NuTRITIONAL

REsPONSE

DeTERMINATION

OpTIMIZATION

Workbook v. 1.0

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The NuRDO.xls workbook is a tool to help researchers maximize the efficiency of experiments to determine nutritional requirements. By simulating results from different combinations of nutrient levels and replicates per level, researchers can explore the relationship between the arrangement of different inputs and the confidence in the determined requirement.

Click here to download the NuRDO workbook, which runs on all modern versions of Microsoft Excel with the Visual Basic function. Before starting, enable macros and activate the solver add-in.

UGA
extension

Why is it important to simulate nutritional response experiments?

It is always important to maximize the use of resources, particularly when animals are involved. When designing experiments to determine nutritional requirements, researchers are faced with a number of options. The researcher should consider the following questions:

- Should the experimental units be individual animals or pens of animals?
- How many animals or pens should be used for each feeding level (treatment group)?
- How many levels need to be fed to get a good estimate of the nutritional requirement?
- What is the optimal range of nutrient levels needed to best estimate a requirement?

The researcher also has to define the "best estimate of a requirement." In other words, the researcher must determine an acceptable confidence interval for his or her requirement estimate.

Which regression models are typically used to estimate nutritional requirements?

Researchers often start analyzing response data by fitting a quadratic polynomial (QP) to it. Figure 1 shows data from a lysine requirement study conducted with broiler chickens fed seven levels of crystalline lysine (from 0.6 to 1.2 percent).

The input in this case was lysine, but it could be any nutrient or feed additive. The output, or response variable, is body weight gain, but could be many other measured quantities, such as feed efficiency, bone ash blood parameters, etc.

When quadratic polynomials are fitted to response data, there is a maximum (or minimum) that might be interpreted as the "requirement." The QP model assumes that there is no plateau and that the nutrient becomes toxic immediately after the maximum response is reached.

Another approach is a spline fit, where there is believed to be an ascending portion of the response and then a plateau that is reached when the nutrient in question neither increases nor decreases the animal's response. The requirement for this approach is the intersection of the two lines. The ascending portion may be either linear (Figure 2, the broken-line linear or BLL) or quadratic (Figure 3, the broken-line quadratic or BLQ).

Functional Form

$$y = b_0 + b_1 x + b_2 x^2$$

The quadratic polynomial plot shows "Observed and Predicted Output" with equation $y = -559.52x^2 + 1460.7x - 505.48$
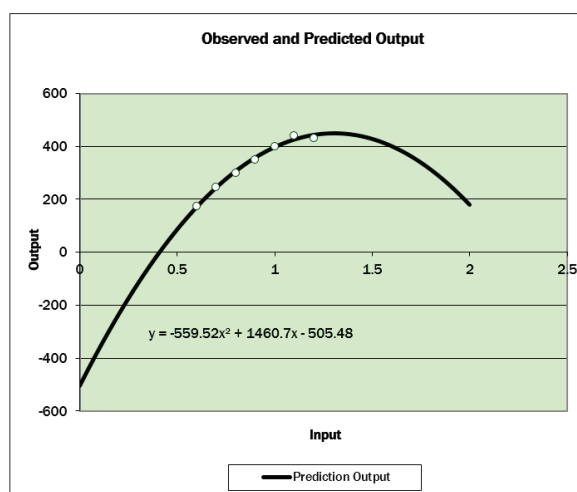
Figure 1. The quadratic polynomial approach to estimating nutritional requirements.

