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J.G.C. da Silva
Universidade Federal de Pelotas

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Experiment: Conceptual Basis

Joao Gilberto Correa da Silva[1*]

[1]*Department of Mathematics and Statistics, Federal University of Pelotas, Pelotas, RS, Brazil.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Texts and teaching of Experimental Statistics emphasize the statistical analysis of experiments and superficially consider the conceptual basis of experimental research. Definitions of basic concepts are imprecise, incoherent and ambiguous. This is the case, for example, with the concepts of experimental material, experimental factor, experimental unit and experimental error. In particular, the lack of distinction between experimental factor and unit factor and between the two classes of experimental factor: treatment factor and intrinsic factor leads to flaws in the plan and analysis of experiments that originate biased inferences. This approach gives rise to ignorance and misunderstanding of these concepts, underestimation of the importance of the planning of the experiment and, consequently, the inefficiency of many research. This paper reviews and reformulates important concepts with the purpose of contributing a rational basis for experimental research and, in particular, for Experimental Statistics. These revised concepts provides a basis for the formulation of an experiment structure that provides appropriate inferences for the achievement of the objectives of the experiment.

Keywords: Experimental statistics; experimental material; experimental factor; experimental unit; unit factor; experimental error.

1. INTRODUCTION

A logical procedure for inferences about a causal relationship of two characteristics is to contrast the variation of a source of the observed values of the variable that expresses the effect and that comprises a component due to the cause with the variation of a source with the same

**Corresponding author: E-mail: jgcs1804@yahoo.com.br;*

components, except for the absence of this particular component. If the common components and the difference between these two sources of variation are random, inferences can be derived by appropriate statistical procedures.

Desirable properties of these inferences require that the definitions of the design of the experiment, the statistical model and the inference procedures be drawn up in the plan of the experiment in an appropriate manner and consistent with the objectives of the experiment and that they satisfy some important properties. These requirements are often not met. The main source of this failure is the precarious conceptual basis of the experimental research presented in the texts and transmitted by teaching. Consequently, the components of the experimental error that affect effects of experimental factors are not correctly identified, which implies bias of inferences derived from the experiment [1].

The precariousness of the concepts formulated in the literature is remarkable, even in the renowned texts and reference books. The following definitions of the experimental unit are illustrative: "the total amount of material to which one treatment is applied in a single replicate" (Federer, [2]); "the group of material to which a treatment is applied in a single trial of the experiment" (Cochran and Cox, [3]); "the smallest subdivision of the experimental material such that any two different experimental units might receive different treatments" (Cox, [4]; Cox and Reid, [5]); "The smallest unit to which one treatment is applied" (Federer, [6]); "the piece of experimental material, to which treatment is assigned and applied" (Hinkelmann and Kempthorne, [7]); and "the "material" to which the levels of the treatment factor(s) are applied." (Dean and Voss, [8]); "the physical entity or subject exposed to the treatment independently of other units" (Kuehl, [9]); "Experimental units are the things to which we apply the treatments." … "One way to determine the experimental unit is via the observation that an experimental unit should be able to receive any treatment." (Oehlert, [10]). These definitions are inaccurate and ambiguous, partly by relying on concepts, such as material, experimental material, treatment and replicate that are not defined.

Bailey [11] admitted the difficulty of the definitions of basic concepts when she states the following definitions of experimental unit and treatment, which she acknowledges as circular: "An experimental unit is the smallest unit to which a treatment can be applied". "A treatment is the entire description of what can be applied to an experimental unit". Oehlert [10] stresses this difficulty particularly with regards to experimental unit: "Experimentation is so diverse that there are relatively few general statements that can be made about experimental units."

The dissatisfaction with these definitions is manifested in the literature. For example, Blight and Pepper [12] reviewed the literature in an attempt to establish a definition of experimental unit for grazing experiments with beef cattle and conclude that there is much debate about it. Brien and Demetrio [13] commented that it is controversial whether the experimental unit is the individual animal, the field unit or the field unit and its animals. They claimed that the recognition that the error that affects treatment effects involves the variability of field and animal and the hope that this error is determined by the experimental units to motivate the statement that the experimental unit is the field unit and the animals it supports. However, this is not the only source of this error. It also results from effects of environmental characteristics not inherent to the field unit, related to climate, diseases, pests, weeds and predators, of management techniques and measurement process. This scope of the concept of experimental unit was recognized by Cox [4] when complemented his definition: "We consider to be included in the definition of the unit all those aspects of the experimental set-up not involved in the treatments, i.e., those that are independent of the particular assignment of treatments adopted". Kozlov and Hurlbert [14] and Hurlbert [15] expressed a similar definition, in discussions about pseudoreplication in ecological research.

Silva [16] suggested a conceptual basis for experimental research, based on a review and reformulation of concepts presented in the literature. Silva [1] discussed flaws in inferences that may arise from the usual concepts, emphasized the importance of distinguishing two classes of experimental factor: treatment factor and intrinsic factor and considering the structure of the units, and suggested a procedure for identifying this structure and deriving inferences that appropriately take into account the structure of the experiment. Silva [17,18] suggested a rational conceptual basis composed of concepts defined coherently with their real meanings and according to the logical sequence of the experimental process.

This paper reviews and extends these basic concepts, with examples from agricultural experimentation. These concepts constitute a hierarchically interrelated structure. For this reason, they are formulated progressively.

2. APPROACH

The **experiment** is an explanatory scientific research method. Its purpose is to provide inferences about causal relationships of characteristics of the units of a target population; more specifically, about relations of characteristics that express the performance or behavior of the units with characteristics that supposedly affect them and whose control and appropriate alteration can improve performance or behavior. These characteristics are a subset of the class of the **response characteristics** and the class of the **explanatory characteristics**, respectively. The response characteristics are also affected by characteristics of the class of the **extraneous characteristics**, which complements the set of characteristics of the units of the target population.

