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Efficiency of Combined Analysis in Youden and Cross-Over Designs Under Missing Values: Evaluation of the Optimal Method

Mahmood M Taher
Mahmood81_tahr@uomosul.edu.iq
University of Mosul

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Corresponding Author : Mahmood M Taher

Abstract : This study examines the relationship between several composite Youden square designs and a basic crossover design, assessing how well each performs when data are missing. Based on experimental data, the analysis is divided into three primary steps: (1) a complete crossover design analysis; (2) a random deletion of the fifth row, followed by decomposition into composite Youden squares and the development of an analytical methodology; and (3) the estimating of the missing values using Yates' iterative compensation method, and this is followed by a comparative analysis of the results obtained. The outcomes demonstrate that the composite Youden square approach is more efficient and statistically solid than the crossover design in all scenarios. Also, significant distinctions between direct and indirect methods have been discovered, mostly as a result of the effect of correction factors.

Keywords: experimental design, statistical efficiency crossover design, and Youden squares.

INTRODUCTION: In agricultural and animal science research, where biological variability and financial constraints often constitute substantial challenges, designing successful and credible experiments is critical. Robust statistical designs serve as essential for arriving at honest inferences and advising management practices in livestock nutrition trials, such as those examining the effect of numerous feed formulations on dairy cow productivity. [3][8].

This study examines a practical and frequent problem in field experiments: loss of information from uncontrollable causes including disruptive environments, animal health issues, or logistical obstacles during the trial period.[11][12]. So as to provide statistically successful options for analyzing incomplete experimental data and guarantee accurate interpretation of treatment effects despite the event of data loss, the research will focus on the relationship between the crossover design and a set of composite Youden squares.[1][6].

Study Objectives:

The research project aims to explain how to address the crossover design deficiency caused by the loss of a row (period) by breaking the missing crossover design into a set of Youden's squares. This division suggests a relationship between the crossover design and the set of Youden's squares, and it is evident that a combined analysis is helpful in drawing conclusions. The fixed model between the crossover and Youden square designs, as well as how to combine the Youden squares into a single combined analysis, will be used to explain the results.

Study significance and contributions:

This paper makes a significant addition to the statistical analysis and experimental design fields.This study is important because it addresses the issue of missing data in Cross-Over Designs by converting the incomplete design to Youden squares and statistically analyzing it using two different methods. This makes statistical inferences more accurate and helps researchers identify the best approaches to deal with experimental missing data.

Simple Cross-Over Design (C.O.D)

There may be cases in which it is not possible to use the Latin square design, especially when the number of parameters used in the experiment is small, as the degree of freedom for error will be nonexistent or small. Therefore, resort to using a design that combines the characteristics of the Latin square and the completely randomized block design, and it can be It is used when there are two, three, or four treatments at most. [6]

The length of the period must be sufficient to allow the effect of the treatment to be reflected correctly, and the length of the period is sufficient so that the effect of the previous treatment does not extend to the next period. The rest period may be as short as a few hours or as long as a few weeks, and this depends on the nature of the treatment. The effect of the treatment is called the carry-over effect, or the residual effect, which continues after the treatment itself stops,

and this residual effect of previous treatments, if any, must affect the measurements of the effect of the present treatments. Such an effect may be overcome by choosing the appropriate design or by introducing A rest period between treatments. [11]
In this design, as in the Latin square design, two restrictions are imposed on the random distribution of treatments on the experimental units. They are the application of all treatments to each column (replication), so that all treatments appear in it once, and that each column is a single experimental unit that can be represented as a complete sector to which the used treatments are applied. In the experiment, all of A multiple of the number of treatments, which is also a multiple of the number of periods.[2]

Mathematical Model of C.O.D

The mathematical model for the cross over design experiment is similar to the mathematical model for the Latin square design, which is: (Ratkowsky et al., 2022)

$$y_{ij(k)} = \mu + \rho_i + \gamma_j + \tau_{(k)} + e_{ij(k)} \qquad (1)$$

$i = 1,2, \dots \dots \dots r \ , j = 1,2, \dots \dots \dots \dots . c \ , \ \ k = 1,2, \dots \dots \dots t \ , r = t$

Estimation of Missing Values

As for the values of the missing observations, the following equation can be used to obtain an estimated value for the missing value as follows:[6] Ratkowsky et al., 2022)

$$\hat{y}_{ij)k)} = {rY_{.j} + t\left(Y_{i.} + Y_{(k).}\right) - 2Y} \Big/ {(t-1)(r-2)} \qquad (2)$$

