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# Advanced row-column designs for animal feed experiments

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## ABSTRACT

Inappropriate statistical designs may misinterpret results of animal feed experiments. Thus complete statistical designs can make animal feed research more appropriate and cost effective. Usually factorial row-column designs are used when the heterogeneity in the experimental material is in two directions and the experimenter is interested in studying the effect of two or more factors simultaneously. Attempts have been to develop the method of construction of balanced nested row column design under factorial setup. Factorial experiments are used in designs when two or more factors have same levels or different levels. The designs that are balanced symmetric factorials nested in blocks are called block designs with nested row-column balanced symmetric factorial experiments. These designs were constructed by using confounding through equation methods.Construction of confounded asymmetrical factorial experiments in row-column settings and efficiency factor of confounded effects was worked out. The design can be used in animal feed experiment with fewer resources by not compromising the test accuracy.

Key words: Animal feed experiment, Asymmetric factorial experiment, Balanced factorial experiments (BFE), Confounding using equation method

Factorial experiments are widely used in agriculture and several other branches of science. In these experiments, there is an output variable, which is dependent on a number of controllable input variables. These input variables are called factors. For each factor have two or more possible settings known as levels. Combinations of the levels of all the factors under consideration are known as treatment combination. If an experimenter wants to study the effect of two or more fertilizers on a crop and there is heterogeneity in two directions in experimental units then more efficient and informative method is to use a nested factorial row column designs (RC) designs. In these designs some of the treatment effects are confounded in rows and some treatment effects are confounded in columns. These effects cannot be estimated if the experiment is conducted in a single RC design. Let us take an experimental situation.

In an animal nutritional experiment, suppose an experimenter is interested to compare four feeds (i.e. oilseed cakes like groundnut cake, cottonseed cake, sunflower cake and soybean meal) each at two levels, then  $2^4$  factorial experiments has to be conducted. But in this experiment, if there is variability of the calves in two directions, say there

Present address: <sup>1</sup>Scientist (prakash289111@gmail.com), <sup>2</sup>Ex-Principal Scientist (klkalra@gmail.com), <sup>4</sup>Scientist (upen4851 @gmail.com), <sup>5</sup>Scientist (mrinmoy4848@gmail.com). <sup>3</sup>PhD Scholar (anirbanmukjiari@gmail.com), <sup>6</sup>Principal Scientist (prakash1964om@gmail.com), Division of Agricultural Extension, ICAR-IARI, New Delhi. are four age groups and there are four weight groups. Then suitale design is Factorial Experiment for Row-Columns.

Suppose an experimenter is interested to compare five feeds and different feed have different levels (i.e. oilseed cakes like groundnut cake, cottonseed cake, soybean meal, dry fodder and green fodder) three factors at 3 levels and two factors at 2 levels, then  $3^3$  and  $2^2$  factorial experiments has to be conducted. But in this experiment, if there is variability of the calves in two directions, say there are four age groups and there are nine weight groups. Then suitable design is nested factorial experiment with Row-Columns.

In this kind of situation the experimental units are classified into b block use for nesting p rows and q columns where  $p=s_1^{n1-m1}$  and  $q=s_2^{n2-m2}$ . Therefore, we have a total of bpq experimental units.

Block designs with nested rows and columns were introduced by Shah (1960); worked on construction of balanced incomplete block designs (BIBD) with nested rows and columns (Agrawal and Prasad 1982); John and Lewis (1983), Ipinyomi and John (1985) worked on factorial experiments in generalized cyclic row-column designs; Cheng (1986) worked on templates for design key construction; Shamsuddin and Agrawal(1989) worked on confounded s<sup>n-L</sup> fractional factorial design under row column setup; Sreenath (1989), Uddin and Morgan (1991), Morgan and Uddin (1993) worked on nested row-column designs; Chang and Notz (1994), Williams and John (1996), Bagchi (1996) worked on optimality of nested row column design; Parsad *et al.* (2001), Cheng and Tsai (2013), Varghese *et al.* (2014), Cheng (2014), Datta *et al.* (2014) studied the nested row-column designs and factorial designs from different angles. Nilson and Ohman (2015) worked on Youden squares designs. Power of optimization of randomization in designed experiments for small samples (Bertsimas *et al.* 2015). Regression-based inferences for  $2^k$  factorial designs were investigated by Dasgupta *et al.* (2015), Lu (2016). But for the estimation of the effects that are confounded in a single RC design requires more number of row-column designs. There is need to construct block design with nested row-column setup for factorial experiments such that, the effects that are confounded in one RC design can be estimated from other RC designs within the block.

