

Over and Switchback for experimentation on large animals

ABSTRACT

Among the most common designs, the very randomized design, in randomized blocks and in Latin squares, stands out, however, approaches to alternative designs are not as common in basic books that cover experimental statistics, especially in the approach that was given in this article. The study of Change Over and Switchback designs is a separate chapter within statistics. For experimentation on large animals, alternative tests are widely used, that is, obtaining a sequence of treatments that can make the final cost of this operation viable. There are two types of experimental tests: rotational (Changeover) and reversal (Switchback). The objective of this work was to present the Changeover and Switchback designs.

Keywords: Rotational experiments, experiments, ruminants

1. INTRODUCTION

According [1], animal tests can be classified into two types: continuous tests and alternative tests. Continuous trials are those in which each animal in the experiment receives a certain initial treatment, and remains with it until the end of the experiment.

Alternative trials are those in which each animal in the experiment receives, in sequence, two or more treatments during the trial, depending on the sequence of treatments applied. Considers the heterogeneity of experimental material (breed, age, lactation period), as well as the high cost of the experimental unit. They are classified into: rotational or Change Over tests and reversal or Switchback tests, represented by the following schemes:

Rotary or Change Over:

Treatment A => Treatment B => Treatment C

Treatment B => Treatment C => Treatment A

Treatment C => Treatment A => Treatment B

Reversion ou Switchback:

Treatment A => Treatment B => Treatment A

Treatment B => Treatment C => Treatment B

Treatment A => Treatment C => Treatment A

In designs with repeated measures, the purpose is to evaluate the effects of the treatment, and individuals are sequentially exposed to the treatments under study, some of which may be repeated, but only one treatment is applied to each of the experimental units.

Thus, for the comparison of three treatments, when it is not known whether treatment residual effects or variations in the response curves of experimental units are more important, balanced residual breeding is preferred. In practice, however, knowledge of the subject generally indicates which factor is most important. If variations in response curves are considered more important, then a switchback design should be used.

Continuous and alternative trials are not easy to modify once started, and errors in the planning stage can result in major losses in terms of time, money and loss of information. Therefore, it is advisable to dedicate sufficient reasoning and time to the planning phase, defining precise objectives and appropriate experimental design, and measuring response variables that are relevant to the postulated hypotheses, as well as helping to understand the verified animal response.

This work was conducted with the purpose of demonstrating the main ideas contained in the experimental design in Rotational or Change Over Tests and Reversion or Switchback Tests.

2. MATERIAL AND METHODS

2.1 Rotating tests or Change Over (Latin square)

Rotational or Change Over trials are those where the two receive, in sequence, two or more treatments during the experiment. They can be classified as: a) Change Over in balanced Latin squares; b) Change Over in balanced Latin squares with extra period. According to the balance, they can be classified as: a) Complete: a treatment is preceded by itself; b) Incomplete: a treatment is not preceded by itself.

2.1.1 Change Over in balanced Latin squares to estimate the residual effect

Rotational trials are those in which all treatments, applied in sequence to a given animal, are different. The analysis method in rotational tests is based on the assumption that the experimental unit is what is called animal-period, that is, it is the result obtained in each observation period for the animal.

It is used when the interest is in the residual effect, that is, in the cumulative effect of the treatment and/or when it is not practical to use a rest period between one treatment and another for an experimental unit.

2.1.1.1. Experimental models for rotary tests

The experimental model for rotating testing is the Latin square, in which the columns correspond to the animals and the lines to the periods.

A so-called balanced or balanced design can be used, for example, when you want to evaluate three rations: A, B and C, using the following scheme (Table 1):

Table 1 – Scheme of an experimental design for a rotating test in equilibrium or balanced, for an odd number of animals

Sequence of animals			
	1	2	3
Period I	A	B	C

Period II	B	C	A
Period III	C	A	B

It is observed that the variation between animals was isolated. Each cow works as a block, receiving all treatments in sequence. There may then be a residual effect of a treatment for one period on subsequent treatment. It is also noted that treatment A always precedes B and C always after B.