The definition of these three classes of characteristics is a crucial step in the planning of the experiment. The important response characteristics and the explanatory characteristics are the object of the research, which is established by the scientific problem and hypothesis, and are designated based on substantive scientific theories and empirical knowledge.

In general, the class of explanatory characteristics is small and the important response characteristics are a subset of the numerous class of response characteristics. These characteristics must be identified, defined and expressed by variables. The class of extraneous characteristics is innumerable and is defined by exclusion, as the set of characteristics of the units, excluding response and explanatory characteristics. It is generally necessary to identify only the relevant ones, individually or by aggregates.

The inferences are based on the verification of the postulated causal relation in a sample of the target population. The units of the sample, as well as the units of the target population, are complex systems of characteristics that interact dynamically in space and time. This interaction originates confounding of effects of explanatory characteristics on response characteristics with effects of extraneous characteristics, which imply imprecision and bias for inferences. However, in the experiment there is control of the sample: extraneous characteristics are controlled to reduce imprecision and avoid bias.

The basic concepts of experimental research must consider this complexity. This approach requires a comprehensive description of the extraneous characteristics that ensures the identification of those whose effects can be considerably confounded with effects of explanatory characteristics. A strategy for this purpose is to start with a list of the large aggregates of the extraneous characteristics and to decompose them progressively, until the identification of the relevant characteristics, individually or by aggregates.

The following examples illustrate the procedure for identifying and describing the three classes of characteristics in an experiment, which forms the basis for understanding the concepts defined ahead. These examples are later conveniently modified and extended to illustrate these concepts.

Example 1. Experiment: "Effect of energy diet on the body development of male lambs between weaning and slaughter ".

The response characteristics are the characteristics that express the animal's performance or behavior in the experimental period, that is, between weaning and slaughter, performed at the ages of 70 and 154 days, respectively. The important response characteristics for the objectives of the experiment are: body weight, body weight gain, food intake and feed conversion, all measured every 14 days of the experimental period, and the following measured at slaughter: live weight, warm carcass weight and carcass yield.

It is decided to consider diet with metabolizable energy (ME) in the interval between 2.4 and 3.2 Mcal/kg of dry matter (DM). Thus, an explanatory characteristic is defined: diet (energy diet) whose levels in the target population are the interval of ME [2.4; 3.2]. The levels defined for the sample are the extremes and the middle of this interval, that is, 2.4, 2.8 and 3.2. As the effect of diet can vary with the age of the animal, age is considered an additional explanatory characteristic for the responses measured every 14 days of the experimental period; its levels in the target population comprise the interval [70; 154] days and in the sample, the particular moments of measurement of these characteristics: 70, 84, 98, 112, 126, 140 and 154 days.

The description of the extraneous characteristics requires a prior view of the experimental procedure. It is planned to carry out the experiment on a farm in the region, with 30 male animals of the Ideal breed aged close to 70 days and to house them in 15 pens for two animals. These pens are equipped with drinkers and feeders, where the animals will receive the diets, constituting the levels of ME 2.4, 2.8 and 3.2. The 15 pens will be assigned to the three levels of the diet factor so that five pens are associated with each of these three levels. The animals will be weighed at the beginning of the experiment, that is, at weaning, and at the end of every 14-day interval until slaughter. The quantities of food supplied and leftovers will be weighed daily and their values accumulated at the end of each of these intervals to determine food consumption and feed conversion. The following characteristics will be measured at slaughter: live weight, warm carcass weight and carcass yield.

Thus, the extraneous characteristics of the sample are: the characteristics of the animal at the beginning of the experimental period (age, health, weight, etc.); environment (installation, weather, diseases, parasites, predators, etc.); management (provision of food, with exception of the components that make up the explanatory characteristic diet, water and protection against pests, diseases and predators, slaughter, etc.) and measurement.

Example 2. Experiment: “Control of gibberella in wheat crops in the State of Rio Grande do Sul ".

The class of response characteristics comprises the characteristics of the grain and the plant. The important characteristics for the purposes of this experiment are those related to grain production: grain yield, hectoliter weight, weight of a thousand grains and number of spikelets, and to control of gibberella: number of infected spikelet, incidence of gibberella and severity of gibberella.

It is decided to verify the effectiveness of the control of gibberella with fungicide. As the fungicide effect may depend on the cultivar's susceptibility to gibberella, alternative cultivars are considered. Then, two explanatory characteristics are defined: fungicide and cultivar, the first with four levels in the target population: Mancozeb, Cyproconazole, Propiconazole and control (without fungicide), and the second with three levels: cultivars BRS 49, BRS Buriti and BRS 177. The levels of these explanatory characteristics in the sample are the same ones of the target population. As the effect of fungicide can depend on environmental conditions that vary in space and time, site and year are also considered explanatory characteristics. Their levels in the target population are the wheat cultivation sites of that State and the next years, respectively. It is then decided to carry out the experiment in four sites that represent the region, in three successive years. These particular sites and years are the sample levels.

At each site and each year, the experiment will be carried out on a ground that will be divided into 48 plots 5 m long and 1.80 m wide, with nine sowing lines. These plots will be assigned to the 12 combinations of the fungicide and cultivar levels defined for the sample so that each plot is associated with one of these combinations and each combination results in four plots. At the maturation stage, 25 ears from the three central lines of each plot will be collected at random to count the number of spikelets and the number of infected spikelets and to assess the incidence and severity of gibberella. The three central lines of each plot, excluding 0,5 m from each end, will be harvested manually to determine the grain yield, the hectoliter weight and the 1000-grain weight.

Thus, the extraneous characteristics of the sample are the characteristics of the seed (size, vigor, purity, sanity, etc., except those inherent to cultivar, which is an explanatory characteristic); environment (soil, climate, pests, diseases, weeds, predators, etc., except those inherent to site and year, which are explanatory characteristics, and to gibberella, which are response characteristics); cultivation techniques (soil tillage, sowing, application of pesticide, fungicide, except fungicide to control gibberella, which is an explanatory characteristic, harvest, etc.); and measurement.

