Technical note: Designing and analyzing quantitative factorial experiments¹

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ABSTRACT

The response of a biological process to various factors is generally nonlinear, with many interactions among those factors. Although meta-analyses of data across multiple studies can help in identifying and quantifying interactions among factors, missing latent variables can result in serious misinterpretation. Eventually, all influential factors have to be studied simultaneously in one single experiment. Because of the curvature of the expected response and the presence of interactions among factors, the size of experiments grows very large, even when only 3 or 4 factors are fully arranged. There exists a class of experimental designs, named central composite designs (CCD), that considerably reduces the number of treatments required to estimate all the terms of a second-order polynomial equation without any loss of efficiency compared with the full factorial design. The objective of this technical note is to explain the construction of a CCD and its statistical analysis using the Statistical Analysis System. In short, a CCD consists of 2^k treatment points (a first-order factorial design, where k represents the number of factors), augmented by at least one center point and $2 \times k$ axial treatments. For 3 factors, the resulting design has 16 treatment points, compared with 27 for a full factorial design. For 4 factors, the CCD has 25 treatment points, compared with 81 for a full factorial design. The CCD can be made orthogonal (no correlation between parameter estimates) or rotatable (the variance of the estimated response is a function only of the distance from the design center and not the direction) by the location of the axial treatments. In spite of the reduced number of treatments compared with a full 3^k factorial, the CCD is relatively efficient in estimation of the quadratic and interaction terms. Blocking of experimental units is often desirable and is sometimes

required. Randomized block designs for CCD are found in some statistical design textbooks. The construction of incomplete, balanced (or near-balanced) designs for CCD experimental layouts is explained using an example. The Statistical Analysis System statements used to analyze a CCD, to identify the significant parameters in the polynomial equation, and to produce parameter estimates are presented and explained.

Key words: factorial experiment, central composite design, randomized incomplete block design, mixed model

Experimental research generally follows a classic path. Initially, experiments are designed to study the effects of 1 or 2 factors while holding all other factors constant. This is the domain of the 1-way and 2-way treatment designs. These experiments help uncover the fundamental processes involved, but quantification of the relationships remains problematic because of the implicit linearity in the resulting estimated relationships. The difficulty in identifying and quantifying interaction effects when one is pooling results across many experiments, such as in quantitative meta-analyses (St-Pierre, 2000), is often misjudged. Any hidden, latent variable can bias all such analyses and their interpretation. Eventually, scientists need to design experiments in which multiple factors are considered simultaneously to quantify the curvature (nonlinearity) in relationships as well as the interactions among factors. This is especially true for certain components of nutritional systems such as energy and microbial production, for which it is important that robust quantitative relationships be developed, thus requiring the inclusion of curvature and interaction terms. Approximating curvature through quadratic polynomials necessitates at least 3 levels for each factor. If a conventional factorial arrangement of treatments is used, the number of experimental treatments required grows geometrically with the number of factors considered. For 2 factors, the resulting 3×3 factorial requires 9 different treatments. For 3 factors, the $3 \times 3 \times 3$ factorial arrangement requires 27 treatments; k factors result in 3^k treatments. In addition to the enormous and generally unpractical number of treatments involved, many experimental designs cause parameter estimates (the regression coefficients in a

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Figure 1. Three-dimensional representation of a factorial treatment arrangement for 3 factors ($k = 3$; number of treatments = 27). The 3 factors (Z_1, Z_2, Z_3) are coded so that their minima, central values, and maxima take values of $-1, 0$, and 1 , respectively.

polynomial equation) that are correlated, thus lowering the power of the experiment. For example, the coefficients for the squared terms of a polynomial function are estimated with relatively low precision with 3^k factorial experiments (Box and Wilson, 1951). There exists a class of experimental designs, named central composite designs (CCD), that can considerably reduce the number of treatments required to estimate all parameters in the model while producing parameter estimates that can be made independent (i.e., orthogonal) of each other (Myers et al., 2009). Unfortunately, the CCD has received little attention in the statistical literature related to biology and agriculture; most textbooks on statistical designs in biology do not even mention CCD. Our objectives are to explain how a CCD works, how to construct a CCD with incomplete blocks, and how to conduct the analysis of biological data from CCD experiments using SAS.

