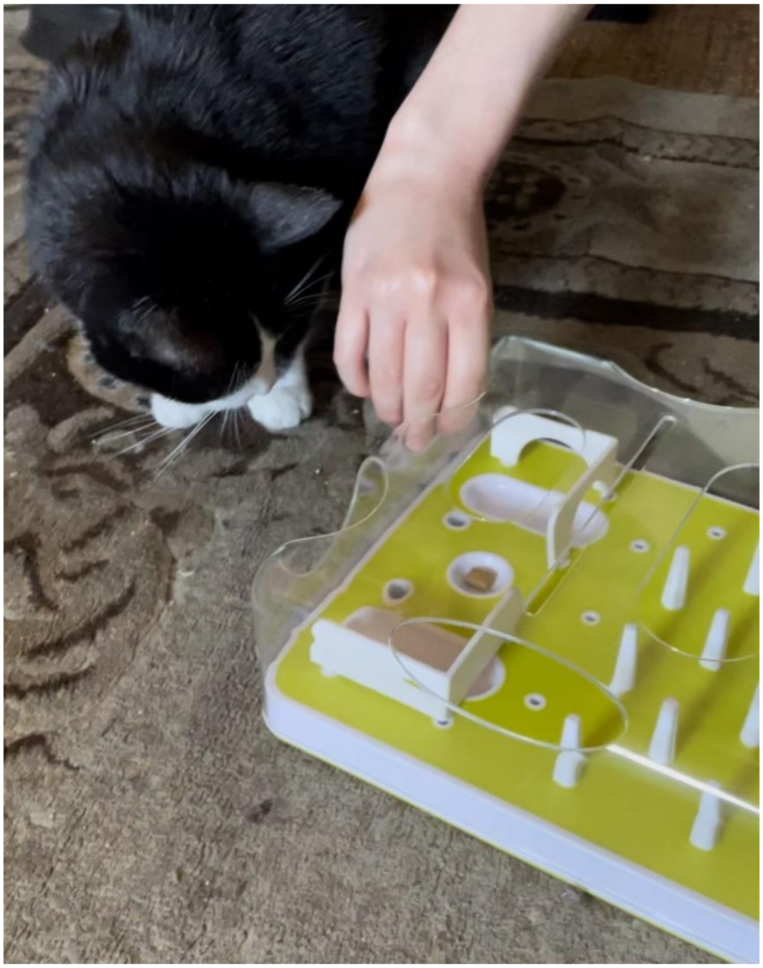
STAT 530 Experimental Design and Analysis

Dr. Zhou

Effects of Treat Flavors on Feline Puzzle-Solving Speed



Created By

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Abstract

This study investigates how different treat flavors affect the cognitive performance of a senior domestic male tuxedo cat engaging in feeding puzzles. The time required for the cat to consume treats of seafood, chicken, dairy, and tuna flavors while completing puzzles was measured. Using a 4 x 4 replicated Latin Square design, we analyzed the time taken to discern if flavor preferences influenced the cat's puzzle-solving efficiency. Pre-planned contrasts were also explored to address specific questions regarding treatment means. The findings indicate no significant impact of treat flavor on the time to solve puzzles. However, the level of puzzle difficulty was a notable factor affecting performance. These results offer valuable considerations for future studies on feline behavior and puzzle interaction.

Introduction

The use of puzzle feeders allows cat owners to control the eating speed of their cats and provide a challenging and engaging experience for their cats. Readily available treats in large quantities can negatively impact a cat's digestion, resulting in regurgitation or an upset stomach. The curiosity and ingenuity of cats, along with their insatiable appetite for savory treats, encourages them to solve the puzzle feeders.

The puzzle feeder used in the study is the Interactive Cat Puzzle Feeder created by the company ALL FOR PAWS. The puzzle feeder consists of a transparent cover with holes, small vertical pegs, four medium-sized L-shaped pegs, and two large dividers with openings that increase the difficulty of the puzzle.

The puzzle design used in the study is as follows. The use of the puzzle feeder without any dividers is defined as an easy puzzle, the use of one divider on the left side is defined as a medium puzzle, and the use of two dividers (one on the left and another on the right) is defined as a hard puzzle.