In this sense, if we replicate this plan four times, four Latin squares will be obtained, each of them applied to three cows that must be very similar to each other. For an odd number of animals, within the Latin square, the square must be repeated, using the following scheme (Table 2):

Table 2 – Scheme of an experimental design for a rotating test in equilibrium or balanced, for an odd number of animals.

	Sequence of animals					
	1	2	3	1	2	3
Period I	A	B	C	A	B	C
Period II	B	C	A	B	C	A
Period III	C	A	B	C	A	B

It is observed that any treatment is preceded, in an equal number of times, by each of the other treatments. A design with this property is called balanced or balanced, although the balance is incomplete, as a treatment is never preceded by itself.

According to [2], if the number of treatments is even, this type of balance can be achieved with a single Latin square, but when it is odd, two Latin squares are necessary for balancing. According to the author, since the 4 x 4 Latin square provides only 3 degrees of freedom for error, we must use at least two Latin squares.

Additionally, [3] consider that, with 3 or 5 treatments, the number of squares must be a multiple of two, where treatments are assigned to letters at random and cows are drawn to sequences. If the experiment has more than one square, we must decide whether to remove the periods separately in each of them, or whether to isolate the general effect of the periods.

For [4], Latin square schemes suffer from limitations regarding the number of repetitions and periods. There are, however, incomplete block models that can be balanced for residual effects, useful when the number of treatments exceeds the number of periods.

[5] used six crossbred male cattle, with 420 kg of live weight, equipped with a rumen fistula, to compare the degradability of cottonseed meal and raw soybean grain, in rations containing 60% soybean matter dry as roughage of sugarcane bagasse treated by: A) 2% soda solution; B) 30% wood ash solution; and C) water. The treatments were carried out by immersing bagasse in liquids and the chosen design was change-over, with two groups of 3 animals each. The results showed that there was no effect of the roughage treatment on the degradability of protein sources, and that the effective degradability of the protein, with a ruminal effluent rate of 0.02, was equal to 58.13% for soybean meal and 90.64% for raw soybeans. The authors concluded that the treatment of sugarcane bagasse with soda, ash or just water did not interfere with the ruminal degradation rates of cottonseed meal or raw soybean, in terms of dry matter and crude protein.

[6] used goats and cattle in a change over 3 x 3 design, to evaluate the degradability of treatments: A) meat and bone meal with 50% crude protein (FCO 50); B) meat and bone meal with 40% crude protein (FCO 40) and C) soybean meal (FS) using the in situ nylon bag technique. Sugarcane was the only roughage offered. DM (dry matter) and CP (crude protein) degradability rates were similar between goats and cattle, in all treatments. The degradability values of FCO-40 and FCO-50 were similar, but lower than FS after 1.5 hours of incubation. The effective degradability of MS was 77.4% for FS; 39.0%

for FCO-40 and 39.1% for FCO-50. At times 1.5 h, 3 h, 6 h and 12 h, FCO-50 had less degraded PB than FCO-40. The FS presented its PB more degraded than the FCO, only at 12 h and 48 h of incubation. The effective degradability of PB was 72.4% for FS; 54.4% for FCO-40 and 49.8% for FCO-50. The experiment allowed us to conclude that FCO can provide greater amounts of CP to the intestines, compared to FS, after 12 hours of incubation.

2.1.2. Change Over in balanced Latin squares with extra period

In the experimental model for rotating trial with extra period, all treatments are preceded by all other treatments and by itself in an equal number of times. They are considered less efficient when the absence of residual treatment effect is admitted. They are not recommended for patients when the number of treatments is equal to or greater than six. It has equal precision for direct and residual effects, due to the extra period.

Models with an additional period are also less efficient, that is, they provide less information per animal and per period than regular Latin squares when assuming that residual effects do not exist.