The value of the sum of squares of the treatments can be corrected by applying the following equation

$$SSt^{'} = SSt - C^{'} \qquad (3)$$

$$C^{'} = {[Y_{..} - Y_{i.} - Y_{.j} - (t-1)Y_{(k).}]^2} \Big/ {[(t-1)(t-2)]^2} \qquad (4)$$

Youden Square Design (Y.S.D)

If a row or a column is missing from a Latin square design, the number of treatments remains equal to either the number of rows or the number of columns. The resulting structure is referred to as a Youden square, named after Youden, who made significant contributions to the development of such designs. Youden squares differ from all Latin squares since not all treatments will be allowed by the same blocking factor, normally rows or columns.[5][10] .
When fully randomization is not acceptable, the Youden square design delivers a reliable and effective solution by combining elements of the Balanced Incomplete Block Design (BIBD) and the Latin square design.[1][4].
For example, creatures could be split into four age groups (rows) for an experiment to examine the effects of four dietary treatments on milk production. There may only be three animals of different sizes (columns) available within each age group.. As a result, only three of the four treatments can be assigned within each row, illustrating the incomplete structure typical of a Youden square. Despite the limitation, this design maintains balance and comparability among treatments. Figure 1 illustrates this experimental configuration [12].

ages (classes)	sizes (columns)		
	1	2	3
1	A	B	C
2	D	A	B
3	B	C	D
4	C	D	A

Figure (1): Yoden square design with size (3×4)

Calculate the sum of squares of the weighted treatments SSt(adj)

When analyzing data in a Youden square design, the sum of the squares of the weighted treatments must be calculated by calculating the following amount:-

$$W_i = KY_i - B_i \qquad (5)$$

W_i: The weighted value of the treatment.
K: Sector size (number of treatments within the sector).
Y_i: Total treatment.
B_i: The total number of blocks in which the treatment appears.

After calculating values (W_i) for all treatments, we calculate the total weighted treatments through the following law: [1][12].

$$SS_t(adj) = \sum_{i=1}^{t} W_i^2 \Big/ kt\,\lambda \qquad (6)$$

λ : The number of times each pair of treatments appears together in the blocks and is calculated as follows:[7][10].

$$\lambda = r(k-1) \Big/ (t-1) \qquad (7)$$

Mathematical Model of Youden

The mathematical model for the Youden square design experiment is similar to the mathematical model for the latin square design and the crossover design, which is: [1]

$$y_{ij(k)} = \mu + \rho_i + \gamma_j + \tau_{(k)} + e_{ij(k)} \qquad (8)$$

$i = 1,2, \ldots \ldots \ldots r\ , j = 1,2, \ldots \ldots \ldots \ldots .c\ ,\ k = 1,2, \ldots \ldots \ldots t\ , r = k\ , t = b$

Latin compound squares

The experiment conducted with the Latin square design may be repeated more than once in the same location or different locations. When the number of parameters exceeds four, the degrees of freedom are large enough so that in this case a group of Latin squares is preferred, and the analysis method used differs from what it is in Analysis of a single Latin square is called the combined statistical analysis of a group of latin squares. [7][9].

Mathematical Model of Latin Compound Squares:

In the case of compound Latin designs, the observation value is affected by multiple sources of variation: row effect, column effect, treatment effect, and block (block or location) effect. The mathematical model that represents the observation value in a row (i) and column (j) that took the treatment (k) and located in the box (l) as follows:-[9][10]

$$y_{ij(k)l} = \mu + \rho_{il} + \gamma_{jl} + \tau_{(k)l} + e_{ij(k)l} \qquad (9)$$

$i = 1,2, \ldots \ldots \ldots r\ , j = 1,2, \ldots \ldots \ldots \ldots .c\ ,\ k = 1,2, \ldots \ldots \ldots t\ ,\quad l = 1,2, \ldots \ldots ..s$

ANOVA Table of Latin Compound Squares

This table is used when combining multiple Latin squares in a single analysis. This table includes all relevant sources of variance, their corresponding degrees of freedom (df), sum of squares (SS), mean squares (MS), and P-Value.[10]