Four easy puzzles, four medium puzzles, and four hard puzzles were used in this experiment. All puzzles, regardless of difficulty, used the four larger L-shaped pegs which stayed fixed in four corners of the feeder. Examples of four puzzle configurations are displayed in Images 1 through 4 in the Appendix. These four configurations are slight variations of an easy puzzle. The four larger L-shaped pegs stay fixed in Image 1 and 4, then slightly change their position for Image 2 and 3. Likewise, the small pegs stay fixed in four corners on the top and bottom edges of the feeder in Image 1 and 2, but then shift to surround the center of the feeder in Image 3 and 4. The addition of one divider with one opening (Image 5, right side) occupying a length of three pegs on the left side is used for the medium puzzles. The addition of both dividers in Image 5, with the second divider with two openings occupying a length of three pegs on the right side of the feeder is used for the hard puzzles.

Two modifications were made to assist the cat in solving the puzzle. First, the number of small vertical pegs were restricted such that only four pegs were used in the center of the feeder and one small peg was used on the right side of the feeder. One limitation of the study arose from the inability to utilize the transparent cover due to its hindrance on the cat's ability to be engaged while solving the puzzle. Having the cover removed throughout the experiment prevented the cat from being too hesitant to eat the treats.

We are interested in investigating whether the choice of treat flavor significantly influences a cat's ability to solve feeding puzzles. The allure of a particular flavor might motivate the cat to approach a puzzle with greater enthusiasm, potentially leading to faster puzzle-solving times. Our study employs a 4 x 4 replicated Latin Square design to assess the effects of four distinct treat flavors—seafood, chicken, dairy, and tuna—on the time it takes for this cat to complete various feeding puzzles. We analyze the relationship between treat flavors and feline puzzle-solving speed.

A preference for the chicken flavor over the dairy flavor was observed in prior observations of this cat's eating habits. Observations hinted at a potential preference between the two fish flavors, tuna and seafood. Moreover, distinctions between fish-flavored and non-fish-flavored treats, as well as between dairy and non-dairy treats prompted the following research questions:

1. Does the dairy treatment mean differ significantly from the chicken treatment mean?
2. Does the tuna treatment mean differ significantly from the seafood treatment mean?
3. Is there a significant difference between the fish-favored means and non-fish flavored means?
4. Is there a significant difference between the dairy mean and non-dairy means?

We investigate these questions in our study to gain a deeper understanding of the cat's treat preferences.

Experimental Factors:

Treatment Factor: The experiment's treatment factor consists of four distinct treat flavors: tuna (A), seafood (B), dairy (C), and chicken (D). These flavors were methodically presented to the cat to evaluate their impact on puzzle-solving efficiency. The primary response variable is the duration, measured in seconds, that the cat takes to consume the flavored treats. Time measurements were meticulously recorded using the Clock app on an iPhone. The units of observation in this study were individual feeding trials, with a total of 32 units observed across the different puzzle configurations.

Blocking Factor 1 (Row): The first blocking factor is the puzzle configuration. Twelve distinct puzzle configurations are used, ensuring that the experimental conditions vary for each replicate in the row.

Blocking Factor 2 (Column): The second blocking factor consists of four different days. The recording time for each treatment flavor remains consistent across these four days. The four days are: Friday, December 1, 2023, Saturday, December 2, 2023, Sunday, December 3, 2023, and Monday, December 4, 2023.

Replicates: Three replicates represent variations in puzzle difficulty. These replicates encompass easy, medium, and hard versions of the feeding puzzles as defined in the introduction of this study.

Methods

Limitations and Assumptions:

The study assumes that the error terms are normally distributed and that all observations are independent of one another. It is important to note that the exclusion of the transparent cover due to the cat's difficulty may limit the generalizability of the results to situations where no visual barriers are present in puzzle feeders. In other words, it is difficult to generalize a normally distributed error per observation made.

Model Configuration:

The linear model $p\ x\ p$ replicated Latin Square:

$$y_{ijkl} = \mu + \rho_{i(l)} + \tau_j + \beta_k + \delta_l + \epsilon_{ijkl}$$

where

$\mu = the\ grand\ mean$

$i = 1, 2, \ldots, 12\ (row = puzzle\ configuration)$

$j = 1, 2, 3, 4\ \big(j = d(i,k)\big)\ (treatment)$

$k = 1, 2, 3, 4\ (column = day)$

$l = 1, 2, 3$ *(rep = puzzle difficulty)*

$\epsilon_{ijkl} \sim iid\ N(0, \sigma^2)\ (error\ term)$.

