Case study 8: An analysis of the number of goats needed in a feeding experiment

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Summary

This case study reports on a goat feeding experiment that went wrong and attempts to rectify it. As the experiment was run in batches it was possible to use data that had already been collected to decide on an appropriate course of action. Various options were possible and the sample size estimation formula is used to evaluate the likely outcomes of each alternative.

The case study provides a good example of the art of making compromises between what is statistically desirable and experimentally possible. Another interesting feature of the design used for this study is the inclusion of a pre-treatment period when milk yield was recorded for use as a covariate in the statistical analysis.

Background

Legume tree browses are increasingly being used for livestock feeding by smallholder dairy farmers. Leaves from the calliandra tree are especially important in the eastern province of
Kenya but the amounts that farmers have available are generally low and often only available intermittently. Farmers are therefore faced with the question of whether to feed small quantities daily over an extended period, e.g. throughout lactation, or to use alternative feeding patterns such as providing larger amounts at particular points, e.g. early in lactation. Another question that can be asked is what effect does fluctuating dietary feed supplies from fodder trees have on milk production?

These different questions are investigated in this case study. Dairy goats were used in a trial at Naivasha in Kenya's Rift Valley to investigate the effect of pattern of feeding on efficiency of utilisation of limited amounts of feed. The experiment examined different patterns of feeding of fixed amounts of calliandra as a supplement to basal forage of average quality

Objectives

The experiment was planned to address the following questions:

1. What is the effect on milk yield of allocating all the Calliandra fed daily to indigenous dairy goats over a 30-day period early in lactation, with none fed over the next 30-day period, compared with half the amount fed on every day of the 60 day period? The latter diet is considered as the control diet.

2. What is the effect on milk yield of feeding a small amount of Calliandra every day versus that of feeding twice the amount for five days and none for five days over the 60-day period?
Study design

The experimental design was planned as follows.

Three alternative patterns of feeding, as described below, were to be assigned at random to 30 goats that had recently kidded.

The goats were to be fed the control diet for 15 days after kidding prior to the experimental 60-day period starting.

It was planned to use average milk yield determined during this initial period as a covariate to adjust for the differences in the milk potentials of the different goats.

The three different diets were planned as follows. The diets are arranged so that after 60 days each goat on each diet was to have received the same overall amounts of hay, calliandra and maize.

1. Grass hay supplemented with 100g maize bran plus 100g Calliandra to form the control diet (diet C) given daily over 60 days.
Experimental design

<table>
<thead>
<tr>
<th>Goats</th>
<th>Pre-experimental period</th>
<th>Experimental period</th>
<th>Patterns of feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(15 days)</td>
<td>Day 1 - 30</td>
<td>Day 31 - 60</td>
</tr>
<tr>
<td>1 - 10</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

Supplemented with 100g calliandra throughout experimental period

| 11 - 20| C | A | D |

Supplemented with 200 g calliandra in first 30 days only

| 21 - 30| C | B | B |

Supplemented with 200 g calliandra for every alternate 5-day periods

2. Grass hay supplemented with 100g maize bran plus 200g calliandra daily (represented as diet A) given over 30 days, and followed by another 30 days supplemented with 100g maize only (represented as diet D). This was to answer the first objective.

<table>
<thead>
<tr>
<th>Goats</th>
<th>Pre-experimental period</th>
<th>Experimental period</th>
<th>Patterns of feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(15 days)</td>
<td>Day 1 - 30</td>
<td>Day 31 - 60</td>
</tr>
<tr>
<td>1 - 10</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

Supplemented with 100g calliandra throughout experimental period

| 11 - 20| C | A | D |

Supplemented with 200 g calliandra in first 30 days only

| 21 - 30| C | B | B |

Supplemented with 200 g calliandra for every alternate 5-day periods

3. Grass hay plus 100g maize bran fed daily, and supplemented or unsupplemented with 200g calliandra per day fed over alternating periods of five days. This is represented as diet B. This was to answer the second objective.
The experiment was planned for execution in three batches to accommodate goats that kidded at different times. Each batch on its own can be considered as a completely randomised design. Together they form a randomised block design with batch taking the role of block. The planned structure for the analysis of variance takes the following form:

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>2</td>
</tr>
<tr>
<td>Diet</td>
<td>2</td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
</tr>
<tr>
<td>Residual</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
</tr>
</tbody>
</table>

After the completion of the first two batches with 12 goats in the first batch and 6 in the second it was discovered that the animal technician had unfortunately misunderstood the instructions. The six animals that had received 200g Calliandra + 100g maize per day from days 1 to 30 (diet A) were put on diet B, not D, by mistake for the subsequent 30 days.