Table (1): Analysis of variance for the design a group of Latin squares

Source of Variation	Degrees of Freedom	Mean square
Between Squares	(s-1)	$SS_{(s)} = \sum_{l=1}^{s} Y_{..l}^2 / rc - CF$
Rows/ Squares	s(r-1)	$SS(r/S) = \sum_{i=1}^{r}\sum_{l=1}^{s} Y_{i.l}^2 / c - \sum_{l=1}^{s} Y_{..l}^2 / rc$
Colums/Squares	s(c-1)	$SS(C/S) = \sum_{j=1}^{c}\sum_{l=1}^{s} Y_{.jl}^2 / r - \sum_{l=1}^{s} Y_{..l}^2 / rc$
Treat/Squares	s(t-1)	$SS(t/S) = \sum_{k=1}^{t}\sum_{l=1}^{s} Y_{(k).l}^2 / r - \sum_{l=1}^{s} Y_{..l}^2 / rc$
Treat	(t-1)	$SS(t) = \sum_{k=1}^{t} Y_{(k)..}^2 / rs - CF$
Treat×squ	(t-1)(s-1)	$SS(t \times S) = \sum_{k=1}^{t}\sum_{l=1}^{s} Y_{(k).l}^2 / r - \sum_{i=1}^{k} Y_{(k)..}^2 / rs - \sum_{l=1}^{s} Y_{..l}^2 / rc + CF$
Error	s(r-1)(c-2)	$SSe = SST - SS_{(s)} - SS(R/S) - SS(C/S) - SS(t/S)$
Total	rcs-1	$SST = \sum_{i=1}^{r}\sum_{j=1}^{c}\sum_{l=1}^{s} Y_{ij(k)l}^2 - CF$

Analysis of Squares Individually:

To explore the variations in treatment within one specific Latin square, it is frequently helpful to perform a separate analysis of each square. utilizing this technique, researchers might determine whether the effects of a treatment are constant or vary depending on the setting, location, or replication. Before conducting a combined analysis, examining each square independently will help in identifying possible sources of heterogeneity, as follows:- [10]

1. The sum of squares between rows within squares.

$$SS(r/S) = SSr(s_1) + SSr(s_2) + \cdots \ldots\ldots + SSr(s_s) \quad (10)$$

2. The sum of squares between columns within squares.

$$SS(c/S) = SSc(s_1) + SSc(s_2) + \cdots \ldots\ldots + SSc(s_s) \quad (11)$$

3. The sum of squares between the treatments within the squares.

$$SS(t/S) = SSt(s_1) + SSt(s_2) + \cdots \ldots\ldots + SSt(s_s) \quad (12)$$

4. Sum of squares between (treatments x squares).

$$SS(t \times s) = SS(t/S) - SSt \quad (13)$$

5. Sum of squares experimental error (residuals).

$$SS(e) = SSe(s_1) + SSe(s_2) + \cdots \ldots\ldots + SSe(s_s) \quad (14)$$

6. Total sum of squares.

$$SST = [SST(s_1) + SST(s_2) + \cdots \ldots\ldots + SST(s_s)] + SS(s) \quad (15)$$

The following diagram describes the general analysis of the research.