Three constraints are necessary to obtain estimates for the parameters in the linear model:

$$\sum_{i=1}^{np=12} \alpha_i = 0\ , \sum_{i=1}^{p=4} \beta_j = 0\ ,\ \ \sum_{i=1}^{p=4} \tau_k = 0$$

The method of contrasts is utilized to analyze comparisons between treatments. Let $C = \sum_{i=1}^{a} c_i\, \mu_i$, where $\sum_{i=1}^{a} c_i = 0$ is a contrast and so the unbiased estimator of the contrast is:

$$C_{est.} = \sum_{i=1}^{a} \left(c_i \cdot \overline{y}_{i\ldots}\right).$$

Therefore, the following contrasts for the four research questions are:

- Dairy vs. Chicken:

$$c_1 = \mu_3 - \mu_4$$

- Tuna vs. Seafood:

$$c_2 = \mu_1 - \mu_2$$

- Fish-flavor vs. Non-fish flavor:

$$c_3 = \frac{1}{2}(\mu_1 + \mu_2) - \frac{1}{2}(\mu_3 + \mu_4)$$

- Dairy vs. Non-dairy:

$$c_4 = \mu_3 - \frac{1}{3}(\mu_1 + \mu_2 + \mu_4) \; .$$

The point estimates for the four contrasts above are:

$$c_{1(est.)} = \bar{y}_{1\ldots} - \bar{y}_{4\ldots}$$

$$c_{2(est.)} = \bar{y}_{1\ldots} - \bar{y}_{2\ldots}$$

$$c_{3(est.)} = \frac{1}{2}(\bar{y}_{1\ldots} + \bar{y}_{2\ldots}) - \frac{1}{2}(\bar{y}_{3\ldots} + \bar{y}_{4\ldots})$$

, and

$$c_{4(est.)} = \bar{y}_{3\ldots} - \frac{1}{3}(\bar{y}_{1\ldots} + \bar{y}_{2\ldots} + \bar{y}_{4\ldots}).$$

Results

I. Data Processing

The data processing stage involved preparing the standard Latin Square for randomization. The data processing was conducted with R. The $p \; x \; p$ square with p representing the number of treatments was initially arranged in alphabetical order for both rows and columns. This setup served as the basis for subsequent randomization processes. Each treatment appeared only once in each row and column.

The randomization process of the Latin Square is as follows:

1. A standard square was randomly selected for the number of treatments. A standard square is defined as a Latin Square with treatments assigned to the first row and the first column in an alphabetical sequence.

2. The order of the rows was randomized by selecting a random permutation.

3. The order of the columns was randomized by selecting a random permutation.

4. The random permutations to the row and column headers of the standard square were assigned and the rows and columns were ordered according to the headers.

5. The treatments were randomized to the letters. In our randomization for this study, Tuna was assigned to treatment A, Seafood was assigned to treatment B, Dairy was assigned to Treatment C, and Chicken was assigned to Treatment D, as shown in Table 4 in the Appendix.

The first four randomization steps were repeated twice to obtain three Latin Squares, one for each replicate. The R code used to generate these Latin Squares is referenced in R Code Sections I and II in the Appendix.

II. Model Results

SAS code was used to produce the following tables for the pre-planned contrasts and the ANOVA table.

Table 1 - Planned Contrasts ANOVA

Contrast Name	df	Contrast SS	MS	F	p-value
Dairy vs. Chicken	1	154.40	28.17	0.35	0.5588
Tuna vs. Seafood	1	246.56	126.04	1.56	0.2208
Fish-flavor vs. Non-fish flavor	1	101.56	0.19	0.00	0.9618
Dairy vs. Non-dairy	1	1508.67	16.67	0.21	0.6525

Table 2 – Replicated Latin Square ANOVA

Source	df	SS	MS	F	p-value
Treatment (Treat Flavors)	3	154.40	51.47	0.64	0.5691
Rows (Puzzle Configuration)	9	246.56	27.40	0.34	0.9540
Columns (Days)	3	101.56	33.85	0.42	0.7399
Replicates (Puzzle Difficulty)	2	1508.67	754.33	9.36	0.0007
Error	30	2417.79	80.59		
Total	47	4428.98			