The Structure of a CCD

We start with a simple example of a 3-factor experiment. The objective is to estimate the curvilinear response surface of a dependent variable Y using a quadratic polynomial function of 3 factors, X_1, X_2 , and X_3 . The statistical model is

$$Y_i = b_0 + b_1X_1 + b_2X_1^2 + b_3X_2 + b_4X_2^2 + b_5X_3 + b_6X_3^2 + b_7X_1X_2 + b_8X_1X_3 + b_9X_2X_3 + e_i, \quad [1]$$

where Y_i are the values of the dependent variable, b_j are the regression parameters to be estimated, and e_i are the residual errors, assumed independent, normal, with a mean of zero and a variance of σ_e^2 . The treatment design for a conventional $3 \times 3 \times 3$ factorial experiment is illustrated in Figure 1. A total of 27 treatments is required to maintain a balanced design and independent parameter estimates. Least squares (and maximum likelihood) estimates of the b_i are easily calculated (Draper and Smith, 1998).

Designs for fitting second-order response surfaces such as in equation [1] must involve at least 3 levels of each variable so that all the coefficients in the model can be estimated. The CCD is a first-order factorial design augmented with additional points to allow the estimation of the coefficients of a second-order surface. A 3-factor CCD is illustrated in Figure 2. With 3 factors, the CCD requires only 15 individual treatments to provide estimates of all 10 parameters in equation [1], compared with 27 treatments for the full $3 \times 3 \times 3$ factorial. With a CCD, it is often desirable to replicate

the center point (or treatment) to attain certain design properties (orthogonality, rotatability) and to allow the splitting of the residual error into lack-of-fit and pure error components. These are discussed below.

In Figure 2, the first 8 points located at the vertices (corners) are the usual factorial points for fitting a first-order model. The ninth point is the center point of the design, which, as we just mentioned, is often repeated n_2 times (n_2 is the number of replicates of the center point). The 6 remaining points are called the axial points. Notice that with a CCD, each independent variable is actually measured at 5 levels: $-\alpha$, -1 , 0 , 1 , $+\alpha$. The choice of α results in different design properties, such as orthogonality and rotatability. The design can be made orthogonal, resulting in uncorrelated estimates of the response model coefficients. Table 1 reports the values of α for an orthogonal CCD with a different number of factors (k) when $n_2 = 1$. The design can also be made rotatable, where the variance of the estimated response is a function only of the distance from the center and not the direction (Myers and Montgomery, 1995). Table 1 also reports the values of α to obtain a rotatable CCD when $n_2 = 1$.

The CCD can be compared with the 3^k factorial design on the basis of efficiency in estimating particular coefficients in the response model. Many efficiency

Table 1. Values of α for an orthogonal or rotatable central composite design (CCD) if a full-factorial design is used with a single center point

k^1	α^2	
	Orthogonal	Rotatable
2	1.000	1.414
3	1.216	1.682
4	1.414	2.000
5	1.596	2.378
6	1.761	2.828
7	1.910	3.364
8	2.045	4.000

¹ k is the number of factors.

²In a CCD, α represents the coded distance between the maximum level of a factor and its central (median) value (see Figure 2).

criteria have been developed, such as A-, D-, and G-optimality criteria (Myers and Montgomery, 1995), each emphasizing a different desirable property of designs. An efficiency criterion was developed to simultaneously take into consideration the precision with which coefficients are estimated and the number of experimental treatments required (Keifer, 1975). Table 2 gives the relative efficiencies for estimating the mixed and pure quadratic coefficients for the orthogonal CCD compared with the 3^k factorial design. In this table, coefficients greater than 1.0 indicate that the CCD has a greater

Figure 2. Three-dimensional representation of a central composite design for 3 factors ($k = 3$; number of treatments = 15). The 3 factors (Z_1 , Z_2 , Z_3) are coded levels of the 3 factors. Note that the minima and maxima take values of $-\alpha$ and α , respectively.