3. BASIC CONCEPTS

3.1 Experimental Material

Experimental material is the specific designation of the sample in the experiment. It comprises the three classes of characteristics: responses, explanatory and extraneous that make up the sample, from its initial constitution to the completion of the measurement of the response characteristics.

It is desirable that the experimental material is valid and representative of the target population, that is, that it be a random sample and have structure consistent with the structure of the target population, respectively. However, the experimental material is usually built during the execution of the experiment; therefore, the first condition is generally not met. It should be emphasized that the inferences derived from the experiment are valid for the population from which the experimental material can be considered a random sample, that is, for the **sampled population**. Extending these inferences to the target population requires a subjective judgment stage. It is essential for this judgment that the characteristics of the experimental material are described clearly and completely in the dissemination of the results of the experiment.

3.2 Experimental Factor

Experimental factor is the variable that expresses an explanatory characteristic in the experiment. Each value that this variable assumes in the target population or the sample is a level of the factor. The levels of an experimental factor defined for the sample are called **experimental conditions** or, simply, **conditions**.

The experimental factor is distinguished by the following properties of its levels in the sample: 1) they are chosen and defined for each unit; 2) constitute a small set; 3) form a partition of the experimental material; and 4) their relationships with the levels of the other experimental factors constitute a significant structure for the objectives of the experiment, called **structure of the experimental conditions** or **condition structure**.

In the experiment, the researcher has control over the presence of the levels of one or more experimental factors in the sample. An experimental factor whose levels are associated with units of the experimental material by random process is an **extrinsic factor** or **treatment factor**. The extraneous characteristics not affected by this randomization are controlled so that their effects do not affect treatment factors biasedly.

Each specific level of a treatment factor defined for the sample is a **treatment**. Thus, treatment is the specific condition designation for treatment factor.

A treatment factor is characterized by the following attributes of its levels: 1) they can be associated with any of the units defined for the factor; 2) they are associated with these units by random process; and 3) are explicitly distinct stimuli.

The experiment can also comprise experimental factors whose, presence of levels in the sample is not controlled or is subject to limited control. Experimental factor in this circumstance is inherent in the experimental material or associated with a relevant extraneous characteristic that causes biased confounding of effects. It is called **intrinsic factor** or **classification factor**.

In the experiment of Example 1, the three diets are assigned to the 15 pens by a random process. Therefore, diet is treatment factor and these diets are conditions and, more specifically, treatments. For the characteristics measured repeatedly at 14-day intervals of the experimental period, age is also experimental factor. This is an intrinsic factor because the seven instants of measurement are inherent to the experimental material; therefore, they are not subject to control. This is an example where the identification of experimental factors depends on the response characteristic. In the experiment of Example 2, at each site and each year, the 12 combinations of the four levels of the fungicide factor with the three levels of the cultivar factor are randomly assigned to the 48 plots. Thus, fungicide and cultivar are treatment factors. Each of these levels of the fungicide and cultivar factors, as well as each of their combinations, is a treatment. The four sites and the three years of execution of the experiment are, respectively, geographic positions and a sequence of intervals in time, which means that these factors are inherent to the experimental material. Therefore, site and year are intrinsic factors. The levels in the sample of each of the four experimental factors and the combinations of the levels of two, three and four of these factors are conditions.

Example 1a. Suppose a change in the plan of the experiment of Example 1 that considers race as an experimental factor, in addition to the diet factor for responses measured at slaughter and also to the age factor for responses measured repeatedly in the experimental period. For this new experimental factor, two levels are chosen: the Ideal and Suffolk races. Now, the experiment is carried out with 15 male animals from each of these two races, with an average age of 70 days, in a facility with 15 pairs of pens for one animal, each pair equipped with common feeder and drinker. These 15 pairs of pens are associated at random with the three levels of the diet factor so that five pairs result with each of these levels, and the two pens in each pair are randomly associated with the two levels of the race factor. Thus, races are randomized to pens. However, race is an inherent characteristic of the animal; therefore, it is not possible to randomize races to animals. Moreover, the effects of the race factor are confounded with effects of extraneous characteristics of environment and management prior to the experimental period. Thus, race is intrinsic factor.

The combinations of the levels of experimental factors have the same properties required of the levels of experimental factor. Therefore, they are the levels of an experimental factor, called **complex factor** or **generalized factor**. A complex factor is denoted by the juxtaposition of the symbols of the simple factors that generate it intercalated with "^". A complex factor originated from only treatment factors is also treatment factor; if one of the simple factors is intrinsic, the factor is intrinsic. In Example 1, the combination of the experimental factors diet and age, respectively treatment factor and intrinsic factor, generates the intrinsic factor diet^age, whose levels in the sample are the 21 combinations of the three levels of the diet factor with the seven levels of the age factor. In Example 2, the combination of the treatment factors fungicide and cultivar generates the treatment factor fungicide^cultivar. The combination of the intrinsic factors site and year generates the intrinsic factor site^year. The combination of one of these two factors with any of the fungicide and cultivar factors is also an intrinsic factor.

The levels of each experimental factor that are defined for the target population and the levels chosen for the sample must be clearly specified in the plan of the experiment. The set of levels of the target population is the collection of levels that is the object of inferences. The set of levels in the sample can be the same of the target population or a subset chosen arbitrarily or randomly to provide inferences for the target population. Thus, the set of the levels of the target population must be established clearly and precisely before choosing the levels for the sample. This procedure is necessary for inferences derived from the sample for the target population to be adequate to the objectives of the experiment.