III. Inference Section

The following hypotheses are of interest for the treatment means:

$$H_0\text{: } \mu_1 = \mu_2 = \mu_3 = \mu_4$$

$$H_1\text{: } at\ least\ one\ \mu_i \neq \mu_j$$

and following hypothesis is of interest for the planned contrasts:

$$H_0\text{: } C = 0$$

$$H_1\text{: } C \neq 0$$

Setting the significance level at α = 0.05, the p-values for the ANOVA analysis, apart from the Replicates factor, exceed this threshold. This indicates that there is no statistically significant evidence at the α = 0.05 level to support the statement that treat flavors, days, or puzzle configurations significantly influence the cat's time to solve puzzles. Conversely, the statistically significant p-value for Replicates suggests that puzzle difficulty does have a significant effect on the cat's performance time in solving puzzles.

For the planned contrast p-values, none of the p-values for the four contrasts are statistically significant at the $\alpha = 0.05$ level. Although none are statistically significant, the p-value for $c_{3(est.)}$is the highest (0.9618) and the p-value for $c_{2(est.)}$(0.2208) is the lowest.

Since the Treatment p-value of 0.5691 is high and is not statistically significant, no post-hoc analyses are conducted.

The 95% percent confidence interval on the i^{th} treatment mean μ_i is:

$$\overline{y_{i\ldots}} - t_{0.025,30} \cdot \sqrt{\frac{MS_E}{n}} \leq \mu_i \leq \overline{y_{i\ldots}} + t_{0.025,30} \cdot \sqrt{\frac{MS_E}{n}}$$

Table 3 – Treatment Mean Confidence Intervals

Treatment Name	Treatment Sample Mean	Lower Bound	Upper Bound
Tuna	13.250	-151.343	177.843
Seafood	12.167	-152.426	176.760
Dairy	10.000	-154.593	174.593
Chicken	8.667	-155.926	173.260

We can assert with 95% confidence that the actual mean times for each treatment are encompassed within the range specified by the confidence intervals presented in Table 3.

The 95% percent confidence interval on the i^{th} treatment mean contrast is:

$$C_{i(est.)} - t_{0.025,30} \cdot s\{C_{i(est.)}\} \leq C_i \leq C_{i(est.)} + t_{0.025,30} \cdot s\{C_{i(est.)}\}$$

$$s\{C_{est.}\} = \sqrt{MS_E \cdot \frac{\sum_{i=1}^{a} c_{i(est.)}^2}{n}}$$

Table 4 – Contrast Confidence Intervals

Contrast Name	Contrast Estimate	Lower Bound	Upper Bound
Dairy vs. Chicken	-0.25	0.009	-0.509
Tuna vs. Seafood	-2.17	-0.011	-4.329
Fish-flavor vs. Non-fish flavor	4.58	0.005	9.155
Dairy vs. Non-dairy	4.08	2.289	5.871

We are 95% confident that the actual values of the contrasts fall within the intervals outlined in Table 4.

Conclusion

The outcomes of this study were notably unexpected. The Latin Square ANOVA and the Contrasts ANOVA both revealed that there was no significant preference for any specific treat flavor by the cat. Interestingly, it appeared that the cat's hesitation during the task was more influenced by the difficulty in accessing the treat rather than its flavor.

Future studies might benefit from an extended training period for the cat with the puzzle feeder, incorporating the transparent cover gradually. This could potentially increase the time required for the cat to solve the puzzles, offering deeper insights into its learning curve and puzzle-solving strategies. Further data collection and analysis, possibly through regression models, could then predict the cat's performance more accurately.

There are two other replicated Latin Square designs that could be used with a similar setup in this study. In Version 1, the puzzle configurations could be restricted to only four types for each replicate and the difficulty could be limited to one among easy, medium, or hard. In Version 3, the experiment would need to be conducted for 12 days rather than only conducting the experiment across 4 days as in this study.

Alternative experimental designs could also be explored. One option could involve a factorial design where elevation of the puzzle feeder could be introduced with two levels. Additionally, the presence of catnip with two levels could make the experiment more interesting since it may alter the cat's behavior, possibly making him more erratic or frantic to eat the treats. Investigating behavioral differences across various cat demographics, such as age groups and gender, could provide additional valuable insights.