2.1.2.1. Experimental models for rotational tests with extra period

If the treatment number is odd, it must be repeated so that the number of squares is even. If the number of treatments is even, the number of squares can be odd or even, according to the following scheme (Table 3):

Table 3 – Treatment scheme an experimental design for a rotating trial with an extra period, for an odd number of animals.

	Sequence of animals					
Period I	A	B	C	A	B	C
Period II	B	C	A	B	C	A
Period III	C	A	B	C	A	B
Extra Period	C	A	B	C	A	B

2.2. Reversal or Switchback Tests (Sequence)

Simple reversal or Switchback trials are those in which each animal receives an initial treatment in a first period, changes treatment in a second period and receives the same initial treatment again in a third period. It is a pre-determined sequence that estimates the direct effect of the treatment, without using periods of adaptation to the subsequent treatment.

In the case of dairy cows, we must only make sure that the cows have passed their lactation peak when starting an experiment, and that none of them have reached the middle of their gestation period at the end of the experiment. During peak lactation, a cow's milk production initially increases and then declines. This drop in production varies from animal to animal. In this sense, the simple reversal or Switchback design eliminates this cause of error.

2.2.1. Experimental models for simple reversal or switchback tests

To compare two treatments, three periods are used: half of the animals receive the sequence A – B – A; the other half the sequence B – A – B, according to the following scheme (Table 4):

Table 4 – Treatment scheme an experimental design for simple reversal or Switchback testing.

	Sequence of animals	
	1	2
Period I	A	B
Period II	B	A
Period III	A	B

The comparison of p treatments requires $p(p - 1)$ sequences (complete design), but if p is odd and greater than or equal to 5, schemes that use $p(p - 1) / 2$ sequences of treatments (reduced design) can be used, according to the following scheme (Table 5).

Table 5 – Treatment scheme an experimental design for testing simple reversal or reduced Switchback.

	Sequence of animals					
	A	B	C	A	B	C
Period I	A	B	C	A	B	C
Period II	B	C	A	B	C	A
Period III	C	A	B	C	A	B

[7] presented the extension of the simple reversal design to more than two treatments. Thus, the complete design can be divided into up to $p(p - 1)$ blocks of p sequences each. The reduced design can be divided into up to $p(p - 1) / 2$ blocks of p sequences each.

[8] used twelve lactating crossbred cows in a switchback design to analyze the possibility of partial or total replacement of disintegrated corn with dry citrus pulp, in the concentrate mixture. The cows remained on pastures, and the results obtained allowed us to conclude that, during the rainy season, cows with average production levels equal to 13.0 kg of milk per day, can receive concentrated mixtures with up to 67% citrus pulp, replacing to disintegrated corn, without any inconvenience.

[9] used twelve crossbred cows in a switchback design comparing, in a 2×2 factorial arrangement, the following treatments, concerning the administration of concentrates: A) once a day, quantified by individual production; B) once a day, quantified by the general average production; C) twice a day, quantified by individual production, and D) twice a day, quantified by general average production. Uncorrected milk yields, milk yields corrected for 4% fat and the amounts of fat produced by the different treatments did not differ statistically.

[10] used twelve cows of the Holstein and Brown Swiss breeds producing 23 kg/day on average. plus daily production of the first 8 kg of milk: A) corn silage + 1.50 kg of soybean meal: B) 50% corn silage + 50% sugar cane + 2.25 kg of soybean meal soy; C) sugar cane + 3.00 kg of soybean meal. The daily milk production averages, corrected for fat in the treatments, were: A = 19.63 kg and 3.57%. B) 18.94 kg and 3.50%. C) 18.07 kg and 3.46%. respectively, and did not show significant differences. The consumption of dry matter and total digestible nutrients by the animals decreased with the increase of

sugar cane in the diets and the demands of the cows in terms of DM were met. PB and NDT. It was possible to replace corn silage with sugar cane, guaranteeing similar milk production, around 18.0 kg/animal/day.