In the experiment in Example 1, the set of levels of the diet factor that is objects of inferences and defined for the target population is the interval [2.4; 3.2] of ME in which an effect on the development of the animal's body is expected. The set of levels of this factor in the sample: {2.4, 2.8, 3.2} is a subset of that interval chosen so that the relationship between important response characteristics and this factor in the target population is adequately approximated by the relationship expressed in the sample. The set of levels of the age factor in the target population is the age interval between weaning and slaughter: [70; 154] days; the set of levels in the sample: {70, 84, 98, 112, 126, 140, 154} is a subset of this interval chosen to provide inferences about the effect of the animal's age on the relationships of response variables measured repeatedly in the experimental period with the diet. In Example 1a, the same set of levels of the race factor defined for the target population: {Ideal, Suffolk} is chosen for the sample. In Example 2, the levels of the fungicide and cultivar factors are also the same in the target population and in the sample. In these last two examples, the specific levels of the experimental factors race, fungicide and cultivar in the sample are the only ones that are the objects of inferences. However, in Example 2, the set of levels of the site factor in the target population comprises the sites in the wheat-producing region of Rio Grande do Sul, while the sample levels are the four specific sites chosen to represent that set of sites. The levels of the year factor in the target population are an interval of years in the future for which the inferences will be applied, while in the sample are the specific three years of execution of the experiment. The sites and years chosen to make up the sample are not random samples from the respective sets of levels of the target population, but are supposed to represent the environmental conditions in the wheat-producing region of Rio Grande do Sul.

Effects of experimental factors are always confounded with effects of extraneous characteristics. The magnitude of this confounding and its unbiasedness depends on the feasibility of its control and the researcher's ability to exercise it. These properties should be considered in the planning and execution of the experiment. The confounding of effects of extraneous characteristics with effects of treatment factors has two origins: Extraneous characteristics conveyed with the treatments and extraneous characteristics that manifest in the sample. However, the researcher can exercise control by the choice and preparation of treatments that guarantee their applications essentially in accordance with the definitions established in the plan of the experiment, association of treatments with units of the experimental material by random process, and control of the presence of extraneous characteristics in these units that reduce this confounding and avoid bias. Thus, in the experiment in Example 1, effects of the diet factor, whose levels are particular amounts of metabolizable energy, are confounded with effects of extraneous characteristics of the feed, environment, management and measurement. However, the control can guarantee that the feed is formulated with homogeneous composition of the extraneous characteristics, varying only the amount of metabolizable energy according to the definitions of the treatments. Then, the random association of diets with units of experimental material and the control of extraneous characteristics related to the environment, management and measurement can reduce the confounding of their effects with effects of treatments and avoid the bias of the remaining confounding. The cultivar factor of Example 2 comprises the set of genetic characteristics transmitted by the seed; its effects are confounded with effects of seed characteristics not related to cultivar: sanity, purity, vigor, etc. The researcher can control these extraneous characteristics to ensure that the differences between the seeds of different cultivars are essentially due to the genotypes. He can also associate cultivars with units of the experimental material by random process and control the extraneous characteristics of the environment, cultivation techniques and measurement.

Distinctly, in general, the effects of intrinsic factors are considerably confounded with effects of extraneous characteristics, so that there is no way to discriminate between the effects of these two origins. In Example 1a, the race factor comprises a set of genetic characteristics; the effects of this experimental factor can be considerably confounded with effects of extraneous characteristics of the animal, originating mainly from environment and management effects prior to the experimental period. The age factor comprises the animal's characteristics related to body development; effects of this factor are confounded with effects of extraneous characteristics that manifest during the experimental period, related to the environment, management and measurement. The site experimental factor of the experiment in Example 2 comprises the set of permanent characteristics of the environment and cultivation techniques specific of the sites that constitute the levels of this factor. Thus, the occurrence of a climatic event, disease or pest in a given place will be inherent to the site experimental factor if it is a characteristic of this place; it will not be inherent to this factor if it is an eventual occurrence. Inevitably, occurrences unrelated to the site factor will be confounded with effects of this factor. Cultivation techniques performed in a non-uniform manner, such as planting and harvesting for prolonged and varied periods, can also imply differences between places not due to the site factor, the effects of which will be confounded with the effects of this experimental factor.

Example 1b. Suppose in the experiment in Example 1 it is expected that the effects of diets may vary with sex. Therefore, sex should be considered experimental factor. Suppose also that this experiment is carried out in the installation described in Example 1a, now with 15 males and 15 females. The two pens in each pair of pens are randomly associated with the two levels of this factor: male and female. However, sex is an inherent characteristic of the animal; therefore, its two levels cannot be randomly assigned to animals. But if the animals come from the same herd and their individual characteristics not inherent to sex are essentially homogeneous or controlled, so that the differences of extraneous characteristics between the group of males and the group of females can be considered random, as well as the differences of these characteristics within these groups, sex can be considered treatment factor. However, if the extraneous characteristics differ considerably between these two groups, as may be the case if the groups were previously submitted to different environments or managements, sex should be considered intrinsic factor.

These examples emphasize that there is no clear dichotomy between treatment factor and intrinsic factor. Essentially, what characterizes treatment factor and distinguishes it from intrinsic factor is the association of its levels to the units of the experimental material by random process and its non-association with relevant extraneous characteristic that can cause biased confounding of effects.

3.3 Experimental Unit

Experimental unit is the smallest fraction of the experimental material that is associated with a level of an experimental factor, independently of the other fractions. The association is determined by randomization or is inherent to the experimental material, respectively in cases of treatment factor and intrinsic factor.

Association of a unit of the experimental material with a level of an experimental factor independently of other units means that: a) the variation of extraneous characteristics between units with a same level of the factor is similar to that between units with different levels; b) the characteristics of an experimental unit do not affect the characteristics of other experimental units.

An essential attribute of treatment factor is the random association of its levels with its experimental units. This property implies that the confounding of the effects of this factor with effects of randomized extraneous characteristics is not biased. In addition, it is assumed that extraneous characteristics not affected by randomization are controlled, so that their effects do not affect biasedly effects of treatment factors. On the other hand, the association of the levels of an intrinsic factor with its experimental units constitutes a one-to-one correspondence of these levels with the levels of an aggregate of relevant extraneous characteristics, which implies a biased confounding of the effects of these two origins.