Functional Form

$$y = \begin{cases} \text{Maximum,} & \text{if } x > \text{Requirement} \\ \text{Maximum} + \text{Rate Constant} \times (\text{Requirement} - x) & \text{if } x \leq \text{Requirement} \end{cases}$$
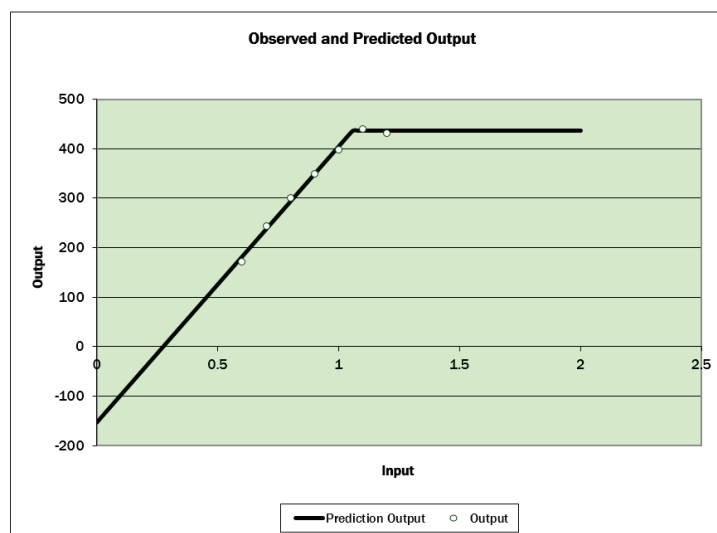

Figure 2. The broken-line linear model, a spline approach to estimating nutritional requirements.

Another approach, and perhaps the most rational, is the Saturation Kinetics Model of Mercer, by Morgan and Flodin, 1975. It may be considered the most rational approach because it is a super set of the widely accepted Michaelis-Mentan model of enzyme kinetics.

Assuming that an enzyme-catalyzed reaction is limiting the performance of an animal when any nutrient is in short supply, it follows that the shape of the response curve should be similar to that of the limiting reaction.

From a practical perspective, with the SK model, a maximum response is never reached, only approached, so there is no requirement per se. As with the BLL and BLQ models, there is no parameter to indicate the level that the nutrient may become toxic.

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Because it is difficult to experimentally or statistically choose between the various models, NuRDO allows the researcher to choose the model he or she thinks will best represent what the actual data will resemble when the experiment is conducted.
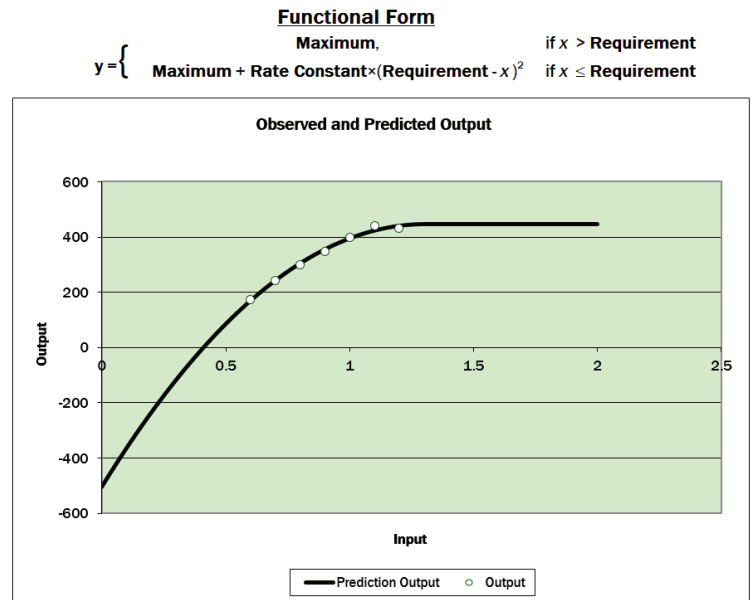
Functional Form

$$y = \begin{cases} \text{Maximum,} & \text{if } x > \text{Requirement} \\ \text{Maximum} + \text{Rate Constant} \times (\text{Requirement} - x)^2 & \text{if } x \le \text{Requirement} \end{cases}$$

Observed and Predicted Output

Figure 3. The broken-line quadratic model, another spline approach to estimating nutritional requirements.