This meant that these goats had been provided with extra supplementation of Calliandra over the last 30 days than had been planned. Advice needed to be sought on what to do with the next batch of 12 animals in order to obtain the desired information on the three alternative feeding regimes that had been planned.

In considering what to do it was realised that the data that had already been collected in the experiment could be used to estimate the total sample size needed to detect statistically significant differences between the three diets, and that this might help with the decision.

The sample size \(n\) needed to detect significant differences between two means is determined from the formula:

\[
t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{2s^2/n}}
\]

Moving the terms around so that \(n\) is on the left hand side, we get

\[
n = \frac{2s^2t^2}{(\bar{x}_1 - \bar{x}_2)^2}
\]

where \(\bar{x}_1 - \bar{x}_2\) is the difference between two means; \(s^2\) is the estimated pooled variance among individuals within groups and \(t\) is the value of the \(t\)-statistic, say for \(P = 0.05\).

An estimate for \(s^2\) could be derived from the first two batches of the experiment. Also, from values obtained for the mean milk yields from the first two batches for the three diets,
preliminary estimates could be obtained for means in the above formula.

Questions to be addressed

The primary question is what can be done to remedy the situation? Secondly, based on data collected so far, can one determine the likely outcome in completing the experiment?

Two suggestions from the researcher were as follows:

Option 1. Six goats in this new batch of 12 animals could be put on diet A, and three each on diets B and C. This would mean that there would be only six replicates overall for diet A+D and nine for C and B, one less than originally planned for these two groups.

Option 2. There was also the possibility of obtaining a further six animals to be included later in the experiment to make up for the loss of the 6 goats, although the availability of six additional goats kidding at the same time was uncertain.

Two alternative suggestions put to the researcher by the biometrician were:

Option 3. Continue the experiment but exclude diet A+B altogether and assign the 12 goats equally to diets C and B to ensure better replication for these two groups. This would mean that objective 1 was dropped.

Option 4. Stay with the altered treatment design and include four goats fed C, B and A+B. This would result in 10 goats in each of these groups as originally planned. It would retain objective 2 but mean that objective 1 would be interpreted somewhat differently. The researcher explained that this would be a desired option since the treatment A+B did not make any sense biologically. Nevertheless, we retain this option for illustration purposes.
Source material

The data collected for the first two batches is given in CS8Data1 and described in CS8Doc1. The data file contains six separate worksheets: two with the raw data collected prior to (Pre_exp_data) and during (Exp_data) the experiment, respectively, three with pivot tables to calculate overall average milk yield per goat, firstly during the pre-experimental period (Pre_exp_means), and, secondly, during the 30-day (Exp_30means) and 60-day (Exp_60means) experimental periods, and a final sheet containing the average values (Mean data) used in the data analysis and copied from the other worksheets.

Data management

The raw data are contained within CS8Data1. The series of spreadsheets show how data can be summarised through use of the Excel pivot table facility and put into a separate sheet for analysis. By tabulating three ways by tag no, block and treatment the pivot table can check that the data coding and data entry are correct. For instance, if the contents of the pre-experiment pivot table are change to 'count' from 'average', it can be seen that every frequency count occurring within the body of the table is 15. Had there been any other values then the user's attention will be drawn to possible mistakes in coding or possible missing records.

This case study also provides an illustration of the level of precision to which data should be recorded. Notice that milk yields were recorded to the nearest half a gram and that the average milk yields and the values for dry matter intake are stored with one decimal place. In retrospect the precision with which the data were recorded was greater than was needed. One only needs to look at the range in values to realise that whole numbers would have been adequate. It was appreciated, once this study had been completed, that it was wasteful to have entered more significant figures than were necessary. The level of precision to which data are recorded is an important point to be considered by the researcher when designing a data recording sheet.