In Example 1, the experimental unit of the diet factor is the pen and the respective animals with the levels of characteristics of the experimental material that correspond to them, that is, level of the experimental factor diet; levels of the extraneous characteristics of the animal (age, weight, health, etc.), environment (pasture, climate, disease, etc.), management (supply of feed, water, vaccines, antibiotics, etc.) and measurement; and of the response characteristics. The experimental unit of the age factor is the set of pens and respective animals at an instant of measurement, with the levels of the characteristics of the experimental material that correspond to them: levels of the experimental factors diet and age, of the extraneous characteristics of the animal, environment, management and measurement, and of the response characteristics. In Example 1a, the experimental unit of the race factor is the set of pens with animals of a race, with the levels of the characteristics of the experimental material corresponding to them. In the experiment in Example 2, the experimental unit of the fungicide and cultivar factors is the plot, comprising the corresponding levels of the characteristics of the experimental material, that is, the specific fungicide and cultivar assigned to the plot; the levels of the extraneous characteristics of the seed (purity, sanity, germination, vigor, etc.), environment (soil, climate, incidence of pests, diseases, weeds, and predators), cultivation techniques (sowing, control of diseases, pests etc. and harvest) and measurement; and the levels of the response characteristics. The levels of the experimental factors site and year are inherent to the geographical positions and the years of execution of the experiment, respectively. Therefore, the experimental unit of the site factor is the set of plots of the three years of a site in which the experiment is carried out; the experimental unit of the factor year is the set of plots of the four sites of a year in which the experiment is carried out.

In some circumstances, a part of the experimental unit is discarded because it is supposed to be affected by effects of treatments or extraneous characteristics of nearby units, or by convenience. In this case, the complementary part is considered as the experimental unit. Thus, in the experiment on control of gibberella in wheat crops with fungicide (Example 2), the fungicide or cultivar in an experimental unit can affect neighboring experimental units, the fungicide by the spreading of the sprayed product and the cultivar by the shading or competition. Likewise, in a soil fertilization experiment for growing barley, the fertilizer applied to a plot can be disseminated to neighboring plots. In these circumstances, it is common to disregard the lateral lines and edges of the plot and to consider the central area, generally called the "useful area of the plot", as the experimental unit. In experiments on the stocking rate of grazing animals, where the number of animals per farm varies, it may be convenient to include "extra animals" to standardize the number of animals; these animals are not considered part of the experimental unit.

It is common to identify the experimental unit in an abbreviated way by one of its basic components. Thus, in Example 1, for responses measured at slaughter, the experimental unit of the diet factor can be identified by the pen; for responses measured repeatedly in the experimental period, the experimental unit of this factor can be identified by the pen at an instant of the measurement, and of the age factor by the instant of measurement. In Example 2, the experimental unit of the fungicide and cultivar factors can be identified by the pen. However, it must always be clear that the experimental unit comprises all the characteristics of the fraction of the experimental material that corresponds to it.

The set of experimental units of an experimental factor forms a partition of the experimental material, called **formation of experimental units**. In an experiment with two or more experimental factors, some factors may have common experimental units and other different experimental units. If factors have a common experimental unit, this unit is also the experimental unit of the complex factor that they constitute; if they have different experimental units, the experimental unit of the complex factor is the intersection of these units.

In the experiment in Example 1, for variables measured at slaughter and accumulated values of variables measured repeatedly in the experimental period, there is only one experimental factor: diet, whose experimental unit is the pen; therefore, there is a unique formation of experimental units. For variables measured in instants of the experimental period there is another formation of experimental units for factor age whose unit is the instant of measurement.

Experiments with two or more treatment factors may comprise one or more formations of experimental units. To illustrate the first situation, consider execution of the experiment in Example 2 in a single site and year. In this circumstance, there are two experimental factors: fungicide and cultivar, with common experimental unit: the plot; therefore, there is a unique formation of experimental units. Example 1c illustrates the situation of treatment factors with different formations of experimental units.

Example 1c. Suppose the plan of experiment in Example 1a is changed to include growth stimulant as an experimental factor, with two levels - with and without stimulant - instead of the race factor, and is carried out in the same facility. Levels of the diet factor are randomly assigned to the 15 pairs of pens and the two stimulant levels are assigned randomly to the two pens in each of these pairs. Therefore, for responses measured at a single moment, there are two treatment factors: diet and stimulant, whose experimental units are a pair of pens and a pen, respectively. Hence, the experiment comprises two formations of experimental units, one for each of these factors. The experimental units of the stimulant factor are partitions of the experimental units of the diet factor. For characteristics measured in more than one instant, there is also the factor age whose experimental unit is different: set of pairs of pens at a particular instant; therefore, for these characteristics, there are three formations of experimental units. The intersection of the experimental units of the factors diet and stimulant is the pen; therefore pen is also the experimental unit of the factor diet^stimulant. The intersections of experimental units of the simple factors that constitute the factors diet^age, stimulant^age and diet^stimulant^age determine their experimental units, respectively: pair of pens at an instant, pen at an instant and pen at an instant.

In the experiment in Example 2, the common experimental unit of the treatment factors fungicide and cultivar: the plot is also the experimental unit of the treatment factor fungicide^cultivar. It is different from the experimental units of the intrinsic factors site and year, which are also distinct. The experimental unit of the site^year factor is the set of plots of a site in one year. The experimental unit of a complex factor consisting of a combination of fungicide or cultivar with site or year is the set of plots with a fungicide or cultivar in one place or year, respectively.

In experiments with two or more formations of experimental units, there is a formation whose unit is the intersection of the units of all these formations. These units are called **elementary experimental units**. Thus, in the experiments of Examples 1, 1a, 1b and 1c the elementary experimental unit is the pen; in Example 2 is the plot.