[11] used twelve cows in a switchback design to evaluate treatments consisting of the following silages, corrected for the same protein level by the addition of different proportions of cottonseed meal, in order to guarantee the nutrients necessary to maintain the females and also for the production of the first 8 kg of milk/animal/day, with supplementation of a concentrated mixture for amounts of milk secreted greater than eight kg: A) grain sorghum silage; B) 50% grain sorghum silage and 50% sugar cane silage; C) sugarcane silage. Milk production per animal and per day were respectively 12.9 kg, 12.3 kg and 11.8 kg, with treatment A being significantly higher than C. The consumption of total dry matter per 100 kg of live weight (3.0 kg, 3.0 kg and 2.8 kg), as well as total digestible nutrients (9.2 kg, 8.8 kg and 8.0 kg), respectively for treatments A, B and C, not were statistically different. Twelve Ideal breed sheep were submitted to an apparent digestibility test with the same treatments applied to lactating cows: the values found for dry matter digestibility were 54.4%; 56.8% and 52.0%; for treatments A, B and C, not considering statistically significant differences.

[12] used ten lactating cows, with 55 days of calving, average weight of 540 kg, distributed in a switchback design with the objective of evaluating the production and composition of milk, consumption and apparent digestibility of dry matter (DM), organic matter (MO), neutral detergent fiber (NDF), crude protein (CP), total carbohydrates (TC) and ether extract (EE), and pH and ruminal ammonia concentration. The animals were fed ad libitum with five diets containing corn silage (SM), alfalfa hay (FA), coastcross grass hay (FCC), $\frac{1}{2}$ FA+ $\frac{1}{2}$ SM, $\frac{1}{2}$ FCC+ $\frac{1}{2}$ SM, in the proportion of 60% of the total ration (dry matter basis). Nutrient intakes were not influenced by diet. The apparent digestibilities of DM, CP and NDF were higher for diets containing corn silage. The pH and ammonia concentration of the rumen fluid were not influenced by the diets, however, a quadratic response was observed for the collection time. Greater milk production was recorded for animals that received corn silage. Crude protein and milk fat contents were not influenced by diet.

[13] evaluated the effect of the particle size profile of dietary particles on the performance of ten dairy cows, distributed in a switchback design, in three consecutive periods. The animals were housed individually and received rations in a complete mixture, consisting of Tifton grass hay (50.4%) and concentrate (49.6%). The hay was crushed in a commercial mill with 3.2 mesh screens; 4.8; 7.9; 15.9; and 25.4 mm, and samples from each milling were submitted to the Penn State model particle separator to determine the particle distribution profile. Five treatments were constituted: T1 – 100% of particles below 8 mm; T2 – 76% below 8 mm and 24% between 8 and 19 mm; T3 – 36.7% below 8 mm, 26.6% between 8 and 19 mm and 36.7% above 19 mm; T4 – 32% below 8 mm, 28% between 8 and 19 mm and 40% above 19 mm; and T5 – 26% below 8 mm, 28% between 8 and 19 mm and 46% above 19 mm. The results indicated that feed and nutrient intakes and feed conversion were not influenced by the different granulometric profiles of the diets, but variations were observed in milk production and composition. It was found that dairy cows fed diets with intermediate particle size profiles produced more milk. The amount of milk fat produced was not affected by the distribution profile of dietary particles in the diet, however, dairy cows fed diets with larger particle sizes produced milk with a higher fat content. The processing of diet ingredients with particle size reductions must be carefully considered, as better performance occurred when animals were subjected to diets with an intermediate particle size profile.