The mean values used for analysis are stored in the worksheet 'Mean data' in CS8Data1. Treatments are codes 1, 2 and 3. In order to facilitate interpretation of the output this file has already been read into GenStat and two new columns Diet30 and Diet60 formed to describe the diets as fed according to the letters A, B and C used in the design table. Factors have already been defined and these are indicated by a ! following the name. This file is stored in CS8Data2 and described in CS8Doc2.
Statistical modeling

To enable us to explore this problem further the following model:

\[ y_{ijk} = \mu + b_i + d_j + \beta x_{ijk} + e_{ijk} \]

was fitted to average milk yield over 1 – 30 days and 1 – 60 days, respectively, stored in CS8Data2, where \( y_{ijk} \) is the average milk yield for goat \( ijk \), \( \mu \) is the grand mean, \( b_i \) is the effect of batch \( i \) (\( i = 1,2 \)), \( d_j \) is the effect of diet \( j \) (\( j = C, A, B \) for the first 30 days; \( j = C, A + B, B \) for the 60 days), \( \beta \) is the regression coefficient on pre-treatment milk yield \( x_{ijk} \) and \( e_{ijk} \) is the residual term.

An analysis of covariance on the average milk yield for the 18 animals from the first two batches can be obtained using the dialogue box below produced via Stats → Analysis of Variance… and changing Design from General Analysis of Variance to One-way ANOVA (in Randomized Blocks).

From the output for the first 30 day-period it can be seen that the residual mean square is 1376 g\(^2\) with 13 degrees of freedom. The covariate pre milk_yld is highly significant and has been very effective in reducing the residual variance for average yield during the experiment (from 7476 g\(^2\), which can be shown to be the residual mean square by re-running GenStat without a covariate term in the model). The analysis of covariance shows that the different feeding patterns had no significant effect on milk yield. Goats receiving diet A had the highest mean milk yield (240.3 g) compared to those on diets B and C (197.9 and 196.7 g, respectively).

Analysis of variance (adjusted for covariate)

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
<td>55822</td>
<td>55822</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch.<em>Units</em> stratum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet30</td>
<td>2</td>
<td>7281</td>
<td>3641</td>
<td>2.65</td>
<td>0.109</td>
</tr>
<tr>
<td>Covariate</td>
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<td>86778</td>
<td>86778</td>
<td>63.08</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>13</td>
<td>17884</td>
<td>1376</td>
<td>5.43</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>180276</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand mean</td>
<td></td>
<td>211.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>197.9</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>240.3</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>196.7</td>
</tr>
<tr>
<td>rep.</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d.f.</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s.e.d.</td>
<td>21.82</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
For the analysis for the complete period of 60 days the second diet is now represented as A+B, not A+D, in view of the mistake by the technician. The residual mean square in this case is 1653 g², slightly higher than for the 30-day period. Also, over the 60-day period the differences between diets were slightly lower than during the first 30 days. In both cases there was no overall significant difference among diets. Indeed, the results for the two diets C and B, which were fed as directed, were very similar.

Analysis of variance (adjusted for covariate)

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch stratum</td>
<td>1</td>
<td>32310</td>
<td>32310</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
<td>85893</td>
<td>85893</td>
<td>51.95</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Batch.<em>Units</em> stratum</td>
<td>2</td>
<td>4313</td>
<td>2157</td>
<td>1.30</td>
<td>0.305</td>
</tr>
<tr>
<td>Diet60</td>
<td>2</td>
<td>21492</td>
<td>1653</td>
<td>4.64</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>13</td>
<td>154607</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>154607</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand mean</td>
<td>198.0</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet60</td>
<td>188.4</td>
<td>A+B</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rep.</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d.f.</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s.e.d.</td>
<td>23.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sample size calculation

Let us now return to the different options proposed earlier.

**Option 1.** Six goats to be put on diet A, and three each on diets B and C - this option will give totals of 9, 6 and 9 goats, respectively, for the 3 groups.

**Comment:** Based on the analysis above mean values for B and C are likely to remain similar and the A+D mean lower than A+B. Will these numbers be sufficient to demonstrate similarity of performance?

