quadratic Model					Quadratic (2nd Order Polynomial)				
	Max/Min	Rate Constant	MSL	R^2	SE of MSL	Max/Min	Rate Constant	MSL	R^2	SE of MSL	Const	Linear	Quadratic	MSL (Calculated)	R^2
Average	2.475	-18.912	14.005	98.8%	0.091	3.173	-1.380	10.892	98.0%	0.236	-14.146	8.639	-0.653	6.618	92.8%
Std. Dev.	0.032	0.850	0.125	0.4%	0.018	0.107	0.105	0.248	0.6%	0.038	0.791	0.337	0.021	0.054	0.4%
Lower 95%	2.469	-19.078	13.981	98.7%	0.088	3.152	-1.401	10.844	97.8%	0.229	-14.301	8.573	-0.657	6.608	92.7%
Upper 95%	2.481	-18.745	14.029	98.9%	0.095	3.194	-1.359	10.941	98.1%	0.244	-13.991	8.705	-0.648	6.629	92.9%
0001	2.4813	-19.5549	14.0536	99.0506%	0.082	3.1589	-1.4425	10.9881	98.3713%	0.210	-14.7114	8.9138	-0.6715	6.6376	93.0585%
0002	2.4914	-19.7111	14.0870	99.0078%	0.083	3.1545	-1.4611	11.0379	98.2894%	0.214	-14.8545	8.9627	-0.6737	6.6518	92.8402%
0003	2.5208	-20.6189	14.2348	97.5306%	0.124	2.9514	-1.6410	11.4493	97.6550%	0.242	-15.8326	9.3435	-0.6943	6.7284	91.3292%
0004	2.4390	-18.7790	13.9674	98.1883%	0.117	3.1533	-1.3571	10.8291	97.2999%	0.276	-14.1041	8.6121	-0.6518	6.6061	92.3440%
0005	2.5177	-18.0122	13.8887	98.4823%	0.110	3.2613	-1.2791	10.6879	97.3933%	0.275	-13.3305	8.2973	-0.6311	6.5740	92.7275%
0006	2.4902	-18.7123	13.8386	98.0715%	0.127	3.3243	-1.3089	10.5802	96.7851%	0.309	-13.9248	8.6322	-0.6589	6.5508	92.3331%
0007	2.4533	-18.9139	14.0539	99.2631%	0.072	3.0895	-1.3984	10.9989	98.5592%	0.197	-14.1855	8.6186	-0.6492	6.6382	93.1641%
0008	2.5041	-18.0617	13.9338	98.9227%	0.091	3.2070	-1.2968	10.7734	97.9345%	0.242	-13.4763	8.3179	-0.6302	6.5989	93.1163%
0009	2.5185	-19.2978	14.0872	98.8849%	0.088	3.0725	-1.4619	11.1267	98.5364%	0.196	-14.5979	8.8148	-0.6615	6.6630	92.9115%
0010	2.5719	-17.8975	13.8065	98.7172%	0.104	3.4607	-1.2280	10.4685	97.1720%	0.293	-13.0344	8.2494	-0.6316	6.5306	92.9263%
0011	2.4541	-17.5316	13.8039	98.1790%	0.124	3.3023	-1.2096	10.4899	96.7562%	0.313	-12.8772	8.0954	-0.6195	6.5337	92.4889%
0012	2.4708	-18.5332	13.9258	98.8172%	0.096	3.2056	-1.3281	10.7577	97.8524%	0.247	-13.8190	8.5145	-0.6463	6.5870	93.0112%
0013	2.4921	-19.7694	14.0909	98.7072%	0.094	3.2230	-1.4485	10.9921	97.8621%	0.241	-14.8047	8.9633	-0.6744	6.6456	92.5251%
0014	2.4551	-20.5105	14.2077	98.9046%	0.083	3.0443	-1.5721	11.2766	98.5070%	0.195	-15.5337	9.2409	-0.6900	6.6966	92.4956%
0015	2.4725	-18.5394	13.9235	99.1665%	0.080	3.1565	-1.3422	10.7993	98.3451%	0.216	-13.9739	8.5571	-0.6484	6.5987	93.4332%
0016	2.5243	-19.1706	14.1356	98.8193%	0.089	3.1489	-1.4342	11.1169	98.1740%	0.220	-14.3047	8.6799	-0.6508	6.6682	92.5526%
0017	2.4516	-18.2511	13.8705	99.2893%	0.075	3.2751	-1.2769	10.6080	97.9945%	0.242	-13.4089	8.3683	-0.6388	6.5504	93.4237%
0018	2.4521	-18.2121	13.9600	99.1333%	0.081	3.2227	-1.2939	10.7502	97.9876%	0.239	-13.5593	8.3464	-0.6323	6.6004	93.1972%
0019	2.4657	-19.3381	14.0532	99.2971%	0.070	3.1989	-1.4061	10.9283	98.3651%	0.211	-14.4780	8.7964	-0.6631	6.6326	93.1842%
0020	2.4335	-18.9436	13.9855	98.4075%	0.109	3.1399	-1.3750	10.8628	97.5676%	0.261	-14.1245	8.6484	-0.6549	6.6027	92.5153%
0021	2.4582	-18.8659	14.0081	99.1912%	0.077	3.1687	-1.3690	10.8812	98.3010%	0.217	-14.1264	8.6217	-0.6513	6.6193	93.2213%

HOME | Instructions | Levels & Reps | **Simulations** | Calculations

Figure 9. The results section, part of the "Simulations" spreadsheet.

How do I read the results?