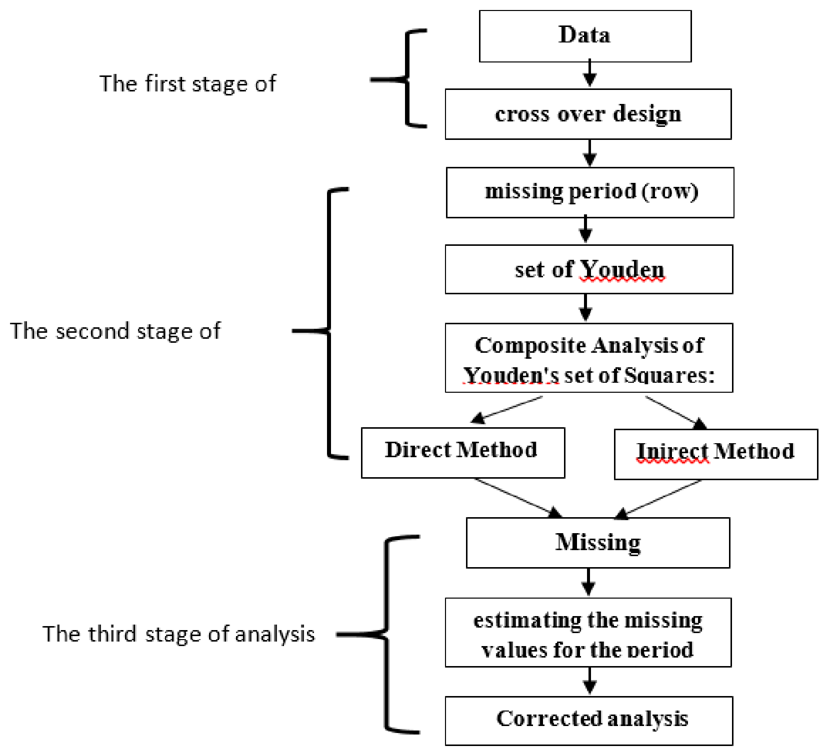

Figure 2: General Scheme for Research

Application:

Using multiple design approaches to analyze experimental data was the focus of the study's applied component. As an outcome, the analysis was organized into three primary stages. Analyzing the entire crossover (transit) design was the first stage. The analysis of data with a single missing period (i.e., a missing row) was the primary focus in the second phase. The third phase involved estimating the missing values identified in the second phase. To evaluate and compare the performance of the different analyses, several statistical measures were employed, including the mean square error (MSE), the mean square for treatments (MST), and the P-value for treatment effects.

The experiment data included five diets (treatments) to study the effect of substituting silage with equivalent nutritional value for dairy cow feed. The experiment data were analyzed using the SAS program, and the following table shows the milk production rate as a result of applying each treatment.

Table (2): Milk production rate as a result of applying each treatment

The first axis: analysis of experimental data in cross over design

B 8.35	C 9.49	C 16.33	D 12.08	E 9.48	E 5.53	B 10.89	A 16.73	C 13.60	D 8.47	E 7.64	A 12.43	D 9.68	A 15.72	B 16.79
A 18.74	B 10.50	B 15.15	C 9.64	D 7.28	D 8.81	A 13.16	E 7.74	B 16.00	C 7.48	D 8.94	E 7.14	C 12.10	E 8.93	A 12.35
D 8.02	E 8.90	E 9.96	A 19.44	B 15.85	B 17.10	D 9.57	C 11.64	E 12.10	A 16.15	B 14.49	C 13.90	A 15.31	C 11.77	D 10.51
E 10.26	A 13.68	A 20.72	B 16.65	C 13.49	C 13.35	E 9.96	D 13.28	A 19.57	B 10.61	C 14.70	D 15.01	B 16.18	D 12.58	E 12.58
C 16.07	D 8.41	D 13.68	E 8.30	A 10.61	A 18.53	C 9.49	B 16.49	D 12.82	E 9.09	A 12.51	B 11.20	E 7.66	B 11.47	C 14.95

Since the design of the experiment is a crossover design and consists of (5) periods (rows) and (15) repetitions (columns), therefore, the data in Table (2) will be analyzed based on (S.O.V) table for the cross-over design. After (S.O.V), the results of the analysis are placed in the table. (3) as follows:-

Table (3): Analysis of variance for the complete crossover design

S.O.V.	D.F.	S.S.	M.S.	p-v
Treatment	4	415.487	103.872	$^{**}9.58 \times 10^{-11}$
Periods	4	96.837	24.209	**0.00137
Replicates	14	150.655	10.761	**0.0151
Error	52	242.870	4.671	
Total	74	905.850		

The second axis: missing period (row) of experiment data

The incomplete experiment data referred to in Table (4) were analyzed due to the loss of the fifth period (row) among the experiment periods in Table 2). The result is a data table consisting of (4) periods (rows) and (15) (column) as follows:-

Table (4): crossover design data resulting from the loss of the fifth period

B 8.35	**C 9.49**	**C 16.33**	**D 12.08**	**E 9.48**	**E 5.53**	**B 10.89**	**A 16.73**	**C 13.60**	**D 8.47**	**E 7.64**	**A 12.43**	**D 9.68**	**A 15.72**	**B 16.79**
A 18.74	**B 10.50**	**B 15.15**	**C 9.64**	**D 7.28**	**D 8.81**	**A 13.16**	**E 7.74**	**B 16.00**	**C 7.48**	**D 8.94**	**E 7.14**	**C 12.10**	**E 8.93**	**A 12.35**
D 8.02	**E 8.90**	**E 9.96**	**A 19.44**	**B 15.85**	**B 17.10**	**D 9.57**	**C 11.64**	**E 12.10**	**A 16.15**	**B 14.49**	**C 13.90**	**A 15.31**	**C 11.77**	**D 10.51**
E 10.26	**A 13.68**	**A 20.72**	**B 16.65**	**C 13.49**	**C 13.35**	**E 9.96**	**D 13.28**	**A 19.57**	**B 10.61**	**C 14.70**	**D 15.01**	**B 16.18**	**D 12.58**	**E 12.58**

Segmentation of the cross over design into a set of Youden Squares.