Functional Form

$$y = \frac{\text{Intercept} \times \text{Rate Constant} + \text{Maximum} \times X^{\text{Kinetic Order}}}{\text{Rate Constant} + X^{\text{Kinetic Order}}}$$

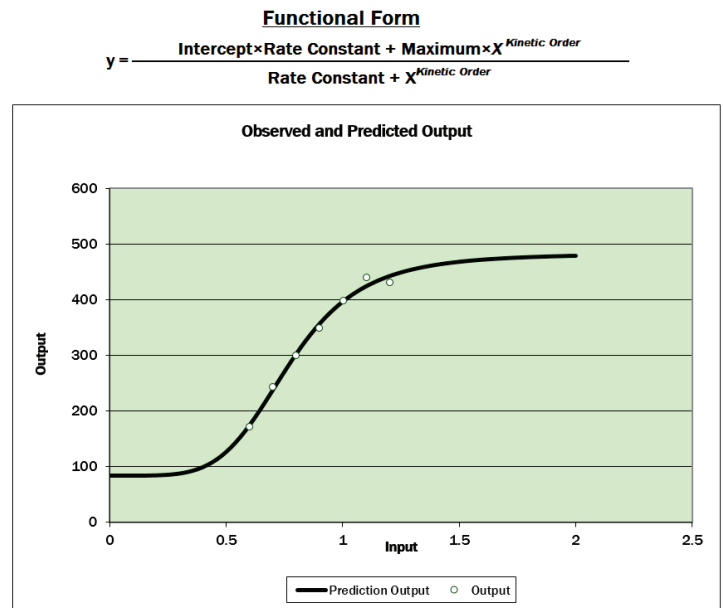**Observed and Predicted Output**

Figure 4. The saturation kinetics model approach to estimating nutritional requirements.

What does the NuRDO workbook do?

The NuRDO workbook allows the research planner to input what he or she estimates the shape of the response curve to be based on history and past experiences. The research planner inputs possible combinations of nutrient levels and the number of observations at each level based on expected variation between replicates.

NuRDO then simulates what the experimental outcomes might be expected to be based on the planner's estimates and Excel's random functions for normal distributions. Once the experimental outcomes are simulated, NuRDO determines what the best-fit models would be, including requirement estimates for each of the models.

Confidence intervals are calculated for the requirement from each simulated experiment. The combination of levels and replications with the lowest confidence intervals for the requirement should indicate the most efficient use of resources.

What does the NuRDO workbook consist of?

The "Levels and Reps" spreadsheet is used to create a grid with combinations of levels and replications for the simulations (Figure 5). The numbers in the grid can be changed manually to have unequal numbers of observations at any level. The cell contents can simply be erased or written over to achieve any combinations up to 25 nutrient levels and 20 replicates.

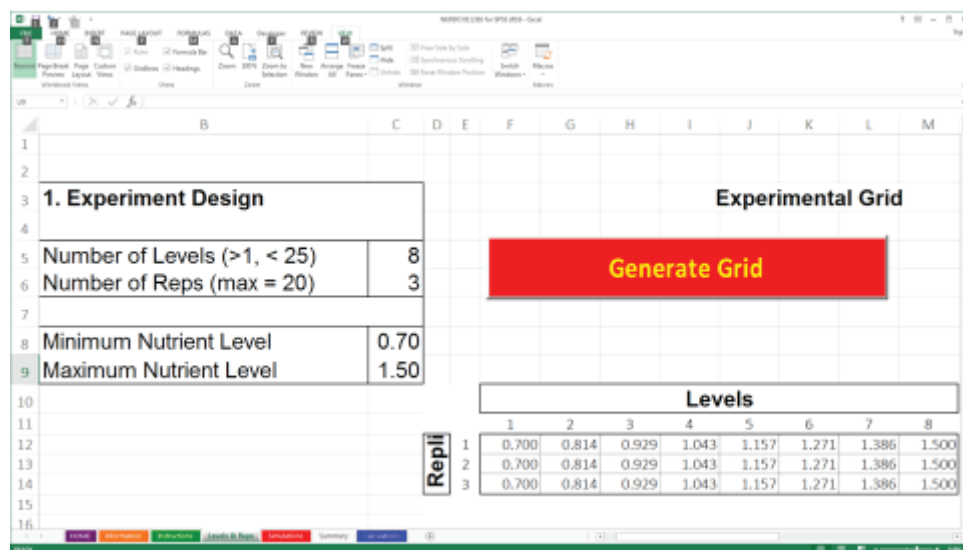## Step 1: Choose the nutrient levels and numbers of replicates.