### MATERIALS AND METHODS

Construction of block designs with nested row-column set-up confounding through equation method: This method follows different approaches and the designs are in different sizes. The construction of the designs is given in sequel with application in some animal nutritional feed experiments: the confounded procedures had also been given by Choi and Gupta (2008) and Agrawal and Shamsuddin (1987) by solving the equations. In the proposed method, we only need to find the first row and the first column for each sub-square; the remaining treatments of the sub-squares are obtained by adding the treatment combinations in the first row and the first column. Here, more than one factorial row column designs will be constructed such that the effects that have been confounded in the first row-column design can be estimated from the other row-column designs. It will be a situation of partial confounding. The complete set of replicates will be constructed so that the effects that have been partially confounded are balanced.

For the construction of these designs, it is assumed that the number of rows (r) and the number of columns (c) are  $r = s^p$  rows,  $c = s^q$  with  $rc = s^{p+q}$  and n = p+q where s is levels of p and q factors. Let  $RC_1$  be the first row-column design which is nested in block design D for a  $s^n$  factorial experiment and  $A_1, A_2, ..., A_{n-q}$  denote the independent interactions confounded  $s^p$  rows of  $RC_1$ . A total of  $s^{n-q-1}$ treatment degrees of freedom are confounded in  $r = s^p$  rows of  $RC_1$ . The total number of effects confounded between rows, each effect having  $s^{n-q-1}$  degrees of freedom are  $[S^{n-}$ q-1)/(S-1)]. The number of generalized interactions confounded between rows of  $RC_1$  is  $I_1 = [S^{n-q}-1)/(s-1)]-$ (n-q).

The generalized interactions are denoted as

 $A_{n-q+1}, A_{n-q+2}, A_{n-q+1i}$ 

Let  $B_1$ ,  $B_2$ ,..., $B_{n,p}$  denote the independent interactions confounded between  $S^q$  columns of  $RC_1$ . Then, following the procedure as above, the number of generalized interactions confounded with  $s^p$  columns of  $RC_1$  is given by  $I_2 = [(s^{n-p}-1)/S-1] - (n-P)$ . These  $I_2$  generalized interactions will be denoted by  $B_{n-p+1}$ ,  $B_{n-p+2}$ ,...,  $B_{n-p+12}$ . The factorial effects  $A_1$ ,  $A_2$ , ...,  $A_{n-p+1}$ ,  $B_1$ ,  $B_2$  ...,  $B_{n-p+12}$  are chosen such that they all are distinct.

For the general procedure of the construction, first construct the key or the principal block for rows by confounding *n*-*q* independent interactions  $A_1, A_2,..., A_{n-q}$  between rows of  $RC_1$ . Let this row key block be denoted by  $(a_1^i, a_2^i, ..., a_m^i, i = 1, 2,...,s^q)$  where  $a_l^i a_1^i \{0, 1,..., s^{"1}\}$  with  $a_l^1 = 0, l = 1, 2, ..., m$ . The column key block is similarly obtained by confounding *n*"*p* independent interactions  $B_1$ ,  $B_2,...,B_{n-p}$  between columns of  $RC_1$ . Let the column key block be denoted by  $(b_1^i, b_2^i, ..., b_m^i; j = 1, 2,...,s^p)$ , where  $b_l^i a_1^i \{0, 1,..., s^{"1}\}$  with  $b_l^i = 0, l = 1, 2,..., m$ . Then the treatment combination in the  $l_1^{\text{th}}$  row and  $l_2^{\text{th}}$  column of  $RC_1$  is denoted by  $c_1, c_2,..., c_m$ , with  $c_i = a_l^{12} + b_l^{11}, t = 1, 2,..., m$ , where the addition is done on the basis of taking mod(s).

For the sake of brevity, we give the construction of block designs nested in factorial row-column designs with two examples.

Construction of confounded asymmetrical factorial experiments in row-column settings: For the construction of confounded asymmetrical factorial experiment, we start with the factorial experiment with two different levels with *n* factors at level  $s_1$  and *m* factors at level  $s_2$ , we can say,  $s_1^n$  $\times s_2^m$  in row column design. Here  $s_1$  and  $s_2$  both are prime or prime power and  $s_1 \neq s_2$ . In row column factorial design the two levels will be on row and column, respectively. Let  $s_1$  levels are on row and  $s_2$  levels are on column, then for the construction of design,  $(s_1^n - 1)/(s_1 - 1)$  and  $(s_2^p - 1)/(s_2 - 1)$ effects will be confounded in row and column, respectively. Here we obtain in general the rectangular row column designs because  $s_1 \neq s_2$ . The complete confounded structure will also be given. For confounding we use equation method, after getting rows and column we concatenate the corresponding setup of a particular cell with rows elements and column elements. Asymmetric factorial row-column designs constructed by using row as  $s_1^n$ , which is confounded or whole factorial setup is taken at level  $s_1$  and column as  $s_2^m$ , similarly which is confounded or whole factorial setup is taken at level  $s_2$ . Attached both corresponding row and column for each and every cell of the row-column design then we can get our desired designs.

