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Determination of Sample Size Using Resource Equation Methods in Analysis of Variance Models in Animal Studies

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Abstract

The Resource Equation Method is a method developed as an alternative to power analysis for the calculation of sample size in animal studies. With this approach, the sample size is calculated based on the error degrees of freedom (DF) in the variance analysis model. In this study, one and two-factor variance analysis models, which are commonly used in animal studies, are discussed. The minimum and maximum sample size required for these models were calculating and presented in tables. While a minimum of 12 and a maximum of 21 animals in total are sufficient for two independent groups (Design 1), and the total number of animals goes up as the number of groups increases. In one factor, repeated-measures experiment design (Design 2), it was observed that the number of animals to be included in the study decreases significantly as the number of repetitions increases. For all factorial designs (Designs 3 and 4), 2 or 3 animals per subgroup were found to be sufficient. Repeat measure experimental designs can be chosen to increase the power of the study without increasing the number of animal/subjects. Statistical power was calculated for different numbers of groups in Design 1, based on their respective minimum and maximum samples sizes and Cohen's effect sizes, and for most cases, power was found to be much lower than 0.80. Statistical power exceeded 0.80 only in the case of very large effect sizes. Therefore, the researcher can test her hypotheses with larger effect sizes to reach 80% power with sample sizes in independent group comparisons. A determined effect size value for animal studies is not available in the literature. According to the results obtained in our study, the effect sizes for the 2 groups are 1.2; 1.5 and 2.0 for 3 or more groups 0.5; 0.7 and 0.9 can be recommended to take as small, medium and large effect sizes, respectively.

Key Words: Analysis of variance models, animal study, degrees of freedom, effect size, resource equation method

Hayvan Çalışmalarında Kaynak Eşitlik Yaklaşımı ile Örneklem Büyüklüğünün Belirlenmesi

Öz

Kaynak eşitlik yöntemi, hayvan çalışmalarında, örneklem büyüklüğünün hesaplanmasında, güç analiz yöntemine alternatif olarak geliştirilen bir yöntemdir. Bu yaklaşım ile örneklem büyüklüğü varyans analizi modelinde yer alan hata serbestlik dereceleri üzerinden hesaplanmaktadır. Bu çalışmada, hayvan çalışmalarında oldukça sık kullanılan tek ve iki faktörlü varyans analizi modelleri ele alınmıştır. Bu modeller için gerekli olan minimum ve maksimum örneklem büyüklükleri hesaplatılarak tablolar halinde sunulmuştur. 2 bağımsız grupta (Design 1) toplam minimum 12, maksimum 21 hayvan yeterli iken grup sayısı arttıkça toplam hayvan sayıları bir miktar artmaktadır. Tek faktörlü ve tekrarlı ölçüm deneme tasarımlarında (Design 2), tekrar sayısı arttıkça çalışmaya dahil edilecek hayvan sayısının büyük oranda azaldığı gözlenmiştir. Tüm faktöriyel tasarımlar için (Design 3 ve 4), her bir alt grupta bulunan hayvan sayılarının 2 veya 3 ile yeterli olduğu tespit edilmiştir. Deney hayvanı sayısını artırmadan tekrarlanan ölçümlü deneme düzenleri seçilerek çalışmanın gücü artırılabilir. Bunun yanısıra, Design 1'deki grup sayılarına göre minimum ve maksimum örneklem büyüklükleri için Cohen'in uygun etki büyüklüklerine göre istatistiksel güç değerleri hesaplanmış ve gücün 0.80'nin çok altında olduğu gözlenmiştir. Gücün 0.80 ve üzeri bir değere sahip olması ancak etki büyüklüklerinin çok büyük olması durumunda söz konusudur. Dolayısıyla araştırmacı, bağımsız grup karşılaştırmalarında, örneklem büyüklükleri ile %80 güç düzeyine ulaşabilmek için hipotezlerini daha yüksek etki büyüklükleri ile test edebilir. Hayvan çalışmaları için belirlenmiş bir etki büyüklüğü değerine literatürde rastlanılamamıştır. Çalışmamızda elde edilen sonuçlara göre, 2 grup için 1.2, 1.5 ve 2.0, 3 ve daha fazla grup için ise 0.5, 0.7 ve 0.9 sırasıyla küçük, orta ve büyük etki büyüklükleri olarak alınması önerilebilir.