Figure 5. The "Levels & Reps" spreadsheet used to create the grid for simulating experiments.

The "Simulations" spreadsheet is used to input what the shape of the response is expected to be. One of the response models must be chosen to represent what is believed to be the true shape of the response (Figure 6).

Step 2: Input your best guess of what the response curve really is.

Figure 6. The "Simulations" spreadsheet section used to describe the model for simulating experiments.

Fitting non-linear models is an iterative process involving initial parameter estimates. Initial parameter estimates for the BLL and BLQ models are fairly straightforward. Visual inspection of the data should allow fair estimates of the maximum response and the break-point requirement. The rate constants are more difficult, but can usually be found fairly quickly by trial and error. The QP model parameters can be found by graphing the data in Excel, fitting a second order polynomial, and asking for the equation to be displayed. The SK model maximum, intercept and rate constant (input level at half maximal response can be estimated by visual inspection, and the rate constant found by making guesses. The parameter estimates in Figure 7 are the same as those in Figure 6.

For this example, the four models were already fitted to the data, but that is not necessary. One or all four models can be fitted to the same data. But if you fit different models at different times, there will be different simulation data each time the program is run.

Step 3: Choose the model(s) and input initial parameters for each model to be run.

Figure 7. The "Simulations" spreadsheet section used to input the initial model parameters for fitting curves to the simulated experimental data.
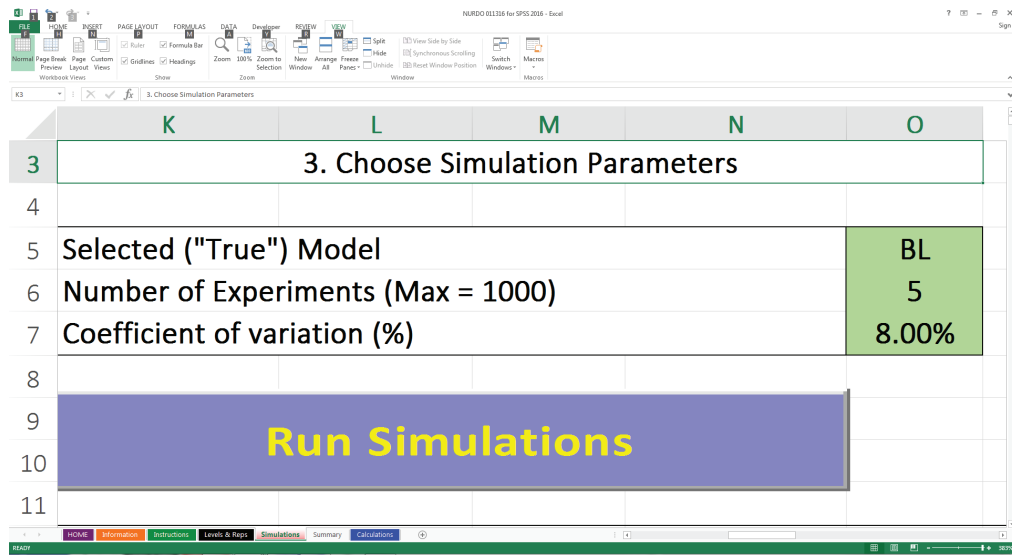
Figure 8. The "Simulations" spreadsheet section used to input the number of experiments to be simulated and the coefficient of variation expected, or normally observed, for the genetic strains to be used and the environmental conditions they will be kept in.