Table 2. Relative efficiencies of orthogonal central composite designs (CCD) compared with 3^k factorials¹

Item	k = 2	k = 3	k = 4	k = 5 ²	k = 6 ²
b _{int} 3	1.00	1.00	1.00	1.00	1.00
b _{squ} 3	1.00	1.07	1.00	1.43	1.62

¹k is the number of factors.
²Denotes CCD containing 1/2 fraction of a 2^k plus appropriate axial points and center points.
³b_{int} and b_{squ} are the regression coefficients for the interaction terms (mixed coefficients) and for the squared terms (pure quadratic coefficients) in the polynomial equation, respectively.

efficiency than the factorial design (i.e., 1.07 indicated a 7% greater efficiency of the CCD). Clearly, according to this efficiency criterion, the CCD is always at least as efficient as the 3^k factorial. Other efficiency criteria would yield different results, but in general, CCD is quite efficient (Myers and Montgomery, 1995).

Blocking of Treatments

The CCD is, in fact, a treatment arrangement that can be arranged in a variety of experimental designs. The randomized incomplete block design (**RIBD**) is generally an attractive statistical design for CCD to decrease the number of treatments needed. Some arrangements of CCD in RIBD have been given by Box and Hunter (1957), and the methods to conduct statistical analysis when blocks are considered to have a fixed effect have been fully described (Cochran and Cox, 1957). The CCD has frequently been used in the design of industrial experiments, in which blocks are more appropriately considered as having a fixed effect. In biological experiments, however, blocks are often the outcome of a random process; their effects should be considered random (Mead and Pike, 1975). Using a mixed model with random blocks allows the recovery of interblock as well as intrablock information (Khuri, 1992). Regardless of the type of block effects, it is always desirable that the design be divided into blocks in such a manner that the block effect does not affect the estimates of the polynomial terms in the model. That is, blocking should be orthogonal or as close to it as possible. Some design plans that ensure block orthogonality are found in numerous textbooks such as that by Cochran and Cox (1957). Often a layout has to be designed to accommodate specific restrictions imposed by a given experiment because of facilities, costs, animal availability, and so on. An example of such a construction is presented in the next section.

Data Analysis Using SAS

The analysis of CCD data using the Statistical Analysis System (**SAS**) is explained using a recent

trial (Weiss, W. P., N. R. St-Pierre, and L. B. Willett, unpublished data; Weiss, W. P., L. B. Willett, N. R. St-Pierre, D. C. Borger, T. R. McKelvey, and D. J. Wyatt, unpublished data) as an example. Prior research identified numerous nutritional factors influencing the fraction of total manure N that volatilizes as NH₃. In nearly all instances, prior research had focused on one factor at a time. Our interest was in the quantitative estimation of the response of several production variables including manure NH₃ volatilization to the levels of 3 dietary factors: starch (% of DM), MP (% of DM), and alfalfa (% of forage DM). A full-factorial arrangement that would allow the estimation of quadrature would have required 3³ = 27 different diets. In contrast, a CCD required 2³ + (2 × 3) + 1 = 15 different diets. The experiment involved total collection of feces and urine. The variance between cows for numerous metabolic variables is substantial. Thus, a crossover-type design was highly desirable to minimize the number of cows and total collections to be done. A full Latin square was clearly out of the question: using a multiple of 15 cows over 15 collection periods of 3 wk each would

Table 3. Treatment layout for a 3-factor orthogonal central composite design (CCD) with n₂ = 4 central points¹

Treatment	Factor ²		
	X ₁	X ₂	X ₃
1	1	1	1
2	1	-1	-1
3	-1	1	-1
4	-1	-1	1
5 ³	0	0	0
6 ³	0	0	0
7	1	1	-1
8	1	-1	1
9	-1	1	1
10	-1	-1	-1
11 ³	0	0	0
12 ³	0	0	0
13	-1.4142	0	0
14	1.4142	0	0
15	0	-1.4142	0
16	0	1.4142	0
17	0	0	-1.4142
18	0	0	1.4142

¹Coefficients in the table are the coded levels for each of the three factors (X₁, X₂, X₃).
²In the example used in the text, X₁, X₂, and X₃ refer to the percentage of alfalfa (A), the percentage of starch (S), and the percentage of MP (P) in the diet, respectively. One unit level in X₁ (A) corresponded to 17.68% of alfalfa (% of forage DM); 1 unit of X₂ (S) corresponded to 2.83% starch (% of DM), and 1 unit of X₃ (P) corresponded to 1.6% of MP (% of DM). Treatments are centered (coded value of 0) at 50% alfalfa, 26% starch, and 10.4% MP. Thus, treatment 1 corresponds to a diet with 67.7% alfalfa, 28.8% starch, and 11.5% MP.
³Treatments 5, 6, 11, and 12 are the same treatment; the different treatment numbers simply reflect replication of the center point treatment.