Appendix

Table 1 - Latin Square for Replicate 1 (Easy Puzzles)

	Day			
Puzzle Configuration	1	2	3	4
1	D=3	A=6	B=3	C=16
2	A=6	B=5	C=3	D=12
3	B=7	C=7	D=18	A=5
4	C=4	D=7	A=7	B=10

Table 2 - Latin Square for Replicate 2 (Medium Puzzles)

	Day			
Puzzle Configuration	1	2	3	4
5	D=5	A=6	C=4	B=6
6	B=12	C=4	D=6	A=6
7	C=10	B=6	A=21	D=5
8	A=5	D=4	B=4	C=3

Table 3 - Latin Square for Replicate 3 (Hard Puzzles)

	Day			
Puzzle Configuration	1	2	3	4
9	A=6	B=25	D=5	C=27
10	C=12	A=52	B=5	D=24
11	D=31	C=7	A=20	B=10
12	B=11	D=26	C=23	A=19

Table 4 – Randomly Assigned Treatments to Latin Letters

Letter	Treatment
A	Tuna
B	Seafood
C	Dairy
D	Chicken

Image 1 - Puzzle Configuration – Easy, Row 1

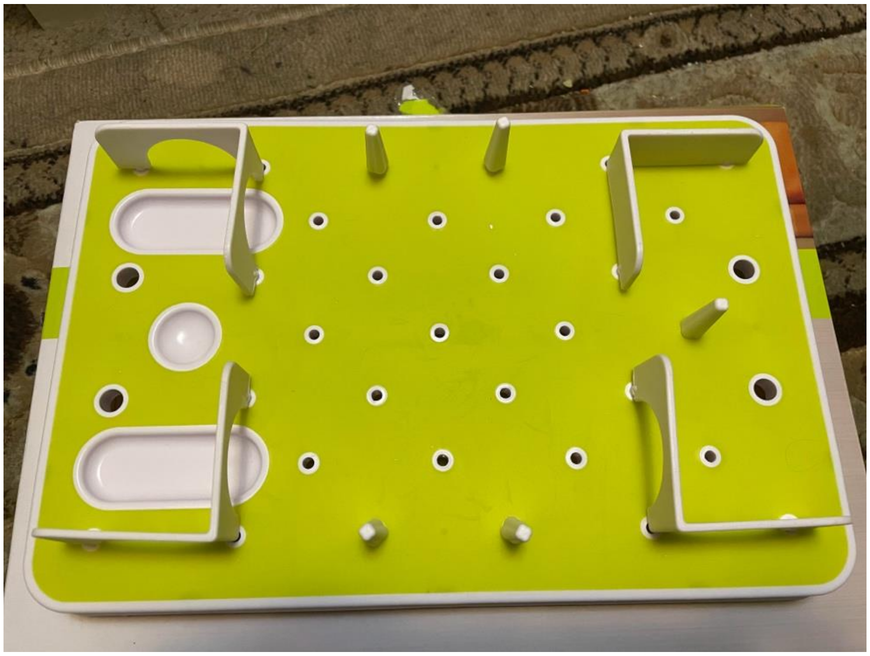


Image 2 - Puzzle Configuration – Easy, Row 2

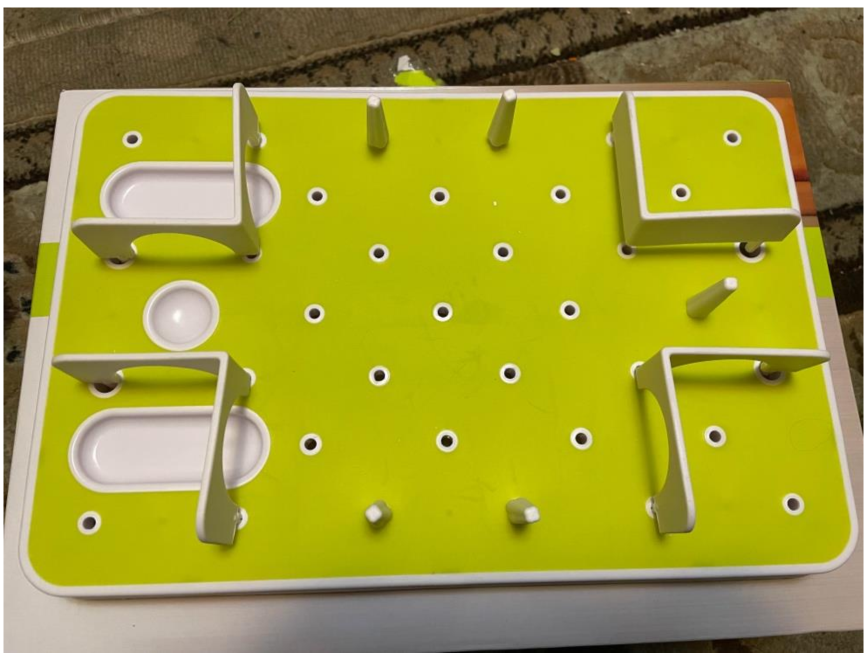


Image 3 - Puzzle Configuration – Easy, Row 3

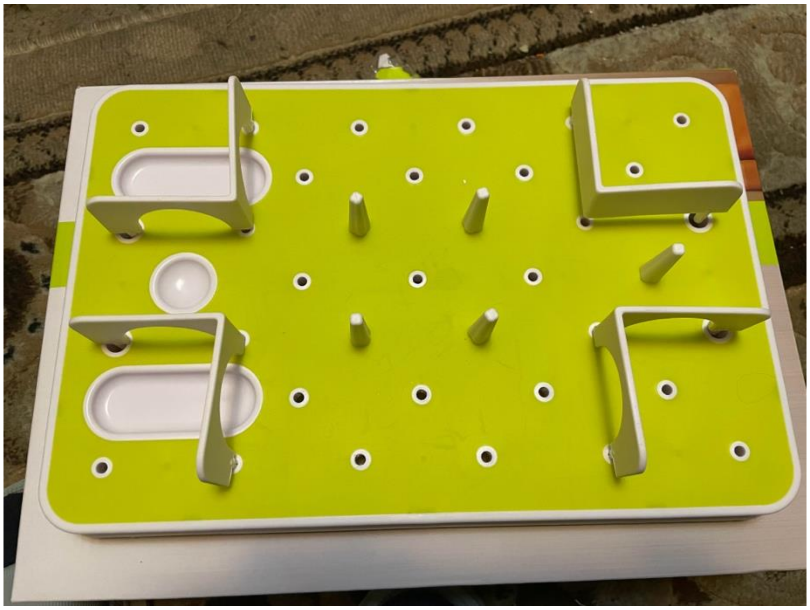


Image 4 - Puzzle Configuration – Easy, Row 4

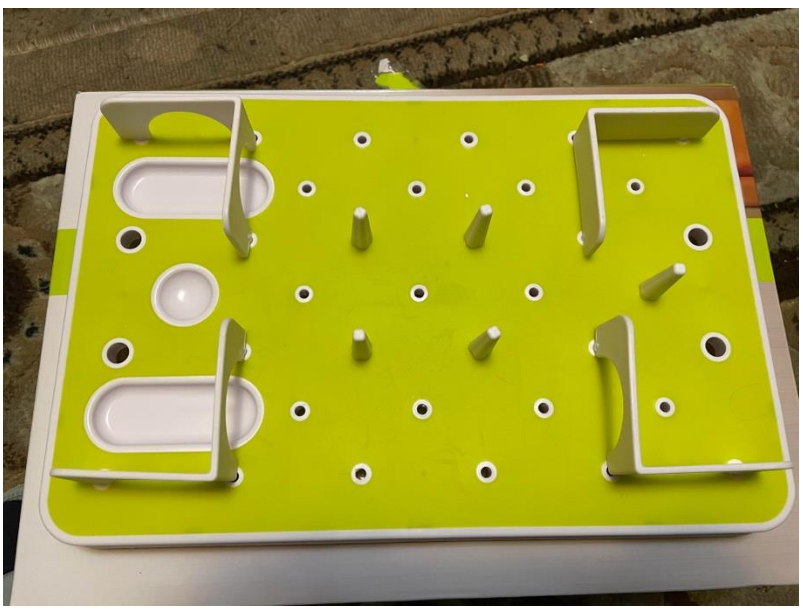
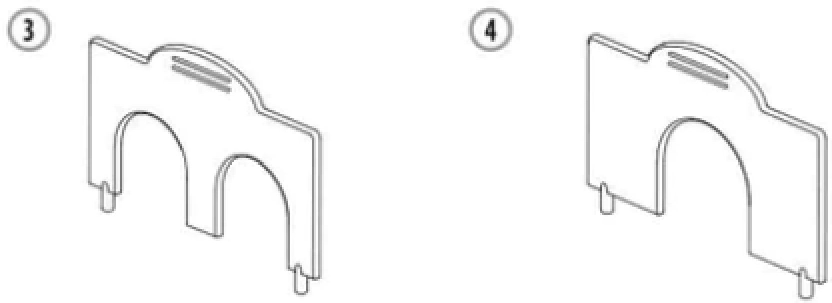