Anahtar Kelimeler: Etki büyüklüğü, hayvan çalışmaları, kaynak eşitlik yöntemi, serbestlik derecesi, varyans analiz modelleri

INTRODUCTION

As a result of Louis Pasteur's animal research, experiments on animals have become standard practice for medical research. In addition, the anatomical and physiological similarities between humans and animals (mammals in particular) have led investigators to conduct experiments primarily on animals in the scientific research associated with human health. Therefore, despite all the current advances in the field, the use of experimental animals is vital in investigations for medical interventions and drug research, in order to adapt the results to human use. In this way, any possible risks that may emerge in humans should be minimized (1,2). However, while reducing the possible risks to humans, in order to observe animal rights and keep their depredation to a minimum, animals should not be used in these studies unless it is mandatory and it is imperative to follow experimental study ethics and obey standards during planning if animals are to be used. The planning phase of the experiment is one of the most important parts of each investigation. The correct determination of experimental design at this phase and determining the sample size correctly is critical economically, ethically and scientifically (3).

One of the main ethics rules is the 3R rule used widely in the planning and implementation of animal studies. The second R (Reduce); expresses that the experiment should be conducted with a small number of animals (4,5). Therefore, the Reduce rule of the 3R rules demonstrates to us, the investigators, that the sample size is very important and one should be careful when deciding the sample size and be attentive when calculating it.

The calculation methods of sample size in clinical studies are quite an enlightened subject in the literature; there are, however, not so many options for animal studies. The sample size can be calculated in clinical studies by different formulations according to the design of the research, the type and number of dependent variables, to the one or two-way hypothesis test, and to the status of assignment to the groups. In addition, the sample size can be calculated using paid or free of charge package programs and online websites. The sample size can even be calculated using the nomogram calculated by entering the effect size and power values by Altman (3, 6-11).

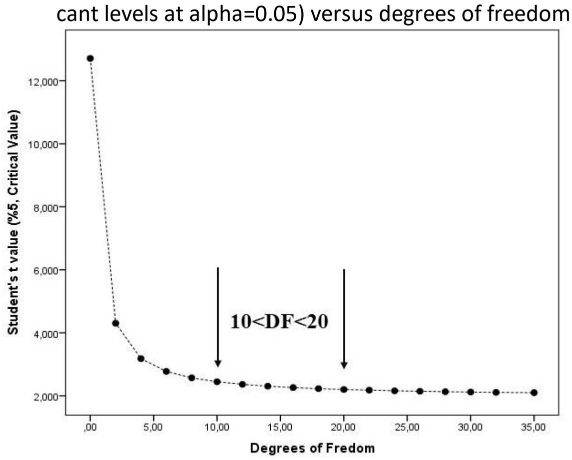
A small number of alternatives are present in the literature for the calculation of sample size in animal studies. Four different sample size methods are encountered in the literature search, namely the traditional methods, power analysis, the Resource Equation Method and the Keep It Simple Stupid (KISS) approach. According to traditional methods, researchers use the sample size in previous studies which are similar to theirs in their own studies. Although this approach seems to be a good approach, it may be a misleading or unsatisfactory approach for researchers designing a new study. Additionally, the method does not have a statistical basis. The power analysis approach is a very popular, powerful, and scientific approach to determine the sample size. However, some information -such as the mean, standard deviation or effect size- is needed for power analysis. Researchers can obtain this information from previous studies as well as conducting a pilot study. If there is not any literature information available or a pilot study cannot be done, alternatively, power analysis can be performed by using the standardized effect size values determined by Cohen (12) for appropriate experimental designs. The addition, the sample sizes obtained by power analyses are quite high for animal studies (12-17).

The KISS approach, is a simple approach suggested by Festing (13) by combining the traditional approach and the Resource Equation Method. With this approach, effect sizes are calculated again (13). As a result, in order to use this method, it is also necessary to have the mean and standard deviation values.

The Resource Equation Method was first described by Mead in 1988 as an alternative to power analysis for the calculation of sample size in animal studies (18). Its calculation is easy; however, it is not accepted as being a robust a model as the power analysis approach however, it is the favored method when a smaller number of subjects/animals are to be used. The Resource Equation Method may be preferred for the conditions that are given, respectively; firstly, no information about the effect size or standard deviation, secondly, difficulty in determining the effect size, thirdly, big number of dependent variables, lastly, not be able to apply power analysis or complex experimental designs (10, 12-16). If the researcher finds interpreting the results more sufficient than the statistical test or the power of the test, this method can also be used easily (10).

The number of animals to be included in the study is calculated based on the degrees of freedom (DF) in the variance analysis model (ANOVA) in this method. It should be a value between $10 \leq DF \leq 20$ (19,20). The addition of more animals to the study when the degree of freedom is less than 10 would increase the possibility of obtaining a more meaningful result. Even so, the addition of more animals would not increase the possibility of obtaining a more meaningful result when the degree of freedom is higher than 20. Therefore, the margins of the degrees of freedom should be considered sufficient for the sample size. This situation is seen quite clearly in Graphic 1. The Resource Equation Method is preferred in all animal studies, although it requires quite a large effect size (1,21).

