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A Sorting Method to Reduce Variation in Starting Pen Weights in Poultry Research Studies Utilizing Microsoft Excel

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Abstract: Variability within nutrition experiments can be problematic, especially when one is trying to pick up small differences between experimental treatments. A variety of methods have been used over the years to reduce starting animal weight such as weighing animals into different weight groupings followed by selection of animals from the different group in an attempt to obtain smaller differences in starting weights. This paper describes a computer based sorting method that will substantially reduce variation of starting animal weights. In the example used, 700 turkey poults were sorted in three ways at one week of age into 70 pens of 10 poults per pen. Initially 800 poults were weighed and banded with top and bottom weight poults removed to the 700 poults needed. Random assignment of poults resulted in pen weights that ranged from 122.12 to 151.94 g/bird, a difference of 24.4%. Using a 3 weight grouping resulted in an average pen weight range of 130.22 to 140.4 g/bird, a difference of 10.18 g/pen or 7.8%. Using the sorting program resulted in average pen weights ranging from 134.88 to 135.56 g/bird, a difference of 0.68 g or 0.5%, substantially lower than the group sorting. It is believed that this type of sorting of animals at the beginning of an experiment will result in substantially reduced variation.

Key words: Nutrition experiments, poultry, variation, computer sorting

INTRODUCTION

One of the important aspects of completing a successful experiment is reducing variability within the experiment. Variation that is not controlled can reduce the power of an experiment to detect the effects of treatment. Variability in poultry trials can be controlled in a number of ways. For example, researchers can reduce heterogeneity in the environment by utilizing batteries or pens that maintain homogenous temperatures, lighting schedules, ventilation, air quality and accessibility to feed and water. Reducing heterogeneity in the birds themselves is another vital step toward reducing variation in experiments. This begins with using birds of the same strain, breeder flock, hatch and age. In addition, it is also essential to start with uniform pen weights. Several methods for accomplishing this are available. For example, it is not uncommon to weigh animals into several groups consisting of light, moderate and heavy body weights and select animals from each group in an attempt to get relatively similar pen weights, although in reality this may result in pen weights that show significant discrepancies. Our lab developed and has used this method of sorting birds for nutritional experiments for many years. The sorting methodology described below using Microsoft Excel removes a great deal of this variation and results in more uniform starting pen weights, thus removing an important source of variability in nutrition experiments.

MATERIALS AND METHODS

A poultry model was used to develop this program a number of years ago. An experiment consisting of 70 pens of one-week-old turkey poults is used for description of the procedure. A total of 800 poults were used to begin and 700 poults (10/pen) were utilized for the trial. It is important to note that the larger the starting number of birds, the more uniform pen weights will be. Additionally, it should be noted that this is more effective on animals that have been allowed to grow for a few days as hatch weight can be affected by a variety of factors that have disappeared within a few days post hatch.

Sorting steps: The first step in this method of sorting birds involves weighing and individually banding each bird with consecutive band numbers. This is important for the individual identification of each bird. First on your Excel spread sheet label a column with band number and enter your band numbers in consecutive order. Next, weigh each bird individually and enter the weight on the spreadsheet next to the corresponding band number. Label the heading of this column as body weight (Table 1).

Next sort body weight in Excel by ascending order (Table 2). Generally, better results will be obtained when extra birds are used. This allows for removal of the obviously underweight or overweight birds. In this

Table 1: Input of band numbers and body weights

Band #	Body wt (g)
1	174.8
2	169.1
3	167.1
4	134.5
5	143.7
continued	continued

Table 2: Sorting of birds by body weight high to low before removal of outliers

Band #	Body weight (g)
166	98.6
369	98.8
559	99
474	99.1
762	99.8
81	100.5
619	100.5
652	100.7
479	100.8
463	101.1
347	101.2
542	101.2
249	101.3
174	101.4
330	101.6
Continued	Continued

example, 700 birds were needed and 800 were weighed. We would generally remove an equal number of birds from the lightest and the heaviest, but if birds are sexed as in this example, one could select more birds for removal from the bottom group as 5% or 40 of the birds may be incorrectly sexed and are actually females. Thus in this example we removed 30 from the top weights and 70 (40 assumed females + 30 from the bottom) from the bottom weights. Some discretion may be used in eliminating animals from the extremes. This eliminates the lightest and the heaviest birds from being included in your pen weights.

We now have the 700 birds that will be included in the experiment and the sorting process. Next, a new column is added called pen #. Starting with the first bird, begin labeling the birds with pen numbers 1 to 70 and the next 70 birds in reverse order 70 to 1 (Table 3). Label the entire group of birds in this fashion until all birds are assigned a pen # from 1 to 70 or 70 to 1.