have exceeded the life expectancy of too many cows, if not that of the authors themselves. In addition, our research facilities allow for a maximum of 6 cows at a time in the metabolism stalls. This imposed a maximum block size of 6 cows. Prior experience with full fecal and urinary collection experiments indicated that 3 periods of 3 wk each resulted in a very good state of animal welfare with a minimal loss of data. Thus, we had to set multiple blocks of 6 cows, each with 3 periods of collection. That is, each cow would go through a sequence of 3 diets out of the total 15 diets. To ensure balance and orthogonality, the number of treatments must be a multiple of the number of cows per block. In a CCD, this is achieved by adding measurements at the center point. In our case, this meant that the center point diet would be repeated 4 times (i.e., $n_2 = 4$), for a total of 18 treatments, with the understanding that 4 of these treatments would be identical (i.e., treatments 5, 6, 11, and 12 in Table 3).

In the design of this experiment, orthogonality of the parameter estimates (the b_i in equation [1]) is desirable because nonsignificant terms are to be removed from the equation and orthogonality ensures that the tests on the estimates are independent of each other. Thus, issues associated with sequential tests are avoided. Because $n_2 = 4$, Table 1 cannot be used to determine the correct value for α . It has to be calculated as follows (Myers and Montgomery, 1995). Let F be the number of factorial treatment points (i.e., 2^k if a complete factorial design is used, where k is the number of factors), and $T = 2k + n_2$ (recall that n_2 is the number of center points). First, calculate

$$Q = [(F + T)^{1/2} - F^{1/2}]^2. \quad [2]$$

Then α is given by

$$\alpha = (QF/4)^{1/4}. \quad [3]$$

In our application, $k = 3$, $F = 8$, $T = 10$, and $Q = 2$; therefore, $\alpha = 1.4142$. The resulting treatment layout is reported in Table 3.

Because no such experimental design exists in the literature, we constructed a crossover RIBD, which could also be described as a replicated, incomplete (truncated) Latin square with 6 blocks, each of 6 cows and 3 periods, and with 18 treatments. To ensure near orthogonality and to balance for any possible carryover effects, the following rules had to be followed during its construction:

- *Rule 1:* Each treatment must be replicated exactly 6 times and appear once in each block.
- *Rule 2:* Each treatment must appear equally often in each of the 3 periods across blocks.
- *Rule 3:* Each treatment is followed by another treatment only once.
- *Rule 4:* Each block is to contain exactly 4 central point treatments (i.e., treatments 5, 6, 11, and 12).
- *Rule 5:* There must be no more than 2 central point treatments in a given period.
- *Rule 6:* The frequency of 2 central point treatments in a period must be the same in each of the 3 periods across blocks.
- *Rule 7:* A central point treatment is not to follow another central point treatment.
- *Rule 8:* Treatment sequences should minimize the frequency when 1 or more of the 3 factors is either below or above the center point in all 3 periods.
- *Rule 9:* There must be no sequence in which a factor is at the same level across 3 periods.

To avoid a fastidious manual search, we used a genetic algorithm (St-Pierre and Cobanov, 2006) to identify a layout that matched these rules. The resulting experimental layout is shown in Figure 3.