The results of the simulation problem for the current example (Moghaddam *et al*, 2012) are displayed in section five of the "Simulations" spreadsheet (Figure 9). The descriptive statistics displayed in rows 37:40 are the results of the 100 experiments simulated for each model. For the BLL model, the maximum safe level of the sunflower meal as an average for the 100 simulated experiments (runs) -/+ SD was 14.005% ± 0.125 (95% CI = 13.981 - 14.029%) for an estimated maximum weight gain of 2.475 ± 0.032 kg. The SE of the maximum safe level was calculated to be 0.091. The R2 of the fitted BLL model function was estimated to be 98.8 percent, which implies a good fit. Similarly, the results of the BLQ model are displayed in columns J to N of section 5. For the 2OP model, the calculated maximum safe level was 6.618 ± 0.054. The estimated regression coefficients were -14.146, 8.639 and – 0.653 as the constant, linear term and the quadratic term, respectively. Poor estimates of the results for any model may require more accurate guesses of the coefficients as they influence the goodness of fit.

What are other uses of the Maximum Ingredient Level Optimization Workbook?

The MIOW can also be used to decide the best combinations of the ingredient levels and replications when designing feeding trials. The combination with the smallest SE of the MSL mean should be the most efficient combination. As the replication number increased from two to 20, the SE of the MSL decreased for both models (Table 3). The SE of the MSL couldn't be estimated with four levels and a minimum of fice levels was required for the estimation of the SE under the conditions (true parameters and initial guesses) of the current simulation example (Table 4).

Table 3. Simulations of increasing the number of replications on estimating the maximum safe level of sunflower meal by broken-line models, based on 100 simulated experiments with a 10 percent CV and five ingredient levels.

	Broken-Line Linear					Broken-Line Quadratic				
	95%					95%				
N[1]	MSL (%)[2]	± SD	± SE	Confidence Lower	Confidence Upper	MSL (%)[2]	± SD	± SE	Confidence Lower	Confidence Upper
1	13.94	0.42	0.02	13.85	14.02	10.39	0.86	0.09	10.23	10.56
2	14.00	0.24	0.23	13.95	14.04	10.49	0.55	0.51	10.39	10.60
4	14.00	0.15	0.21	13.97	14.03	10.50	0.34	0.47	10.43	10.57
6	13.96	0.13	0.19	13.94	13.99	10.42	0.30	0.43	10.36	10.48
8	14.00	0.11	0.15	13.98	14.02	10.50	0.24	0.35	10.45	10.55
10	14.01	0.11	0.14	13.99	14.03	10.53	0.24	0.31	10.48	10.58
12	14.00	0.09	0.13	13.99	14.02	10.51	0.20	0.30	10.47	10.54
14	14.00	0.08	0.12	13.98	14.01	10.49	0.19	0.28	10.45	10.53
16	14.00	0.08	0.11	13.99	14.02	10.50	0.18	0.26	10.47	10.54
18	14.00	0.07	0.11	13.98	14.01	10.49	0.17	0.25	10.46	10.53
20	14.00	0.08	0.10	13.98	14.01	10.50	0.17	0.23	10.46	10.53

[1] Number of simulated experiments
[2] Maximum safe level of the test ingredient

Table 4. Simulations of increasing the number of Levels on estimating the maximum safe level of Sunflower meal by broken-line models, based on 100 simulated experiments with a 10 percent CV and four replications.

	Broken-Line Linear					Broken-Line Quadratic				
	95%					95%				
N[1]	MSL (%)[2]	± SD	± SE	Confidence Lower	Confidence Upper	MSL (%)[2]	± SD	± SE	Confidence Lower	Confidence Upper
2	13.98	0.31	NA	13.92	14.04	13.49	0.13	NA	13.47	13.52
3	13.98	0.26	NA	13.93	14.04	13.49	0.12	NA	13.46	13.51
4	14.12	0.16	NA	14.09	14.15	13.90	0.11	NA	13.88	13.92
5	14.00	0.17	0.22	13.96	14.03	10.49	0.38	0.51	10.42	10.57
6	13.99	0.34	0.24	13.93	14.06	10.27	0.39	0.49	10.20	10.35
8	14.00	0.14	0.16	13.97	14.03	10.57	0.35	0.43	10.50	10.64
15	14.01	0.14	0.12	13.98	14.04	10.79	0.28	0.30	10.74	10.85
20	14.00	0.10	0.09	13.98	14.02	10.85	0.22	0.26	10.81	10.90
24	14.01	0.12	0.09	13.98	14.03	10.89	0.25	0.24	10.84	10.94

[1] Number of simulated experiments
[2] Maximum safe level of the test ingredient

What can be concluded from the Maximum Ingredient Level Optimization Workbook (MIOW)?

The workbook offers a method to estimate the maximum safe level of test ingredients and the related statistics (CI, SD, SE and R^2). Unlike the multiple range and the orthogonal contrast approaches, the broken-line and quadratic polynomial models of the MIOW treat the independent variable as continuous and offer estimations of the descriptive statistics of the means. The SD provides information on the dispersion of the data while the SE tells how accurate the estimate of the mean is. The accuracy of fit as represented by the R^2 should help users to determine how well the model fits the data. The MIOW can be used to find the best combination of levels and replications when designing feeding trials. The combination with the smallest SD and SE should be the most efficient design.

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[1]Workbook completed during graduate studies in the Department of Poultry Science, University of Georgia.

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Bulletin 1469 **January 2017**

Published by the University of Georgia in cooperation with Fort Valley State University, the U.S. Department of Agriculture, and counties of the state. For more information, contact your local UGA Cooperative Extension office.