Image 5 – Medium Divider (right) and Hard Divider (left)



R Code

Section I

```
# Step 1: Define the number of treatments (p=4)

p <- 4

# Create a standard Latin Square with treatments assigned alphabetically

standard_square <- matrix(c(1,2,3,4,2,1,4,3,3,4,2,1,4,3,1,2),nrow=p,ncol=p,byrow=TRUE)

# Step 2: Randomize the order of rows

rows_permutation <- sample(1:p)

# Step 3: Randomize the order of columns

cols_permutation <- sample(1:p)

# Step 4: Assign random permutations to row and column headers

randomized_square <- standard_square[rows_permutation, cols_permutation]

# Step 5: Randomize treatments

treatments <- sample(letters[1:p])

# Create the Latin Square with randomized treatments

latin_square <- matrix(treatments[randomized_square], ncol = p, nrow = p)

print(latin_square)
```

Section II

```
# Define the four treat flavors
food_items <- c("Seafood", "Chicken", "Dairy", "Tuna")
 # Randomly shuffle the treats
random_food_items <- sample(food_items)
# Create a data frame to store the treatment and assigned food
assignment_df <- data.frame(Treatment = treatments, Food = random_food_items)
print(assignment_df)
```

SAS Code

```
data cat_treats;
input rep row col trt resp;
datalines;
1 1 1 4 3.0
1 1 2 1 6.0
1 1 3 2 3.0
1 1 4 3 16.0
1 2 1 1 6.0
1 2 2 2 5.0
1 2 3 3 3.0
1 2 4 4 12.0
1 3 1 2 7.0
1 3 2 3 7.0
1 3 3 4 18.0
1 3 4 1 5.0
1 4 1 3 4.0
1 4 2 4 7.0
1 4 3 1 7.0
```

1 4 4 2 10.0

2 5 1 4 5.0

2 5 2 1 6.0

2 5 3 3 4.0

2 5 4 2 6.0

2 6 1 2 12.0

2 6 2 3 4.0

2 6 3 4 6.0

2 6 4 1 6.0

2 7 1 3 10.0

2 7 2 2 6.0

2 7 3 1 21.0

2 7 4 4 5.0

2 8 1 1 5.0

2 8 2 4 4.0

2 8 3 2 4.0

2 8 4 3 3.0

3 9 1 1 6.0

3 9 2 2 25.0

3 9 3 4 5.0

3 9 4 3 27.0

3 10 1 3 12.0

3 10 2 1 52.0

3 10 3 2 5.0

3 10 4 4 24.0

3 11 1 4 31.0

```
3 11 2 3 7.0
3 11 3 1 20.0
3 11 4 2 10.0
3 12 1 2 11.0
3 12 2 4 26.0
3 12 3 3 23.0
3 12 4 1 19.0
;

proc glm data=cat_treats;
class rep row col trt;
model resp=rep row(rep) col trt;
contrast "Dairy vs. Chicken" trt 1 1 -1 -1;
contrast "Tuna vs. Seafood" trt 0 0 1 -1;
contrast "Fish-flavor vs. Non-fish flavor" trt 1 -1 0 0;
contrast "Dairy vs. Non-dairy " trt 1 1 -3 1;
estimate "Dairy vs. Chicken" trt 1 1 -1 -1;
estimate "Tuna vs. Seafood" trt 0 0 1 -1;
estimate "Fish-flavor vs. Non-fish flavor" trt 1 -1 0 0;
estimate "Dairy vs. Non-dairy " trt 1 1 -3 1;
means trt  / alpha=0.05 lsd;
run;
quit;

proc means data=cat_treats fw=8 maxdec=2 alpha=0.5 clm mean std;
run;
quit;
```

Sources Used for SAS Code Generation

[1] - https://www.stat.purdue.edu/~yuzhu/stat514fall05/Lecnot/latinsquarefall05.pdf

[2] - https://stats.oarc.ucla.edu/sas/faq/how-can-i-do-anova-contrasts/

[3] - https://documentation.sas.com/doc/en/pgmsascdc/v_019/proc/n19r5d7g9eae2on1rdcljoxjhp5i.htm