Factors X_1 , X_2 , and X_3 in Table 3 refer to the coded levels of alfalfa (A), starch (S), and MP (P) in the diets. The coding worked as follows. First, minimum and maximum concentrations of each factor were set, based on typical ranges observed in the field, feed cost considerations (e.g., MP maximum), and cow health considerations (e.g., starch maximum). For alfalfa, the minimum and maximum levels were set at 25 and 75%. A $1-\alpha$ unit of X_1 (i.e., A) corresponds to $(75 - 50) = 25\%$ of alfalfa (% of forage DM). Recalling that $\alpha = \sqrt{2}$ in our application, a coded 1 unit of A corresponds to $25 \div \sqrt{2} = 17.68\%$ alfalfa. Thus, the coded levels for X_1 (A) in Table 3 correspond to 25.0, 32.3, 50.0, 67.7, and 75.0% alfalfa in the diet. For starch, the minimum and maximum levels were set at 22 and 30% of DM, respectively. Thus, 1 unit of S corresponds to 2.83% starch in diet DM, and the 5 levels of starch used were 22.0, 23.2, 26.0, 28.8, and 30.0% starch. For MP, the minimum and maximum were set at 8.8 and 12.0% of DM. Therefore, 1 unit of P corresponds to 1.1% MP in diet DM, and the 5 levels of MP used were 8.8, 9.3, 10.4, 11.5, and 12.0% MP. A complete data set is provided for total manure output (kg/cow per day) in the Appendix section (see Figure A1) to allow readers to replicate the calculations and procedures explained in this article. Data are also available as a downloadable Excel spreadsheet (<http://dairy.osu.edu>). Readers should note that cow 4137 in block 3 had to be removed from the experiment in period 2 and was replaced by cow 4523 in period 3. This created

Block 1						
Period	Cows					
	A	B	C	D	E	F
I	5	4	7	10	13	16
II	1	17	11	14	8	2
III	18	12	15	9	3	6

Block 2						
Period	Cows					
	A	B	C	D	E	F
I	11	17	14	12	7	9
II	1	13	5	15	4	18
III	10	2	3	8	16	6

Block 3						
Period	Cows					
	A	B	C	D	E	F
I	10	1	3	15	18	12
II	2	6	14	4	11	16
III	13	17	7	5	8	9

Block 4						
Period	Cows					
	A	B	C	D	E	F
I	13	6	1	16	3	9
II	5	2	17	12	15	7
III	4	14	10	8	11	18

Block 5						
Period	Cows					
	A	B	C	D	E	F
I	18	8	15	5	2	11
II	4	6	3	13	9	16
III	1	14	10	7	12	17

Block 6						
Period	Cows					
	A	B	C	D	E	F
I	2	14	6	8	4	17
II	12	7	18	13	10	3
III	9	1	16	11	15	5

Figure 3. Experimental layout for a 3-period crossover, randomized, balanced incomplete block design with 6 blocks of 6 cows and 18 treatments. Cells in each block refer to the treatment numbers of the central composite treatment design of Table 3. Cows and periods are nested within blocks (i.e., cow A in block 1 is different from cow A in block 2; period I in block 1 is different from period I in block 2).

a slight imbalance in the data, with the consequence that the coefficient estimates in the polynomial equations were not perfectly orthogonal.

The statistical model for data from this experiment includes the fixed effects of A, S, P, their squared terms, and their single interactions terms (i.e., equation [1]), whereas blocks, period within blocks, and cows within blocks were considered random effects. This model is solved using the MIXED procedure (SAS Institute, 2004). In a first step, the complete model is fitted using the coded values (A, S, P) for the 3 factors (output is in Figure 4):

```
PROC MIXED COVTEST;  
CLASSES Block Period Cow;  
MODEL Manure = S P A  
S*S P*P A*A  
S*P S*A P*A/CORRB DDFM = KR;  
RANDOM Block Period(Block) Cow(Block);  
RUN;
```

[4]

The CORRB option in the MODEL statement calculates and reports the approximate correlation matrix of the fixed-effects parameter estimates. If the design were perfectly orthogonal, all its off-diagonal elements would be equal to zero (i.e., the correlation between all pairs of estimates would be equal to zero). Because of the loss and replacement of 1 cow, the design was no longer perfectly orthogonal, but the approximate correlation matrix showed that all correlations were, in fact, very small (all were less than |0.06| and most were less than |0.01|). Figure 4 presents the SAS output relevant to the fixed effects in the model. An orthogonal design implies that the test of a given effect is completely independent of the other effects. That is, the test on the linear effect of S (starch) is completely independent of whether S² is included in the model. Our design was

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
S	1	66.4	6.72	0.0117
P	1	66.1	0.01	0.9128
A	1	66.9	15.06	0.0002
S*S	1	61.9	0.19	0.6614
P*P	1	61.9	0.06	0.8021
A*A	1	65.3	0.18	0.6690
S*P	1	62.9	0.25	0.6155
S*A	1	70.9	0.96	0.3307
P*A	1	65.2	0.99	0.3245