Graphic 1. Critical values of Student's t-test (two-sided test, significant levels at alpha=0.05) versus degrees of freedom.



In this study, one and two-factor variance analysis models, which are commonly used in animal studies, are discussed. The minimum and maximum sample size required for these models were calculating using the Resource Equation Method and presented in tables. Moreover, this study is extremely important for the manuscript evaluation process of the reviewers and editors of journals in which animal studies are published, in order to conduct a standard evaluation. It is also essential that researchers can report their studies in a standard format. In addition, this aim of this study is to be a helpful guide for researchers conducting animal studies, ethics board members and for the reviewers and editors of journals in which animal studies are published.

MATERIAL AND METHODS

The present study, with the help of simple formulations suggested by Arifin et al. (19) will present steps for the calculation of the minimum and maximum sample sizes required, based on the degrees of freedom for one or two-factor variance analysis models widely used (19). Source of variations and degrees of freedom for each trial design are summarized in Table 1 (22).

Table 1. Sources of variation and degrees of freedom for each trial design

		Source	DF
One Factor	One Way ANOVA (Design 1)	Total	N-1
		Between Groups	(k-1)
		Error	k(n-1)
	Repeated Measures ANOVA (Design 2)	Total	(nr-1)
		Between Subject	(n-1)
		Within Subject	n(r-1)
		Period	(r-1)
		Error(SubjectXPeriod)	(n-1)(r-1)
Two Factor	Repeated Measures on one factor (Design 3)	Total	(nkr-1)
		Between Subject	(nk-1)
		Factor1	(k-1)
		Error1	k(n-1)
		Within Subject	nk(r-1)
		Factor2	(r-1)
		Factor1XFactor2	(k-1)(r-1)
		Error2(Factor2XError1)	k(n-1)(r-1)
	Repeated Measures on two factor (Design 4)	Total	(nrs-1)
		Between Subject	(n-1)
		Within Subject	nk(r-1)
		Factor1	(r-1)
		Error1(SubjectXFactor1)	(n-1)(r-1)
		Factor2	(s-1)
		Error2(SubjectXFactor2)	(n-1)(s-1)
		Factor1XFactor2	(r-1)(s-1)
		Error3(SubjectXFactor1XFactor2)	(n-1)(r-1)(s-1)

DF: degrees of freedom; k: number of groups; r: number of repeated measurement of the first factor; s: number of repeated measurement of the second factor

Design 1- One Way ANOVA

Error degree of freedom is obtained by DF= k(n-1) in One Way ANOVA trial designs. Where k is number of groups, and n is number of animal per group. The number of animals in each group is calculated based on the degree of freedom formula as in the Equation 1 (19,22).

Equation 1: $n = \frac{DF}{k} + 1$

Any sample size keeping the error degree of freedom between the margins of 10 to 20 is accepted as sufficient in animal experiments. The minimum and maximum sample sizes in each group are calculated as Minimum n= (10/k) +1, and Maximum n= (20/k) +1. There is such a rule when the minimum and maximum number of animals calculated in each group is not an integer: The number obtained for the minimum number of animals is rounded up to the first integer larger than itself when it is not an integer (Minimum n = 10/3 + 1 = 4.3 = rounded up to 5 animals/group). The number obtained for the maximum number of animals is rounded down to the first integer smaller than itself when it is not an integer (Maximum n = 20/3 + 1 = 7.7 = rounded down to 7 animals/group). Considering the group numbers, the total sample sizes are multiplied by the group numbers based on animal numbers after rounding up or down (19).

Design 2- Repeated Measures ANOVA

In repeated measure experimental designs, only the measurements of the subjects/animals and the same subjects/animals taken at different times are performed. The degree of freedom of error is DF= (n-1) (r-1) in such a design. Where, n is the total number of subjects/animals, and r is the number of repeated measurements. The number of subjects/animals in each group is calculated based on the degree of freedom formula as in the Equation 2 (19,22).

Equation 2: $n = \frac{DF}{(r-1)} + 1$

The minimum and maximum sample sizes in each group are calculated as Minimum n= (10/k) +1, and Maximum n= (20/k) +1. The number of animals are rounded up or down as mentioned in Design 1 when the number is not an integer. Also, the total sample size is multiplied by the repeat number when the animals are to be sacrificed during different measurements for each animal (19).