When dividing the data for the incomplete cross over design referred to in Table (4), three incomplete latin squares are produced, the size of each square being (5x4) diagram (3), since each of these squares is missing the fifth row, and the result is called the Youden square, as The columns represent the missing sectors, and such squares have their own design and analysis, which has a clear analytical relationship to balanced incomplete block design (BIBD). The statistical analysis of each square will depend on the application of the Youden square analysis of variance table.

Youden Square (1)

B 10.89	C 13.60	D 9.68	A 16.73	E 7.64
A 13.16	B 16.00	C 12.10	E 7.74	D 8.94
D 9.57	E 12.10	A 15.31	C 11.64	B 14.49
E 9.96	A 19.57	B 16.18	D 13.28	C 14.70

Youden Square (2)

C 9.49	E 9.48	D 8.47	A 15.72	B 8.35
B 10.50	D 7.28	C 7.48	E 8.93	A 18.74
E 8.90	B 15.85	A 16.15	C 11.77	D 8.02
A 13.68	C 13.49	B 10.61	D 12.58	E 10.26

Youden Square (3)

C 16.33	A 12.43	B 16.79	D 12.08	E 5.53
B 15.15	E 7.14	A 12.35	C 9.64	D 8.81
E 9.96	C 13.90	D 10.51	A 19.44	B 17.10
A 20.72	D 15.01	E 12.58	B 16.65	C 13.35

Figure (3) missing Latin squares data (Youden squares) resulting from segmentation of the data in table (4)

Composite Analysis of Youden's set of Squares:
The results were analyzed based on the combined statistical analysis of the Youden square set shown in the figure (3) by relying on: -

Direct Method
Based on the formulas in Table (1), the variance analysis table will be as follows:

Table (5): Analysis of variance for the Youden square set design
using the direct method

S.O.V	D.F	S.S	M.S	p.v
Squares	2	40.303	20.1515	0.0031**
Rows./ Squares	9	115.266	12.807	0.0013**
Columns./Squares	12	107.711	8.975	0.0086**
Treatments./squares	12	419.385	34.948	1.47×10^{-7}**
Treatments.	4	377.272	94.318	1.59×10^{-9}**
Treatments×squares.	8	43.012	5.3765	0.1034
Error.	24	67.177	2.7990	
Total.	59	749.842		

Indirect Method
Based on equations (10,11,12,13,14,15), the variance analysis table will be as follows:

Table (6): Analysis of variance for the Youden square set design
by the indirect method

S.O.V.	D.F.	S.S.	M.S.	p.v
Squares	2	40.303	20.1515	0.0132**
Rows./ Squares	9	115.266	12.807	0.007**
Columns./Squares	12	107.711	8.975	0.035**
Treatments./squares	12	392.820	32.735	1.6×10^{-6}**
Treatments	4	377.272	94.318	4.1×10^{-8}**
Treatments×squares	8	16.430	2.0537	0.8252
Error	24	93.742	3.9059	
Total	59	749.842		

The Third Axis: Estimating the Values of Missing Observations
In this paragraph, the values of the missing observations were estimated by using Equation (2) to estimate the values of the missing observations for the crossover design, by following the Yates method of successive compensation and

two cycles. After estimating the values of the missing observations, the estimated values are put in place as in Table (4), and then the Statistical analysis of the data, as usual, will depend on applying the formulas of (S.O.V) table for the cross-over design, noting the decrease of one degree of freedom from each of the degrees of freedom of the experimental error and the total degrees of freedom for each estimated value, so that the number of degrees of freedom(D.F) that must be subtracted is (15) as follows:

Table (7) Cross over design data after estimating the missing values for the fifth period from Table (5)