Figure 4. Statistical tests of the fixed-effect factors reported by the MIXED procedure (SAS Institute, 2004) for the coded factors, where S is the coded level of starch, P is the coded level of MP, and A is the coded level of alfalfa. The Num DF and Den DF columns report the numerator and denominator degrees of freedom used to calculate the probability (Pr > F) of the F-statistics (F Value) under the null hypothesis that the effect is null.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Alf	1	72.4	16.77	0.0001
Starch	1	72.8	7.39	0.0082

Estimates					
Label	Estimate	Standard Error	DF	t Value	Pr > t
Intercept	91.9574	8.8597	76.3	10.38	<.0001
Alfalfa	0.2058	0.05026	72.4	4.10	0.0001
Starch	-0.8545	0.3144	72.8	-2.72	0.0082

Figure 5. Statistical tests and parameter estimates of the fixed-effect factors reported by the MIXED procedure (SAS Institute, 2004) for the final model with uncoded factor effects. The resulting equation is $\text{Manure (kg/d)} = 92.0 (\pm 8.86) + 0.206 (\pm 0.050) \times \text{Alfalfa (\% of forage DM)} - 0.855 (\pm 0.314) \times \text{Starch (\% of DM)}$. Num DF and Den DF represent numerator and denominator degrees of freedom, respectively. Alf = alfalfa.

not perfectly orthogonal, but the correlations between parameter estimates were so small that the effects on the standard error of estimates and, consequently, the statistical test on each estimate were negligible.

Because of the factor orthogonality (or near-orthogonality), it is not necessary to proceed through a questionable sequence in which insignificant terms would be iteratively removed from the model. For example, the prediction of manure excretion must involve only the linear effects of starch and alfalfa levels in the diet (Figure 4). All other fixed effects can be removed simultaneously from the model, and the remaining terms can be expressed in their natural scales, as opposed to the coded ones. The statements (SAS Institute, 2004) to achieve this are as follows (output is shown in Figure 5):

```
PROC MIXED COVTEST;
CLASSES Block Period Cow;
MODEL Manure = Alf Starch/DDFM = KR;
RANDOM Block Period(Block) Cow(Block);
ESTIMATE 'Intercept' Intercept 1;
ESTIMATE 'Alfalfa' Alf 1;
ESTIMATE 'Starch' Starch 1;
RUN;
```

[5]

Figure 5 reports the results. Because the analysis is now conducted in the natural scales as opposed to the coded ones, the design is no longer orthogonal and tests on the fixed effects are no longer of interest; parameter estimates and their standard errors are. In our manure example, the resulting prediction equation can be reported as follows:

$$\begin{aligned} \text{Manure (kg/d)} &= 92.0 (\pm 8.86) + 0.206 (\pm 0.050) \\ &\times \text{Alfalfa (\% of forage DM)} - 0.855 (\pm 0.314) \\ &\times \text{Starch (\% of DM)}. \end{aligned} \quad [6]$$

Each percentage unit increase in alfalfa concentration results in an expected increase of 0.21 kg/d in manure excretion. Likewise, each percentage unit increase in starch concentration results in an expected decrease of 0.8 kg/d in manure excretion.

The treatment replications at the center point can be used to partition the residual standard error into a lack-of-fit and pure error terms (Cochran and Cox, 1957). The lack-of-fit test determines whether the fitted quadratic function is a reasonable approximation to the true but unknown relationship.

In this example, the use of a CCD reduced the number of cow-periods from 186 to 112 while maintaining power and the ability to identify all the parameters independently in the quadratic function. The use of a CCD allowed us to conduct an experiment that essentially would be impossible to conduct using standard experimental designs because of limits on cow availability, facility limitations, and economic considerations. Because, in this type of experiment, most of the costs are directly related to the number of cow-periods, the use of a CCD reduced the experimental costs by approximately 40% compared with a full-factorial treatment design.