A specific experimental condition can be present in one or more experimental units. Different experimental units with the same condition are **repetitions** of this condition. The number of experimental units with a specific condition is the **number of repetitions** of this condition. In Example 1, the number of repetitions for each level of the factor diet is 5 (5 pens) and for each level of the age factor is 1 (one instant of measurement). In Example 2, at each site and year, the number of repetitions for each level of the fungicide factor is 12 (4 plots x 3 cultivars) and for each level of the cultivar factor is 16 (4 plots x 4 fungicides); in the whole experiment (12 combinations of sites and years), the number of repetitions for each level of the fungicide factor is 144 (4 plots x 3 cultivars x 4 sites x 3 years), for each level of the cultivar factor is 192 (4 plots x 4 fungicides x 4 sites x 3 years), for each level of the site factor and each level of the year factor is one. In general, for every condition of an intrinsic factor, there is only one repetition.

3.4 Observation Unit

Observation unit is the fraction of the experimental material in which an individual measurement of a response characteristic is made, that is, where a value of the variable that expresses this characteristic is observed.

The observation unit refers to a specific response characteristic and may not be the same for different responses. For this reason, it must be identified in relation to the response characteristic. In the experiment of Example 1, live weight, warm carcass weight and carcass yield are measured in each animal at slaughter; therefore, the observation unit of these response characteristics is the animal at slaughter. Body weight and body weight gain are measured in each animal every 14 days from weaning to slaughter; thus, their observation unit is the animal at one of these particular moments. Food consumption and feed conversion are measured in each pen at these same times pen at one of those particular moments. In the experiment of Example 2, the important response characteristics related to the grain and the plant are measured in each plot; therefore, the observation unit of these characteristics is the vation unit of these response characteristics is the plot.

The elementary experimental unit may comprise one or more observation units of a response characteristic. In these situations, it is said that the experiment comprises, respectively, **simple observations** or **multiple observations** of this response characteristic. In the experiment of Example 1, body weight, body weight gain, live weight, warm carcass weight and carcass yield are measured in each of the two animals of each pen; therefore, in each of the two observation units of the elementary experimental unit; thus, the experiment comprises double observations for these responses characteristics. Food consumption and feed conversion are measured in the pen; thus, there are simple observations for these characteristics. The experiment considered in Example 2 comprises simple observations for all response characteristics.

A response characteristic may be measured in a unique instant or in more than one instant of the experimental period. In the second case, it is said that there are **repeated observations** for this response characteristic. In Example 1, body weight, body weight gain, feed consumption and feed conversion are measured at the end of each interval of 14 days of the experimental period; therefore, the experiment comprises repeated observations for these response characteristics.

3.5 Experimental Error

Experimental error is the effect of extraneous characteristics on the observed values of the variable that expresses the response characteristic, and, therefore, on the variation of the values of the response variable between the observation units.

The value of the response variable in an observation unit has two origins: experimental factors and extraneous characteristics, that is, experimental error. Therefore, the variation of these values between the observation units has these same origins. Generally, there is no way to discriminate, absolutely, effects of experimental factors from effects of experimental error. These effects are inevitably confounded. The consequences are imprecision and bias of inferences about effects of experimental factors. However, the experimental error can be controlled so that effects of experimental factors are revealed as clearly as possible.

3.6 Experimental Control

Experimental control is the set of actions taken to control the experimental error.

The experimental control aims to reduce and make unbiased the confounding of the effects of extraneous characteristics with effects of experimental factors. It can be implemented by the following procedures: 1) control of experimental techniques, 2) local control, 3) statistical control and 4) randomization.

The definition of the control procedures to be used depends on the target population, the importance of the representation of the target population by the sample, the inferences to be derived and the desirable properties of these inferences, the experience and vision of the researcher and the availability of resources for research.

The efficiency of the experimental control requires the identification, in the planning phase of the experiment, of the extraneous characteristics of the sample that may be important.

3.6.1 Control of experimental techniques

Control of experimental techniques is the set of actions of intervention in the sample to control extraneous characteristics with the purpose of reducing and making unbiased the confounding of their effects with effects of experimental factors.

In the experiment on the effect of energy diet on lambs (Example 1), considerable control of extraneous characteristics can be obtained by constituting the experimental material by animals of similar characteristics and uniform pens; application of the levels of the diet factor according to the definitions; homogenization of management techniques, particularly supply of feed (except energy diet, which is experimental factor) and water, application of the recommended vaccine, antibiotic and anthelmintic, and slaughter; and use of accurate and uniform measurement procedures. In the experiment on control of wheat gibberella (Example 2), in each site and year, the control can be carried out by using healthy seeds, free of impurities and with homogeneous germination and vigor; running the experiment on uniform ground as to soil characteristics, such as depth, fertility and humidity; application of treatments according to their definitions; uniform use of cultivation techniques, such as soil preparation and fertilization, sowing, control of diseases (except gibberella, which is object of control by treatments), pests, weeds and predators; and uniform crop and measurement processing.

The control of experimental techniques has implications for the constitution of the sample: the fractions of the extraneous characteristics that are controlled are excluded from the sample. Therefore, it can cause impairment to the sample's representativeness. This is a restriction on its use: It must be carried out to the point of not having this implication. For instance, if the target population of experiment in Example 1 is composed of heterogeneous animals and there is an indication that the effects of diets may vary with this heterogeneity, the sample should be made up of animals that represent this heterogeneity. Clearly, the control exercised by the homogenization of management techniques, such as water and food supply, application of vaccine, antibiotic and anthelmintic, protection against predators, slaughter and measurement, has no implications for the sample's representativeness.

Procedures of experimental control applicable to extraneous characteristics whose control by experimental techniques is not recommended are very useful. The procedures that do not affect the constitution of the sample are local control, statistical control and randomization.

3.6.2 Local control

Local control is the classification of experimental units according to the levels of relevant extraneous characteristics and their association with treatments so that the effects of these characteristics are not confounded with important effects of experimental factors and are separated from the experimental error affecting these effects.

Extraneous characteristics that can significantly affect response characteristics should be considered for local control. These characteristics are expected to constitute classifications of the observation units in groups so that their variation between groups is considerably greater than the variation within groups. These classifications can be natural or convenient.