#### **RESULTS AND DISCUSSION**

Consider the following two-way  $(2^4, 2^2)$  design with b = 2, row = 4, column = 4 and v = 16 with factors A, B, C and D each at level 2.

For this design, we construct two blocks as Block 1 and Block 2 using the equation method.

Treatment effects confounded in Block 1 (Table 1)

Row wise = AC, BD and ABCD; Column wise = ABD, ACD

Treatment effects confounded in Block 2 (Table 2)

Row wise = AB, CD & ABCD; Column wise = ABC, BCD & AD

Now design contains these two blocks (Tables 1, 2) is Block 1

Confounded row wise = AC, BD & ABCD;

Confounded column wise = ABD, ACD & BC

С	olumn	key blo	ock							R	ow key	/ block							
				0	0	0	0	1	0	1	0	0	1	0	1	1	1	1	1
0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	1	1	1	1	1
1	1	1	0	1	1	1	0	0	1	0	0	1	0	1	1	0	0	0	1
1	0	0	1	1	0	0	1	0	0	1	1	1	1	0	0	0	1	1	0
0	1	1	1	0	1	1	1	1	1	0	1	0	0	1	0	1	0	0	0

Table 1. Confounded treatments effects in Block 1

Table 2. Confounded treatments effects in Block 2

C	olumn	key blo	ck		Row key block   0 0 0 1 1 0 0 1 1 1 1 1														
				0													1	1	
0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	1	1	1	1
0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	1	1	0	0	1
1	1	0	1	1	1	0	1	0	0	0	1	1	1	1	0	0	0	1	0
1	0	1	1	1	0	1	1	0	1	1	1	1	0	0	0	0	1	0	0

Block 2

Confounded row wise = AB, CD & ABCD;

Confounded column wise = ABC, BCD & AD

The parameters of the design D1 are

 $v = 2^4, b = 2, row = 4, column = 4$ 

Efficiency of the effects confounded

A=B=C=D=1; AB=AC=AD=BC=BD=CD=0.50; ABC = ABD = ACD = BCD = 0.50.

Here highest order effect ABCD is completely confounded. Consider the  $(2^5, 2^3)$  factorial experiment in two-way settings of 8×8 row-column design in five blocks with rows = columns = 8 and  $v = 2^5 = 32$  treatments.

The design contains five blocks Block 1, Block 2, Block 3, Block 4, and Block 5 with effects cofounded is Block 1

Confounded row wise = ABD, ACE, BCDE;

Confounded column wise = ACD, BCE, ABDE Block 2

Confounded row wise = ACD, BCE, ABDE;

Confounded column wise = ABC, BDE, ACDE Block 3

Confounded row wise = ABE, CDE, ABCD;

Confounded column wise = ABC, BDE, ACDE Block 4

Confounded row wise = ABE, CDE, ABCD;

Confounded column wise = ADE, BCD, ABCE Block 5

Confounded row wise = ABD, ACE, BCDE;

Confounded column wise = ADE, BCD, ABCE Parameters of the design D2 are

 $v = 2^5, b = 5, row = 8, column = 8,$ 

A = B = C = D = E = 1

AB = AC = AD = AE = BC = BD = BE = CD = CE = DE

ABC = ABD = ABE = ACD = ACE = ADE = BCD = BCE = BDE = CDE = 0.600

ABCD = ABCE = ABDE = ACDE = BCDE = 0.600,

#### ABCDE = 1.

In Design D2 the efficiency of all main effects and two factors interactions is one. The five blocks of design with ten three-factor interactions and five four-factor interactions are partially confounded. This is an example of balanced symmetric factorial experiment nested in blocks. The effects confounded in a particular block are estimable from other blocks. The efficiency factor for three factor and four factor interactions is 0.60.

Five different feed (A, B, C, D, E) where feeds A, B and C have two level and feeds D and E have three levels  $2^3 \times 3^2 / 2^3 \times 3$ .

Here factor A is at 3 (0, 1, 2) levels and factor B, C and D is at 2 (0, 1) levels. For confounded asymmetrical factorial nested row-column designs we use  $3^1$  factorial structure use as row wise and confounded factorial ( $2^3$ ,  $2^2$ ) structure as a column wise.

Here BCD is confounded then the designs given below are:-

Parameters of the design D2 are

 $v = 2^5, b = 5, row = 8, column = 8,$ 

Efficiency of the effects confounded

A = B = C = D = E = 1

AB = AC = AD = AE = BC = BD = BE = CD = CE = DE= 1

ABC = ABD = ABE = ACD = ACE = ADE = BCD = BCE = BDE = CDE = 0.600

ABCD = ABCE = ABDE = ACDE = BCDE = 0.600,ABCDE = 1.