[14] evaluated the effect of different particle sizes of dietary fiber on the average retention time and apparent digestibility of diets from ten lactating dairy cows, distributed in a switchback design over three consecutive periods. The animals were housed individually and fed complete mix rations, consisting of Tifton grass hay (50.4%) and concentrate (49.6%). The hay was crushed in a commercial mill with 3.2 mesh screens; 4.8; 7.9; 15.9 and 25.4 mm. Samples from each milling were submitted to the Penn State model particle separator to determine the distribution profile of the fiber particles. Five treatments consisted of: T1 – 100% of particles below 8 mm; T2 – 76% below 8 mm and 24% between 8 and 19 mm; T3 – 36.7% below 8 mm, 26.6% between 8 and 19 mm and 36.7% above 19 mm; T4 – 32% below 8 mm, 28% between 8 and 19 mm and 40% above 19 mm; T5 – 26% below 8 mm, 28% between 8 and 19 mm and 46% above 19 mm. The granulometric profile had a preponderant effect on the transit of diets, observing that those with larger particles stayed longer in the gastrointestinal tract. The digestibility coefficients of dry and organic matter were higher for diets with smaller particle size compared to those with a coarser profile. There was no effect on the digestibility of crude protein and non-fibrous carbohydrates. Fiber digestibility was greater in diets with a thinner profile. The results suggest that the particle size of the diet may be decisive in retention time and digestibility.

[15] evaluated dry matter intake, milk production and thermal stress indicators of Brown-Swiss cows fed cashew nuts in the semi-arid region of Northeast Brazil. Twelve animals were distributed in a reversion trial, with four treatments: 0, 8, 16 and 24% of chestnut in the concentrate. The cows received sugar cane ad libitum and seven kilos of concentrate per day. Greater consumption of sugarcane dry matter was observed in the treatment with concentrate without nuts (7.70 kgDM/day) in relation to treatments with 16% and 24% nuts (7.35 and 7.05 kgDM/day, respectively). Consumption in the treatment with concentrate without nuts did not differ from consumption in the treatment with 8% (7.59 kgDM/day). There was no effect of treatments on milk production or variables indicative of heat stress.

[16] evaluated the effect of two forage offers (25 and 40 kg DM/cow.day) on forage consumption and milk production in cows grazing annual ryegrass. The rotated method was used, in a double reversal experimental design, with three periods of 12 days and ten Holstein cows in the middle third of lactation. Forage consumption was estimated by fecal production and digestibility of ingested forage and milk production was measured daily. Biomass and canopy height before grazing did not differ between forage offerings. The post-grazing height was greater in the forage supply of 40 kg DM/cow.day, which determined the disappearance of forage equivalent to 49.0% in this forage supply and 69.0% in the 25 kg DM/cow supply. Individual forage dry matter (DM) consumption was 11.9 and 16.6 kg/day and milk production was 18.4 and 21.1 kg/day in offers of 25 and 40 kg DM/cow.day, respectively. Dairy cows on ryegrass pasture with a high forage supply can ingest more than 16.0 kg of DM and produce more than 20 kg of milk/day. Milk production reduces approximately 0.2 kg for each kg decrease in DM of forage offered.

We comment on the advantages and disadvantages of models with an extra period compared to regular Latin squares. It was also highlighted that the Switchback design may be preferable if residual effects are neglected. For [17], the main factors to be considered when choosing between these three types of tests seem to be the following: a) the magnitude of the experimental errors per observation; b) the effective number of repetitions per observation, for direct, residual and permanent effects; c) whether residual effects are present or not; d) whether or not there is interest in interpreting and evaluating the residual and permanent effects, or whether we simply wish to discriminate differences in treatments, without caring about the residual effects.

Current trends in terms of number of publications and the generation of an increasing volume of data organized on the Change Over and Switchback designs lead us to believe that the interest in using and supporting public policy will be an area of intense development in the near future.

4. CONCLUSION

This work showed the main ideas contained in these designs and the use of appropriate layouts to better separate the treatment and residual effects, in addition to presenting some practical examples. These designs appear as a very interesting tool for studies involving animals, allowing the elimination of much of the variability due to individuals.

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