In the simplest situation, the experimental material comprises only one relevant extraneous characteristics and a single formation of experimental units. Then, the experimental units are classified into blocks of units more homogeneous for these characteristics than the set of experimental units, each block with as many units as the number of treatments, and the units of each block are randomly associated with the treatments. Thus, in the experiment on the effect of energy diet on the body development of lambs (Example 1), if the variation in the characteristics of the environment implies heterogeneity of the 15 pens, it may be convenient to exercise local control by classifying the 15 pens in five blocks of three nearby pens and assigning the three pens (with the respective two animals) of each block to the three diets. If the experiment on control of wheat gibberella (Example 2) is carried out, at each place and year, in a heterogeneous terrain as to soil characteristics, it may be convenient to divide the terrain into four blocks of 12 plots, according to one of the following forms: a) on sloping terrain, constituting four blocks each with 12 plots of the same level range; b) on flat terrain, classifying the 48 plots into four blocks of 12 plots according to their proximity. In these circumstances, soil characteristics (depth, texture, structure, fertility and humidity) of plots in the same block can be expected to be more similar than of plots in different blocks. Then, the 12 plots in each block are associated with the 12 combinations of the levels of fungicide and cultivar factors.

The most effective local control encompasses the important extraneous characteristics with the fewest classifications of the experimental units. The following two procedures are particularly suitable for obtaining effective local control: a) association of the classifications of the experimental units according to two or more relevant extraneous characteristics for the constitution of a single classification for local control; b) use of classifications for local control of extraneous characteristics of the initial experimental material to control characteristics that may manifest in the experimental period, particularly in the execution of experimental techniques. Suppose that in the experiment of Example 1 in which local control of the characteristics of the environment is carried out by classifying the 15 pens in five blocks of three nearby pens, the variation of weaning dates of the 30 animals is considerable. In these circumstances, it may also be appropriate to classify the 30 animals in five blocks of six animals with weaning at close dates and then associate the blocks of animals with the blocks of plots, constituting five blocks of three plots, each with two animals. This same classification of the experimental units can be considered in the implementation of management techniques that may imply heterogeneity of the experimental material, executing them block by block. In the experiment of Example 2, the same classification of the plots according to level range or proximity constituted for local control of soil characteristics can be used to control relevant extraneous characteristics related to cultivation techniques, such as planting, applications of fungicide, insecticide and herbicide, and harvesting.

3.6.3 Statistical control

Statistical control comprises the record of the observed values of one or more variables that express relevant extraneous characteristics and their use to adjust the observed values of response variables to separate the effects of these characteristics from the effects of experimental factors and the experimental error that affects these effects.

In the experiment in Example 1, statistical control of the initial lamb weight may be appropriate to control its effect on body weight at instants of the experimental period, and live weight and warm carcass weight, in one of the following two circumstances: a) the animals in each block constituted for local control of the characteristics of the environment or of the amplitude of weaning dates have considerable differences in initial weight; b) the amplitude of the weaning dates is not considerable to require local control, but the variation in the initial weight is relevant. In these circumstances, statistical control can be used, recording the initial weight of the animals and using it to adjust the observed values of response variables.

An extraneous variable considered for statistical control is called **covariate**. This experimental control is performed by the **covariance analysis**. This statistical analysis procedure is based on the existence of a relationship between the response variable and the covariate, which is generally assumed linear. The adequacy of the statistical control can be verified by an appropriate preliminary statistical analysis to test the postulated relationship.

Statistical control is less applicable than local control and in some situations is adopted in addition to local control, as illustrated above considering Example 1. Furthermore, when appropriate, it is generally applicable to only one or a few response variables. It should be pointed out that statistical control only makes sense if there is a relationship between the response variable and the covariate. For example, there may be a linear relationship with the initial weight (at 70 days) of the animal's body weight at the ages of 84 and 98 days, but not of later weighings, live weight and warm carcass weight.

Statistical control should be used with caution when there is a suspicion that the effects of treatments affect the covariate, as in this case inferences may become biased.

Local control and statistical control allow separating the effects of extraneous characteristics from effects of experimental factors and the experimental error that affects these effects. However, these procedures of experimental control have restrictive consequences: They impose loss of information about the experimental error. Thus, they can be used to control only a few extraneous characteristics or their aggregates.

Effects of extraneous characteristics that are not controlled by experimental techniques, local control and statistical control are confounded with effects of experimental actors. The resource to avoid bias that may result from this remaining confounding is randomization.

3.6.4 Randomization

Randomization is the random association of extraneous characteristics with treatment factors.

Randomization is the process of experimental control to render unbiased the confounding of effects of extraneous characteristics not controlled by experimental techniques, local control and statistical control with effects of treatments. It is performed by randomization of treatments and randomization of experimental technique.

3.6.4.1 Randomization of treatments

Randomization of treatments is the random association of the experimental units of a treatment factor with the levels of this factor.

Randomization assigns to the experimental units of a treatment factor equal probability of association with any level of this factor. Consequently, the treatments are equally likely to be affected positively or negatively by the effects of the extraneous characteristics affected by randomization. Thus, the confounding of the effects of these extraneous characteristics with effects of treatments is not biased.

The randomization of treatments is restricted by local control and formations of experimental units to ensure the arrangement of treatments in the experimental units they determine. In the situation of simple local control and a unique formation of experimental units, randomization is performed within each block, separately and independently for each block. In the experiment with lambs (Example 1) with local control of the characteristics of the environment by forming five blocks of three nearby pens, each with two animals, the three pens of each block must be associated with the three levels of the diet factor by random process that must be repeated separately and independently for each block. In the experiment to control wheat gibberella (Example 2) with local control of soil characteristics, in each site of each year, the 12 plots of each of the four blocks should be randomly associated with the 12 combinations of the levels of the factors fungicide and cultivar, separately and independently for each block.