In order to plan an experiment, it is always important to know how much variation normally exists between individuals or pens of individuals subjected to the same experimental treatments (levels). It helps to reduce variation by choosing one gender or equalizing pen starting weights, etc. When the number of experiments to simulate and CV have been inputted, choose "Run Simulations" and see how long it takes to compute the model(s) entered. If the models fit quickly, more "Experiments" can be specified. The more experiments that are simulated, the better the parameter estimates will be.

Step 4: Input the number of experiments to be simulated and the CV (mean/standard deviation), and click "Run"!

The number of simulated experiments is chosen based on the speed of the computer being used and the patience of the operator. With small CVs, stable confidence interval estimates will be achieved with fewer experimental simulations. For most problems, 20 to 100 experiment simulations should result in stable coefficient estimates. But since the calculations are based on random number generation, the results should be different each time the "Run Simulations" button is activated. If you consider them only slightly different each time the "Run Simulations" button is activated, then you are probably simulating enough experiments.

Figure 9. The "Simulations" spreadsheet is also used to display NuRDO results.

NuRDO displays the results of each simulated experiment. In the example in Figure 9, the 5 experiments simulated resulted in nutrient requirement estimates of 1.029 to 1.083 percent (the actual requirement input was 1.057 percent). The results for each simulation are displayed on a different row and the average statistics are displayed on the top. In this case only 5 simulations resulted in an estimate of 1.055 ± 0.034, very close to and including the actual parameter value. The key number displayed is the SE of requirement, indicating the confidence interval of 1.055 ± 0.034. The actual mean has about a 66 percent chance of being between 1.021 and 1.089.

How is the NuRDO workbook used?

The NuRDO workbook can be used to estimate nutritional requirements and their expected confidence intervals from different combinations of nutrient levels and replication, expected variation, and the spacing or range of nutrient levels to use. Figure 10 shows the results from a series of simulations with different numbers of levels and pens per level.

Step 5: Compare the results of using different levels and reps per level on the standard error of the requirement estimate, or other output parameter.

			BLL Model			BLQ Model			QP Model	SK Model
Pens	Levels	Reps	Req.	± SE	R^2	Req.	± SE	R^2	R^2	R^2
							....%			
16	4	4	1.050	**0.041**	89.5	1.216	0.022	89.5	89.1	89.7
24	6	4	1.064	**0.038**	87.1	1.245	0.017	86.3	85.9	87.4
24	4	6	1.053	**0.034**	88.9	1.231	0.018	89.0	88.3	89.2
24	8	3	1.058	**0.040**	85.3	1.237	0.023	84.5	84.2	85.1
48	8	6	1.057	**0.027**	85.4	1.222	0.007	84.4	83.8	84.8
48	6	8	1.060	**0.025**	86.7	1.238	0.008	86.0	85.6	86.9
96	8	12	1.058	**0.019**	84.3	1.227	0.004	83.4	82.8	83.9

Figure 10. Simulations of lysine requirements based on BLL model, 100 simulations, 8 percent CV and a lysine range of 0.7 to 1.5 percent. Actual requirement = 1.057 percent.

When pens of animals are used, the pen-to-pen variation can be reduced by adding more animals to each pen, selecting more uniform animals, etc. The decision of what levels and reps to use depends on the goals of the trial and resources available.

How are the results interpreted?

Since the goal of nutritional requirement studies is to determine the requirement or breakpoint of one of the broken-line models, the standard error of the requirement estimate is the indication of experimental power. The smaller the error in estimating the requirement, the more powerful the experiment is.

In the example in Figure 10, there is some advantage in using 24 pens instead of 16 (Row 1 vs. Row 3) if the number of replicate pens per level is increased (not number of levels; Row 1 vs. Row 4). Doubling the size and expense of the experiment to 48 pens resulted in a decrease in the expected standard error of the requirement, but the results are very similar with greater numbers of reps per nutrient level or more nutrient levels with the same number of reps (Rows 5 and 6 vs. Rows 2 and 3). Further doubling of the size and expense of the experiment resulted in another decrease in the expected standard error (Rows 5 and 6 vs. Row 7). Similar comparisons should be made with range of nutrient levels and the coefficient of variation.

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