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APPENDIX

Block	Period	Cow	Starch	MP	Alfalfa	Trt	A	S	P	Manure	Block	Period	Cow	Starch	MP	Alfalfa	Trt	A	S	P	Manure
1	1	4394	28.83	9.3	67.88	7	1	1	-1	58.662	4	1	4344	28.83	11.5	32.32	9	-1	1	1	88.823
1	1	4473	23.17	9.3	32.32	10	-1	-1	-1	81.084	4	1	4539	28.83	11.5	67.68	1	1	1	1	77.372
1	1	4502	30	10.4	50	16	0	1.4142	0	65.540	4	1	4321	28.83	9.3	32.32	3	-1	1	-1	96.111
1	1	4300	26	10.4	50	5	0	0	0	69.749	4	1	4463	30	10.4	50	16	0	1.4142	0	92.296
1	1	4474	26	10.4	25	13	-1.4142	0	0	66.932	4	1	4540	26	10.4	50	6	0	0	0	73.767
1	1	4493	23.17	11.5	32.32	4	-1	-1	1	85.417	4	1	4325	26	10.4	25	13	-1.4142	0	0	82.903
1	2	4394	26	10.4	50	11	0	0	0	67.388	4	2	4344	28.83	9.3	67.88	7	1	1	-1	99.125
1	2	4473	26	10.4	75	14	1.4142	0	0	98.277	4	2	4539	26	8.8	50	17	0	0	-1.4142	90.907
1	2	4502	23.17	9.3	67.68	2	1	-1	-1	85.793	4	2	4321	22	10.4	50	15	0	-1.4142	0	110.518
1	2	4300	28.83	11.5	67.68	1	1	1	1	77.598	4	2	4463	26	10.4	50	12	0	0	0	90.092
1	2	4474	23.17	11.5	67.68	8	1	-1	1	88.363	4	2	4540	23.17	9.3	67.68	2	1	-1	-1	83.734
1	2	4493	26	8.8	50	17	0	0	-1.4142	93.460	4	2	4325	26	10.4	50	5	0	0	0	94.336
1	3	4394	22	10.4	50	15	0	-1.4142	0	72.228	4	3	4344	26	12	50	18	0	0	1.4142	82.223
1	3	4473	28.83	11.5	32.32	9	-1	1	1	87.864	4	3	4539	23.17	9.3	32.32	10	-1	-1	-1	81.826
1	3	4502	26	10.4	50	6	0	0	0	75.431	4	3	4321	26	10.4	50	11	0	0	0	93.574
1	3	4300	26	12	50	18	0	0	1.4142	104.924	4	3	4463	23.17	11.5	67.68	8	1	-1	1	93.534
1	3	4474	28.83	9.3	32.32	3	-1	1	-1	69.479	4	3	4540	26	10.4	75	14	1.4142	0	0	75.129
1	3	4493	26	10.4	50	12	0	0	0	87.558	4	3	4325	23.17	11.5	32.32	4	-1	-1	1	85.149
2	1	4508	28.83	9.3	67.88	7	1	1	-1	73.545	5	1	4527	22	10.4	50	15	0	-1.4142	0	64.615
2	1	4446	28.83	11.5	32.32	9	-1	1	1	72.948	5	1	4549	23.17	9.3	67.68	2	1	-1	-1	68.048
2	1	4326	26	10.4	50	11	0	0	0	81.067	5	1	4537	26	10.4	50	5	0	0	0	90.305
2	1	4411	26	8.8	50	17	0	0	-1.4142	89.582	5	1	4552	26	12	50	18	0	0	1.4142	76.821
2	1	4464	26	10.4	75	14	1.4142	0	0	89.397	5	1	4374	23.17	11.5	67.68	8	1	-1	1	65.162
2	1	4499	26	10.4	50	12	0	0	0	88.917	5	1	4521	26	10.4	50	11	0	0	0	66.542
2	2	4508	23.17	11.5	32.32	4	-1	-1	1	69.422	5	2	4527	28.83	9.3	32.32	3	-1	1	-1	62.415
2	2	4446	26	12	50	18	0	0	1.4142	78.767	5	2	4549	28.83	11.5	32.32	9	-1	1	1	63.495
2	2	4326	28.83	11.5	67.68	1	1	1	1	88.141	5	2	4537	26	10.4	25	13	-1.4142	0	0	88.200
2	2	4411	26	10.4	25	13	-1.4142	0	0	82.710	5	2	4552	23.17	11.5	32.32	4	-1	-1	1	79.635
2	2	4464	26	10.4	50	5	0	0	0	99.223	5	2	4374	26	10.4	50	6	0	0	0	63.705