If the experiment comprises more than one formation of experimental units for treatment factors, randomization must be performed separately for each formation. In a formation for two or more treatment factors, randomization should be performed for the complex factor generated by the largest number of these simple factors. The randomization of a complex factor implies randomization of the simple factors that generate it; the separate randomization of simple factors implies randomization of the complex factors they form. In the experiment in Example 2, randomization is performed for the factor fungicide^cultivar, which implies the randomization of each of the factors fungicide and cultivar. In the experiment of Example 1c, with two formations of experimental units - one for each of the factors diet and stimulant - randomization is performed separately for these two factors; as a consequence, the factor diet^stimulant is randomized.

3.6.4.2 Randomization of experimental technique

Randomization of experimental technique is the execution of an experimental technique in the experimental units of treatment factors in random order and, consequently, random association of the levels of the extraneous characteristics that they affect with the treatments.

This randomization assigns to the orders of execution of the experimental technique an equal probability of association with any of the treatments. This implies that the confounding of the effects of treatment factors with effects of extraneous characteristics covered by randomization is not biased.

This use of randomization may be convenient in situations where there is an expectation that the order of implementing an experimental technique can give rise to relevant effects of extraneous characteristics that affect biasedly effects of treatments. For example, in the lamb experiment, it may be appropriate to randomize the order of weighing and slaughtering animals, if these operations take a considerable amount of time; in the wheat experiment, randomization of sowing and harvesting orders may be appropriate if they last a long time.

The randomization of treatments and the randomization of experimental techniques are complementary procedures. Both must be carried out by draw that guarantees equal probability to all possible configurations of association between the randomized extraneous characteristics and treatment factors, under the restrictions determined by local control and formations of experimental units.

According to the experimental control procedures that affect them, the extraneous characteristics of the experimental material are classified into three classes: a) controlled - affected by local control or statistical control; b) randomized - affected by randomization; c) potentially disturbing - not controlled and not randomized. This last class comprises two subclasses: c1) irrelevant - characteristics whose effects are not confounded biasedly with effects of experimental factors; therefore, behave as randomized; c2) disturbing - characteristics whose effects are confounded biasedly with effects of experimental factors.

3.7 Unit Factor

Unit factor is an extraneous characteristic that constitute a relevant classification of the observation units whose classes are the observation units, or the groups of these units determined by local control, or the units of a formation of experimental units. These classes are the levels of the unit factor.

The identification of unit factors depends on the response variable. For example, the experiment with lambs (Example 1) with local control that forms blocks of homogeneous animals and pens comprises three unit factors for the response variables measured at slaughter: Animal, pen and block; for response variables measured repeatedly in the experimental period in the animal there is an additional unit factor: Instant of measurement; and for variables measured repeatedly in the pen there are three unit factors: pen, block and instant of measurement. With the consideration of race as an additional experimental factor (Example 1a) a unit factor must be defined that comprises the extraneous characteristics that are common to the animals of a race. The levels of this unit factor correspond one-to-one with the levels of the race experimental factor; therefore, the effects of these unit and experimental factors will be completely confounded. In the experiment on control of the wheat gibberella (Example 2) with the formation of blocks, there are four unit factors: plot, block, place and year.

A unit factor satisfies properties similar to those required of experimental factor: 1) its levels are chosen and defined for each observation unit, 2) they constitute a small set, 3) they form a partition of the experimental material, and 4) their relationship with the levels of the other unit factors constitute a structure that affects the effects of experimental factors, called **unit structure**.

Unit factors stratify the experimental error in as many strata as the number of factors. The fraction of the experimental error corresponding to a unit factor constitutes a **stratum of the experimental error**.

The correct determination of the strata of the experimental error is crucial for the validity of the inferences. The identification of the components of the experimental error that affect the effects of experimental factors depends on it. Failures in this identification can lead to underestimation or overestimation of these errors and, consequently, to biased inferences.

The association of the unit structure with the condition structure, determined by the randomization of the levels of the treatment factors and the manifestation of the levels of the intrinsic factors, determines the **experiment structure**. The levels of the unit factor associated with an experimental factor are the experimental units of this experimental factor.

3.8 Experimental Error that Affects the Effect of an Experimental Factor

Experimental error that affects the effect of an experimental factor is the effect of the extraneous characteristics on the observed values of the response variable that is confounded with this effect of experimental factor.

This experimental error is composed of a subset of the strata of the experimental error and can vary with the effect of the experimental factor. It comprises one or more strata where the effects of the extraneous characteristics that are confounded with this effect of experimental factor are located.

The experimental error that affects effects of experimental factors excludes the fractions controlled by local control and statistical control. Therefore, it originates from randomized and potentially disturbing extraneous characteristics. A basic assumption for valid inferences is that this experimental error is random, which requires that the effects of treatments are not affected by disturbing extraneous characteristics.

3.9 Observation Error

Observation error is the variation of the observed values of the response variable between the observation units within the elementary experimental units.

The observation error can be determined in experiments where the elementary experimental unit comprises more than one observation unit.

It is important to distinguish between the elementary experimental unit and the observation unit. The incorrect use of observation error instead of experimental error gives rise to biased inferences about the effects of experimental factors. This is because, if the elementary experimental unit comprises more than one observation unit, the experimental errors that affect the effects of experimental factors originate from the variation external to the experimental units. Thus, the elementary experimental unit must be considered as a whole.

4. CONCLUSION

- The sample units, as well as the units of the target population, are complex systems that consist of numerous characteristics that interact dynamically in space and time.
- The discernment of a causal relationship of characteristics requires the understanding of this complexity through a complete and sufficiently detailed description of the sample that identifies the characteristics according to their role in this relationship and the confounding of effects of experimental factors with effects of extraneous characteristics.
- Precise and unbiased inferences about causal relationships of characteristics require basis on a conceptual framework that adequately considers this complexity.
- Experimental control can reduce and make unbiased the confounding of effects of extraneous characteristics with effects of experimental factors and, consequently, guarantee the desirable precision and unbiasedness of these inferences.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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