<table>
<thead>
<tr>
<th>Option</th>
<th>C</th>
<th>A + D</th>
<th>B</th>
<th>A + B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9 (1,2,3)*</td>
<td>6 (3)</td>
<td>9 (1,2,3)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10 (1,2,3,4)</td>
<td>10 (3,4)</td>
<td>10 (1,2,3,4)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>12 (1,2,3)</td>
<td>0</td>
<td>12 (1,2,3)</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>10 (1,2,3)</td>
<td>0</td>
<td>10 (1,2,3)</td>
<td>10 (1,2,3)</td>
</tr>
</tbody>
</table>

* batch numbers in which goats occur

Let us now return to the different options proposed earlier.

**Option 2.** Find an extra six goats to be included later. This would achieve the 10, 10, 10
goats originally planned for the three diets.

**Comment:** This would increase the sample size, especially in the A+D group, but how much of an improvement would this be on Option 1? The researcher had also expressed some doubt as to whether he would be able to find a suitable homogeneous group of goats.

<table>
<thead>
<tr>
<th>Option</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>A + D</td>
</tr>
<tr>
<td>1</td>
<td>9 (1,2,3)*</td>
</tr>
<tr>
<td>2</td>
<td>10 (1,2,3,4)</td>
</tr>
<tr>
<td>3</td>
<td>12 (1,2,3)</td>
</tr>
<tr>
<td>4</td>
<td>10 (1,2,3)</td>
</tr>
</tbody>
</table>

* batch numbers in which goats occur

**Option 3.** Continue the experiment but exclude diet A+D altogether and assign the 12 goats equally to diets C and B. This option allows 12 goats to be compared on diets C and B.

**Comment:** The analysis of data collected in the first two batches indicates that there is little difference in milk yields between goats fed diet B or C. So, again how much improvement would this be on Option 1?

<table>
<thead>
<tr>
<th>Option</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>A + D</td>
</tr>
<tr>
<td>1</td>
<td>9 (1,2,3)*</td>
</tr>
<tr>
<td>2</td>
<td>10 (1,2,3,4)</td>
</tr>
<tr>
<td>3</td>
<td>12 (1,2,3)</td>
</tr>
<tr>
<td>4</td>
<td>10 (1,2,3)</td>
</tr>
</tbody>
</table>

* batch numbers in which goats occur

**Option 4.** Stay with the altered treatment design and include four goats fed C, B and A+B. This option would allow the experiment to continue as planned, but with diet A+D rather than A+B.

**Comment:** This would give the opportunity to determine the increase in milk yield expected from feeding A+B compared with B and C, i.e. giving extra feed during the first 30 days. The researcher explained that this would not really be a useful or interesting result, but we include it here for illustration purposes. To determine whether the sample size of 10, 10, 10 will be adequate we would need to apply the formula introduced earlier, namely \( n = \frac{2s^2r^2}{(\bar{x}_1 - \bar{x}_2)^2} \)
Let us calculate the sample size needed to achieve Option 4. As the 60-day period is the primary period of interest we shall use the residual mean square 1653 g² from the analysis of covariance for the 60-day period for the first two batches as the estimate of the residual variance that might occur in the complete experiment. Let the total number of animals to be used per diet be n. From the formula for the t-test shown earlier, namely \( n = 2s^2t^2/(\bar{x}_1 - \bar{x}_2)^2 \), we estimate: \( n = (2 \times 1653 \times t^2) / (219.9 - 188.4)^2 \) where means 219.9 (diet A+B) and 188.4 (diet C) shown in the output are substituted for \( \bar{x}_1 \) and \( \bar{x}_2 \) respectively.