In Design D2 the efficiency of all main effects and two factors interactions is one. The five blocks of design with ten three-factor interactions and five four-factor interactions are partially confounded. This is an example of balanced symmetric factorial experiment nested in blocks. The effects confounded in a particular block are estimable from other blocks. The efficiency factor for three factor and four factor interactions is 0.60.

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Here BCD is confounded then the designs given below are:-

Five different feed (A, B, C, D, E) where feeds A, B and Table 3. Confounding of factorial experiment  $2^3$ 

В	С	D	BCD
0	0	0	0
1	0	0	1
0	1	0	1
1	1	0	0
0	0	1	1
1	0	1	0
0	1	1	0
1	1	1	1

C have two level and feeds D and E have three levels  $2^3 \times 3^2 / 2^3 \times 3$ .

For confounded asymmetrical factorial nested rowcolumn designs we use  $2^3$  factorial structure use as row wise and confounded factorial ( $3^2$ ,  $3^1$ ) structure as a column wise.

Here DE confounded in  $3^2$  factorial experiments then the designs given below are (Table 3):

Let us consider five different feed (A, B, C, D, E) where feeds A, B and C have two level and feeds D and E have three levels  $2^3 \times 3^2 / 2^3 \times 3$  but (DE<sup>2</sup> confounded).

For confounded asymmetrical factorial nested rowcolumn designs we use  $2^3$  factorial structure use as row wise and confounded factorial ( $3^2$ ,  $3^1$ ) structure as a column wise.

Here  $DE^2$  confounded in  $3^2$  factorial experiments then the designs given below are:-

These designs are useful to experimental situation where the study of two or more different factor at different levels

Table 4. Design 3.1 (Confounded effect is ABC	Table 4.	Design 3	.1 (	Confounded	effect is	ABC)
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		0	0	0		1	1	0		1	0	1		0	1	1
0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	1	1
1	1	0	0	0	1	1	1	0	1	1	0	1	1	0	1	1
2	2	0	0	0	2	1	1	0	2	1	0	1	2	0	1	1

D	E	Confounding=DE
0	0	0
0	1	1
0	2	2
1	0	1
1	1	2
1	2	0
2	0	2
2	1	0
2	2	1

Table 5. Confounded DE from 3<sup>2</sup> factorial experiments

simultaneously, when in experimental material there is two ways elimination of heterogeneity is required.

D	Е	DE <sup>2</sup>
0	0	0
0	1	2
0	2	1
1	0	1
1	1	0
1	2	2
2	0	2
2	1	1
2	2	0

#### Table 6. Design constructed after confounded DE

Row key block $\rightarrow$ Columnkey block $\downarrow$							0				1	2				2	1
0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	2	1
1	0	0	1	0	0	0	0	1	0	0	1	2	1	0	0	2	1
0	1	0	0	1	0	0	0	0	1	0	1	2	0	1	0	2	1
1	1	0	1	1	0	0	0	1	1	0	1	2	1	1	0	2	1
0	0	1	0	0	1	0	0	0	0	1	1	2	0	0	1	2	1
1	0	1	1	0	1	0	0	1	0	1	1	2	1	0	1	2	1
0	1	1	0	1	1	0	0	0	1	1	1	2	0	1	1	2	1
1	1	1	1	1	1	0	0	1	1	1	1	2	1	1	1	2	1

	key blo mnkey					0	0				1	2				2	1
0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2	2
1	0	0	1	0	0	0	0	1	0	0	1	1	1	0	0	2	2
0	1	0	0	1	0	0	0	0	1	0	1	1	0	1	0	2	2
1	1	0	1	1	0	0	0	1	1	0	1	1	1	1	0	2	2
0	0	1	0	0	1	0	0	0	0	1	1	1	0	0	1	2	2
1	0	1	1	0	1	0	0	1	0	1	1	1	1	0	1	2	2
0	1	1	0	1	1	0	0	0	1	1	1	1	0	1	1	2	2
1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	2	2

Table 7. Design constructed after confounded  $DE^2$ 

In animal feed experiments, animals are housed in pan and animals are grouped according to their age and weight considered as experimental material containing two way variations. So, in this condition the suitable designs will be Row-Column design. Here, methods of construction of balanced confounded symmetrical factorial nested row column designs have been obtained. The method of constructing nested design gives larger designs but is variance balanced for estimating all the effects. It is not always possible to conduct the experiment with all the factors at the same level i.e. symmetrical factorial experiments. So, asymmetrical factorial experiments commonly used when levels of all the factors are not same. If equal sample sizes are taken for each of the possible factor combinations then the design is a balanced factorial design. Method of construction of balanced factorial nested row column designs for symmetrical factorials has been developed. But in case of asymmetrical factorial experiments it is partially balanced. For analysis of these designs we can use PROC GLM procedure in SAS.

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