B 8.35	C 9.49	C 16.33	D 12.08	E 9.48	E 5.53	B 10.89	A 16.73	C 13.60	D 8.47	E 7.64	A 12.43	D 9.68	A 15.72	B 16.79
A 18.74	B 10.50	B 15.15	C 9.64	D 7.28	D 8.81	A 13.16	E 7.74	B 16.00	C 7.48	D 8.94	E 7.14	C 12.10	E 8.93	A 12.35
D 8.02	E 8.90	E 9.96	A 19.44	B 15.85	B 17.10	D 9.57	C 11.64	E 12.10	A 16.15	B 14.49	C 13.90	A 15.31	C 11.77	D 10.51
E 10.26	A 13.68	A 20.72	B 16.65	C 13.49	C 13.35	E 9.96	D 13.28	A 19.57	B 10.61	C 14.70	D 15.01	B 16.18	D 12.58	E 12.58
C 10.83	D 8.25	D 13.19	E 10.19	A 16.09	A 15.75	C 10.4	B 14.35	D 12.93	E 6.4	A 15.99	B 14.13	E 9.04	B 14.26	C 12.57

Table (8): Analysis of variance for the cross over design after estimating the missing values for the period

S.O.V.	D.F.	S.S.	M.S.	p-v
Treatment	4	469.858	117.465	$**1.1 \times 10^{-10}$
Periods	4	95.598	23.810	**0.0012
Replicates	14	152.759	10.911	**0.0108
Error	37	156.789	4.238	
Total	59	875.004		

It is noted from the variance analysis table (8) that the sum of the squares of the treatments has an upward error. To correct this error, the sum of the squares of the treatments is corrected by calculating the correction value for each estimated value by using equations (4) and (3).

The final variance analysis table after making this correction is as follows:

Table (9): Corrected analysis of variance for crossover design

S.O.V.	D.F.	S.S.	M.S.	p-v
Treatment	4	367.433	91.858	$**2.58 \times 10^{-9}$
Periods	4	95.749	23.937	**0.00118
Replicates	14	153.446	10.960	**0.0101
Error	37	155.767	4.210	
Total	59	772.395		

Compare results:

1. Comparison of Combined statistical analysis of Youden squares with the direct method and the indirect method:

From Tables (5) and (6), it is noted that the differences are in values $(SS(t/s), SS(t \times s), SSe)$ and that the value of the apparent difference between these sums is equal to (26.56) and represents the correction value. The reason for the appearance of this difference represented by the correction value is that if the grouping is used incorrectly, the direct basis will be on the values of the sum of squares of the corrected (SSt) treatments.

It is observed that the p-values obtained through the direct method are significantly lower than those derived from the indirect method. This indicates that the direct method yields more precise and efficient results, as smaller p-values reflect stronger statistical evidence and greater experimental accuracy,as shows The diagram (3) that.

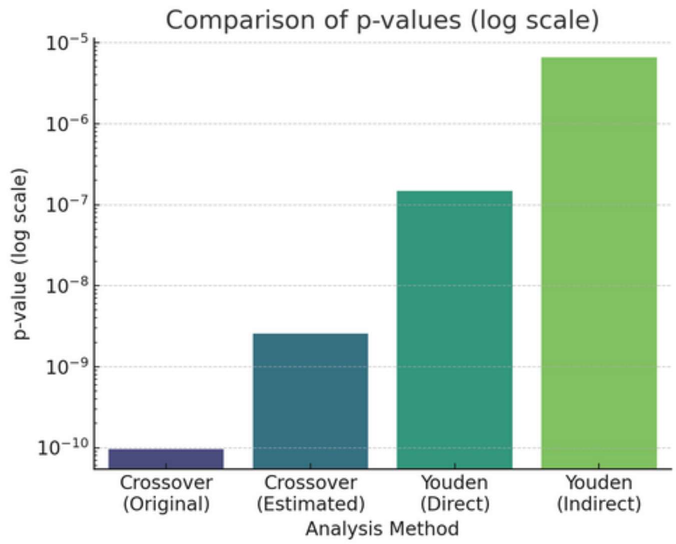

Figure 3:Comparison of p-values

It shows that the direct method (Youden Direct) gives the lowest p-value after the original method of the crossover design, indicating high statistical significance power.

2.A comparison between the crossover design after estimating missing values and the composite statistical analysis of Youden's group of squares using the direct method:

From Tables (5) and (9), it is evident that (D.F) for the error term in the intercept design after estimating the missing values are greater than those in the Youden square set analyzed using the direct method. Moreover, the p-value for the treatments in the Youden square set appears to be more precise compared to that of the intercept design following the estimation of missing values. Additionally, the sum of squares for treatments in the Youden square set is lower than that observed in the intercept design after missing value estimation,The following Figure illustrates this.