Analysis of variance (adjusted for covariate)

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch stratum</td>
<td>1</td>
<td>32310</td>
<td>32310</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
<td>32310</td>
<td>32310</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch.<em>Units</em> stratum</td>
<td>2</td>
<td>4313</td>
<td>2157</td>
<td>1.30</td>
<td>0.305</td>
</tr>
<tr>
<td>Diet60</td>
<td>1</td>
<td>85893</td>
<td>85893</td>
<td>51.95</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
<td>85893</td>
<td>85893</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>13</td>
<td>21492</td>
<td>1653</td>
<td>4.64</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>154607</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet60</td>
<td>C</td>
<td>A+B</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rep.</td>
<td>6</td>
<td>219.9</td>
<td>185.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d.f.</td>
<td>13</td>
<td>23.92</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Using \( t = 2 \) for a first approximation (\( P=0.05 \)), it can be shown that \( n = 13.3 \) or, rounding up to the nearest integer, \( n = 14 \). Sketching out the analysis of variance table this would give:

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>2</td>
</tr>
<tr>
<td>Diet</td>
<td>2</td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
</tr>
<tr>
<td>Residual</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
</tr>
</tbody>
</table>

The 5% \( t \)-value with 36 degrees of freedom is 2.03. Repeating the above calculation but replacing \( t \) by this value, the new result is \( n = 13.7 \). This confirms that 14 animals will be necessary for each of the diets C and A+B in order to demonstrate a significant effect of supplementation with calliandra over the 60 days.

However, only a total of 30 goats are available for all the 3 groups. So what can we do? The analysis of the first two batches suggested that there was no difference between diets C and B in terms of average milk yield. In other words, fluctuating supplementation levels of calliandra appeared to have no negative effect on milk yield.
Analysis of variance (adjusted for covariate)

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>s.s.</th>
<th>m.s.</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch stratum</td>
<td>1</td>
<td>32310</td>
<td>32310</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
<td>85893</td>
<td>85893</td>
<td>51.95</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>154607</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
<td>4313</td>
<td>4313</td>
<td>1.30</td>
<td>0.305</td>
</tr>
<tr>
<td>Diet60</td>
<td>2</td>
<td>21492</td>
<td>10746</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>21492</td>
<td>1653</td>
<td>4.64</td>
<td></td>
</tr>
</tbody>
</table>

Grand mean 198.0

Diet60  C  A+B  B

rep.  6

d.f.  13

s.e.d. 23.92

Suppose that we are prepared to accept that this is likely to be confirmed in the third batch, i.e. that we can combine the results for C and B throughout the 3 batches for a comparison with A+B. The formula for \( t \) now becomes

\[
t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s^2 \left( \frac{1}{n_1} + \frac{1}{n_2} \right)}}
\]

where \( \bar{x}_1 \) and \( \bar{x}_2 \) now correspond to diets C and B pooled and diet A+B, respectively.

Suppose that \( n_1 = 2n_2 \) and let us write \( n_2 \) as \( n \). Using this new formula it can be shown that

\[
n = \frac{(3/2 \ t^2 \ s^2)}{(\bar{x}_1 - \bar{x}_2)^2}
\]

Substituting values from the GenStat output, as before, we obtain

\[
n = \frac{(1.5 \times 2^2 \times 1653)}{(219.9 - 187.25)^2}
\]

where \( t = 2 \) and 187.25 = average of 188.4 for diet C and 185.7 for diet B. Thus, \( n = 9.3 \) or, rounding up to the next integer, \( n = 10 \).

By following the same procedure as before this results in the analysis of variance structure:

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>2</td>
</tr>
<tr>
<td>Diet</td>
<td>2 (C and B pooled)</td>
</tr>
<tr>
<td>Covariate</td>
<td>1</td>
</tr>
<tr>
<td>Residual</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
</tr>
</tbody>
</table>

The 95% \( t \)-value with 25 degrees of freedom = 2.06. Repeating the calculation with \( t = 2.06 \) gives \( n = 9.9 \). This confirms that 10 goats will be necessary for group A and 10 each for the other two groups. This was the number planned at the outset. Thus, if the researcher were to decide to continue the experiment along the lines of Option 4, the prediction is that the sample might be just large enough to determine a statistically significant effect (\( P < 0.05 \)).
But as already indicated this would not provide information of biological interest to the researcher. Other options are unlikely also to yield data that would give results of statistical significance. Thus, there might be little value in continuing the experiment.

Findings, implications and lessons learned

1. A mistake was made in the execution of the experiment. This may have been the fault of the technician who did not listen to his/her instructions or possibly the instructions were not sufficiently clear. Mistakes can occur in any study and so it is important that the person in charge of an experiment keeps a close eye on what is going on and makes sure that experimental data sheets are written neatly and kept up to date.