Next we will use the Random function in the middle grouping of birds. The 4 groups of birds in the middle of the list (280 birds starting after the 210th bird up to the 490th bird) will be used. This can be modified to get the best mix and is based on the number of birds in the pen. In our example we have 10 birds per pen and will use the middle 4 groups of birds. If we had 5 birds in a pen we might use only the middle group. If 6 birds, the 2 middle groups and so forth. This group will then be assigned random values using the Excel program. To do this, enter the formula =RAND() in place of number

Table 3: Bird labeling by Pen # followed by reverse Pen #

Band #	Body wt (g)	Pen #
1	174.8	1
2	169.1	2
3	167.1	3
4	134.5	4
5	143.7	5
continue	continue	continue to 70
71	158.2	70
72	132.6	69
73	123.9	68
74	159.8	67
75	130.5	66
continue	continue	continue to 1

Table 4: Random value assignment

Band #	Body wt (g)	Random #
6	134.9	0.052370837
67	134.9	0.143321005
437	135.5	0.611591964
486	135.5	0.878751376
458	135.6	0.127469083
631	135.6	0.564527964
533	135.7	0.909002987
		continued

one, then drag the formula down for all 280 birds in the 4 middle groups of 1-70 and 70-1. This will assign a different random value between 0 and 1 to each bird in these groups (Table 4).

Select this group of birds, copy the cells and paste them in the same location as the original values (use paste special, select values) to remove the formula. Next for this group of birds, select the band numbers, bird weights and assigned random values for all of the birds in this group and sort ascending by the random number column. This will randomly assort the 280 birds in the selected group. Now, you can reassign your random values the pen numbers 1-70 in consecutive order four times to account for the 280 birds sorted. The final four groups of 70 birds should still be labeled 1-70 and 70-1, respectively.

After the labeling of your pen numbers is accomplished, the next step is to select all of the values on the spreadsheet, including band number, body weight and pen number. Then, sort ascending by pen number. This will show the 10 birds in each pen, labeled by pen number (Table 6). Note that each pen has both a similar average bird weight as well as a similar distribution of sizes within the pen.

From this point, it is possible to look at each bird's band number, compare to the spreadsheet and see exactly which pen that bird is assigned to. Finally, it is also a simple task to sum the body weights for each pen, providing the total pen weights for each of the 70 pens in the trial. These data can then be used as the starting weights for the experiment.

The spreadsheet can then be printed out and brought to the research facility for allocation of birds. First, sort the

Table 5: Random sorting example

Band #	Body wt (g)	Random #	Pen #
6	134.9	0.052370837	1
458	135.6	0.127469083	2
67	134.9	0.143321005	3
630	134.8	0.488381373	4
631	135.6	0.564527964	5
437	135.5	0.611591964	6
486	135.5	0.878751376	7
533	135.7	0.909002987	8

Table 6: Final sorting with average pen weight

Band #	Body wt	Pen #	Average pen weight
54	175.3	1	
435	151.3	1	
24	151.2	1	
342	140.3	1	
460	140.3	1	
44	130.9	1	
541	130.8	1	
191	118.6	1	
635	118.3	1	
166	98.6	1	135.56
1	174.8	2	
331	151.5	2	
519	151.2	2	
320	140.3	2	
488	140.3	2	
798	130.9	2	
349	130.6	2	
548	118.7	2	
186	118.1	2	
369	98.8	2	135.52
Continued		Continued	

Table 7: Spreadsheet printout for allocation

Band #	Pen #
1	2
2	13
3	17
4	56
5	55
6	63
7	30
8	55
9	60
10	34
11	65
12	44
13	60
14	56
15	50
Continued	Continued

spreadsheet by band # (be sure to include pen # in the sort) to arrange the birds in ascending band order such that band # 1 is at the top of the sheet with the associated pen # (Table 7). Print this off so that researchers can pick up the bird, identify the band number, find it on the spreadsheet and place the bird in the correct pen.

RESULTS AND DISCUSSION

Typically animals are randomly assigned to pens or are sorted based on some weight sorting method (low, medium, high). With this data set, random assignment of poults resulted in average weights per pen that ranged from 122.12 to 151.94 g/bird, a difference of 24.4%. Using a three weight grouping (low, medium, high) resulted in a range of 130.22 to 140.4 g/bird, a difference of 10.18 g/bird or 7.8%. Using the sorting program resulted in weights ranging from 134.88 to 135.56 g/bird, a difference of 0.68 g or 0.5%, substantially lower than the group sorting. Additionally, this appeared to provide a similar distribution of body weights to achieve the average body weight. It is believed that this type of sorting of animals at the beginning of an experiment will result in substantially reduced variation. This can be absolutely critical in certain types of experiments. Our laboratory has done a number of amino acid titration experiments with turkeys where this has been used to effectively reduce variation. Again, using a larger number of birds to select from should result in even more uniform pen weights. This program can aid in removing some of the variation associated with beginning pen weights in animal research trials and is especially helpful in trials that utilize a large number of animals.

No similar types of sorting are reported in the literature. Richter *et al.* (2010) and coworkers found that systemic variation (heterogenization) can improve reproducibility of animal experiments, but this does little to reduce variation within an experiment. Most attempts to reduce variation relate to reduction of animal numbers are related to animal welfare concerns. For a review see Boo and Hendriksen (2005). Older papers used the computer to achieve a random sort (Borsini, 1985; Martin *et al.*, 1986). For experiments where reduced variability is needed to determine small differences in response, this methodology can be used with great success.

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