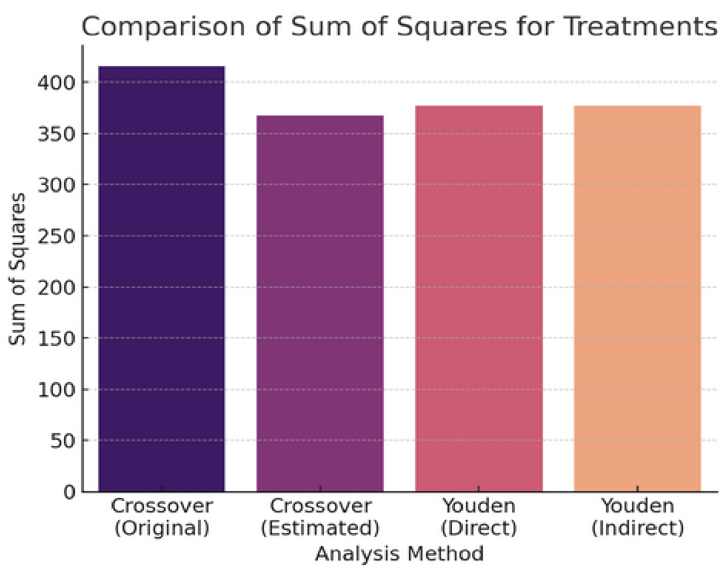

Figure 4: Comparison (SS_t)

It is shown that the crossover estimation method produced a lower sum of squares than the original method, while the Youden values were constant and lower than the original method but slightly higher than the estimation method.

3.Comparison between the crossover design after estimating missing values and the composite statistical analysis of Youden's group of squares using the indirect method:

From Tables (6) and (9), we note that the degree of freedom for error for the intercept design after estimating the missing values is greater than it is for the Youden squares group using the indirect method, and that the calculated p-value for the significance of the treatments for the Youden squares group is less accurate than the intercept design after estimating the missing values, and that the value of the Youden squares sum is less than the value of the intercept design after estimating the missing values,The following Figure illustrates this.

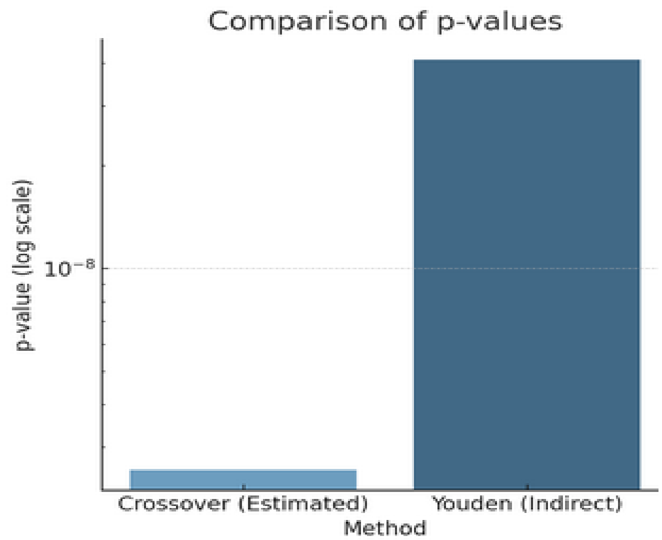

Figure 5: Comparison of p-values(**after estimating missing values)**

The Figure (5) shows the p-values for the indirect Youden design, which yielded a larger p-value, indicating weaker statistical significance compared to the crossover design.

Conclusions:

When a period is missing from a simple crossover design, the most appropriate solution is to decompose the incomplete design into a set of Youden squares and conduct a combined statistical analysis on these components.The direct and indirect methods applied to the composite analysis of Youden squares in the present scenario produce different results, and the primary reason for the variation in the values (SS(t⁄S),SS(t s),SSe) is the adjustment factors that are used to explicate the observed differences in values.Nonetheless, outcomes are largely comparable when juxtaposing the composite analysis of Youden squares with a full crossover design, meaning that the statistical inference approaches are similar.The correction is a contributing factor to why the direct method of composite analysis for Youden squares operates more efficiently than the indirect method.

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