Likewise a biometrician needs to write down clearly (and keep a record of) the design of an experiment and any other instructions he/she wishes the researcher to follow. It is always a good idea for the biometrician to visit the experimental site himself/herself to see the layout of the experiment and to observe measurements being undertaken. He/she will better understand the nature of the experiment and the sources of experimental errors likely to arise.

2. Mistakes can occur at any time or situations arise where the researcher needs to modify the design. A biometrician needs to be able to respond quickly to such situations and advise the researcher accordingly.

3. Bearing in mind that drop-outs can occur in any animal experiment for a variety of reasons unrelated to the treatments being offered it is often advisable to assign one or two extra animals to the experiment at the start.

4. Experimental design is often an art in compromise between that which is statistically desirable and that which is experimentally possible. The biometrician has to be firm when he/she thinks that an experiment that the researcher proposes is not viable and should tell the researcher so. It should always be born in mind that an experiment that may be 'too small' when carried out alone can be replicated. The researcher also needs to be clear of the experimental objectives and what comparisons make sense biologically.

5. An important feature of this experiment is the large reduction in residual variance brought about by introduction of the pre-treatment covariate milk yield. When it is known that there are likely to be large individual variations in the primary response variable being measured it is important to consider either blocking for pre-experimental information or using this information as a covariate. Such variables in animal experiments can be milk yield, body weight, or, in animal health, a blood measurement of particular interest such as haemoglobin or packed cell volume.

6. The researcher chose Option 1 ending up with six goats receiving diet A + D and nine each of B and C. Unfortunately the technician made another mistake in the allocation of feeds and the experiment was terminated.

Study questions
1. Checking of raw data was not done. Plot line graphs across time for each goat and determine whether there are any odd values. If there are, what would you do with them? Do the lines give any indications of any trends in milk yield that may help in understanding the pattern?

2. In this study an estimate of variance $s^2$ was available. Discuss in general terms how you might determine sample size if no estimate of $s^2$ is available.

3. Suppose that Option 4 was chosen but rather than assign 4 goats to each diet, 6 were assigned to the (A + B) group and 3 to each of B and C. Assuming $s^2=1653$, calculate the minimum difference in means between A+B (12 goats) and C and B pooled (18 goats) that will need to be observed for the difference in means to be significant ($P<0.05$). Is this less than the difference that needs to be observed given 10 and 20 goats in the respective groups?

4. Suppose that measurements on milk yield had not been taken before the experiment started. Using the estimate of residual variance without adjustment for covariance, namely 7476 g2, estimate the sample size that would need to be achieved in Option 4 given equal numbers in the three groups.

5. The smaller the variance $s^2$ the fewer animals are needed to detect significant differences between means. Discuss other ways, in addition to the existence of a pre-treatment covariate period, in which the variability among goats might be minimised.

6. Rerun GenStat for 60-day milk yield for the first two batches and calculate the coefficient of variation (100 x standard deviation / mean). Comment on the value you get. What would you tell the researcher? If the researcher were to do another experiment what steps would you advise him/her to take in order to try and reduce the variation?

7. Following analysis of the data from the first two batches, your advice to the researcher is that there seems to be little value in proceeding with the experiment and that it should be aborted. He ignores your advice, completes the experiment and brings the data back to you for analysis. Discuss your role and the consequences of agreeing or refusing to do the analysis.

8. When reporting the results of a study when things do not always go quite to plan it is usually possible to present the study in the way that disguises what really happened without completely hiding the truth. Write a description of the experimental design assuming that Option 1, as carried out, was followed correctly. You will need to pretend that the treatment A + B applied in the first two batches was actually not done.

9. A 2-week pre-treatment period was allowed in this experiment to allow pre-treatment milk yield to be determined for each goat. This was then used as a covariate in the analysis. This, however, required a 15-day control period at the start of lactation. Design an alternative experiment in which the experimental treatments could be applied as the goats kidded. What additional information on the goats would you require for an efficient design?
Acknowledgements

We are grateful to Dannie Romney who advised on the original design of the experiment and to John Rowlands who assisted with the preparation of this case study. Teachers and other users of this case study are welcome to use the data for further analysis but not to publish any results without permission from the Kenya Agricultural Research Institute.