2	2	4499	22	10.4	50	15	0	-1.4142	0	89.612	5	2	4521	30	10.4	50	16	0	1.4142	0	60.765
2	3	4508	30	10.4	50	16	0	1.4142	0	67.221	5	3	4527	23.17	9.3	32.32	10	-1	-1	-1	69.130
2	3	4446	26	10.4	50	6	0	0	0	73.696	5	3	4549	26	10.4	50	12	0	0	0	70.375
2	3	4326	23.17	9.3	32.32	10	-1	-1	-1	84.917	5	3	4537	28.83	9.3	67.88	7	1	1	-1	90.590
2	3	4411	23.17	9.3	67.68	2	1	-1	-1	101.764	5	3	4552	28.83	11.5	67.68	1	1	1	1	73.740
2	3	4464	28.83	9.3	32.32	3	-1	1	-1	78.558	5	3	4374	26	10.4	75	14	1.4142	0	0	76.755
2	3	4499	23.17	11.5	67.68	8	1	-1	1	89.298	5	3	4521	26	8.8	50	17	0	0	-1.4142	59.885
3	1	4525	23.17	9.3	32.32	10	-1	-1	-1	55.917	6	1	4452	23.17	9.3	67.68	2	1	-1	-1	83.295
3	1	4137	22	10.4	50	15	0	-1.4142	0	66.958	6	1	4571	23.17	11.5	32.32	4	-1	-1	1	76.620
3	1	4426	28.83	9.3	32.32	3	-1	1	-1	87.789	6	1	4349	23.17	11.5	67.68	8	1	-1	1	64.820
3	1	4512	26	12	50	18	0	0	1.4142	72.557	6	1	4562	26	8.8	50	17	0	0	-1.4142	69.875
3	1	4428	26	10.4	50	12	0	0	0	73.633	6	1	4395	26	10.4	50	6	0	0	0	60.700
3	1	4404	28.83	11.5	67.68	1	1	1	1	84.836	6	1	4339	26	10.4	75	14	1.4142	0	0	76.160
3	2	4525	23.17	9.3	67.68	2	1	-1	-1	78.642	6	2	4452	26	10.4	50	12	0	0	0	75.000
3	2	4426	26	10.4	75	14	1.4142	0	0	106.672	6	2	4571	23.17	9.3	32.32	10	-1	-1	-1	86.860
3	2	4512	26	10.4	50	11	0	0	0	87.348	6	2	4349	26	10.4	25	13	-1.4142	0	0	55.165
3	2	4428	30	10.4	50	16	0	1.4142	0	74.816	6	2	4562	28.83	9.3	32.32	3	-1	1	-1	85.255
3	2	4404	26	10.4	50	6	0	0	0	74.967	6	2	4395	26	12	50	18	0	0	1.4142	66.245
3	3	4525	26	10.4	25	13	-1.4142	0	0	75.408	6	2	4339	28.83	9.3	67.88	7	1	1	-1	85.505
3	3	4523	26	10.4	50	5	0	0	0	92.275	6	3	4452	28.83	11.5	32.32	9	-1	1	1	78.331
3	3	4426	28.83	9.3	67.88	7	1	1	-1	100.284	6	3	4571	22	10.4	50	15	0	-1.4142	0	100.981
3	3	4512	23.17	11.5	67.68	8	1	-1	1	98.701	6	3	4349	26	10.4	50	11	0	0	0	76.731
3	3	4428	28.83	11.5	32.32	9	-1	1	1	71.585	6	3	4562	26	10.4	50	5	0	0	0	83.057
3	3	4404	26	8.8	50	17	0	0	-1.4142	74.900	6	3	4395	30	10.4	50	16	0	1.4142	0	71.323
											6	3	4339	28.83	11.5	67.68	1	1	1	1	79.291

Figure A1. Complete data set for total manure output (kg/cow per day). Trt corresponds to the treatment number of Table 3. Starch, MP and alfalfa are expressed as percentages of diet DM; A, S, and P are the coded variables for alfalfa, starch, and MP, respectively. One unit of A corresponds to 17.68% of alfalfa (% of diet DM); 1 unit of S corresponds to 2.83% starch (% of diet DM); and 1 unit of P corresponds to 1.6% of DM (% of diet DM). Treatments are centered (coded values of 0 for A, S, and P) at 50% alfalfa, 26% starch, and 10.4% MP.