Design 3- Two Factor; Factor 1: Groups; Factor 2: Repeated Measures ANOVA

Two degrees of freedom, such as between the subjects and within-subjects, are calculated in trial designs including two factors, one of them being a repeated measure. Between-subject error DF is calculated as (k(n-1)), and within-subject error DF in this ANOVA design is calculated as (k(n-1)(r-1)). Based on this, the sum of the two degrees of freedom is calculated as DF= kr(n-1). Where, k is the number of groups, r is the number of repeated measurements, and n is the number of subjects/animals per group. The total number of animals is calculated as in Equation 3 (19,22).

Equation 3: $n = \frac{DF}{kr} + 1$

The minimum and maximum sample sizes in each group are calculated as Minimum n= (10/k) +1, and Maximum n= (20/k) +1. The number of animals are rounded up or down as mentioned in Design 1 when the number is not an integer. Considering the group numbers, the total sample sizes are multiplied by the group numbers based on the animal numbers after rounding up or down. Also, the total sample size is multiplied by the repeat number when the animals are to be sacrificed during different measurements for each animal (19).

Design 4-Two Factor; All Factors: Repeated Measures ANOVA

Three degrees of freedom of error are calculated in experimental designs including two factors, each with repeated measures. The degree of freedom for the first and second factors are calculated as DF1= (n-1)(r-1) and DF2= (n-1)(s-1), respectively. The third degree of freedom of error is calculated as DF3= (n-1)(r-1)(s-1). The total of error degrees of freedom are presented as DF= (n-1)(r-1) + (n-1)(s-1) + (n-1)(r-1)(s-1). Subsequently, it is summarized as DF=(n-1)(rs-1). Where, r is the number of repeated measurements of the first factor, s is the number of repeated measurements of the second factor and n is the number of subjects/animals per group. The total number of the animals is calculated as in Equation 4 (22,23).

Equation 4: $n = \frac{DF}{(rs-1)} + 1$

The minimum and maximum sample sizes in each group are calculated as Minimum n= (10/k) +1, and Maximum n= (20/k) +1. The number of animals are rounded up or down as mentioned in Design 1 when the number is not an integer. Also, the total sample size is multiplied by the repeat numbers in Factor 1 and 2 when the animals are to be sacrificed during different measurements of Factor 1 and 2 for each animal (19).

RESULTS

The results of minimum and maximum sample sizes for Design 1 and 2 are presented in Table 2, the results for Design 3 in Table 3, and results for Design 4 are presented in Table 4. The values in the first column in Table 2 show group numbers in Design 1 and repeat numbers in Design 2. The groups in Design 1 are independent, while the groups in Design 2 are dependent. The number of subjects/animals are seen to be lower in the repeated measure designs (Design 2) when the number of animals in Design 1 and 2 are compared. The study is conducted with a minimum of 6 animals in each group and 12 in total when the number of groups is 2 in Design 1; while using only 11 animals would suffice in a trial design with two repetitions. A minimum of 5 animals in each group and 15 in total is sufficient when the number of groups/repetitions is 3 in Design 1, this number would be a maximum of 11 in Design 2. In two-factor experimental designs (Designs 3 and 4), the largest number of animals to be included in the study were required for the case where both factors had two groups/repetitions. As the number of groups/repetitions increased, so did the number of animals to be included in the study. In particular, when the number of groups/repetitions was three or more, the number of animals in each subgroup was observed to be 2 or 3.

Table 2. Minimum and maximum sample size results of Design 1 and Design 2

		Design 1					Design 2	
				Effect size				
Number of group/repetitions		**The number of subject/animal of per groups (n)**	**Total sample size[a] (N)**	**0.20**	**0.50**	**0.80**	**Total sample size (n)**	**Total sample size (Sacrificed required)[b]**
2	Min	6	12	0.061	0.123	0.241	11	22
	Max	11	22	0.073	0.201	0.431	21	42
				0.10	**0.25**	**0.40**		
3	Min	5	15	0.059	0.110	0.214	6	18
	Max	7	21	0.064	0.143	0.307	11	33
4	Min	4	16	0.057	0.097	0.183	5	20
	Max	6	24	0.062	0.133	0.289	7	28
5	Min	3	15	0.055	0.082	0.142	4	20
	Max	5	25	0.060	0.121	0.259	6	30
6	Min	3	18	0.055	0.086	0.152	3	18
	Max	4	24	0.058	0.106	0.215	5	30
7	Min	3	21	0.056	0.088	0.162	3	21
	Max	3	21	0.056	0.088	0.162	4	28
8	Min	3	24	0.056	0.091	0.171	3	24
	Max	3	24	0.056	0.091	0.171	3	24
9	Min	3	27	0.056	0.081	0.181	3	27
	Max	3	27	0.056	0.081	0.181	3	27
10	Min	2	20	0.053	0.072	0.112	3	30
	Max	3	30	0.056	0.096	0.190	3	30
>11	Min	2	22	0.053	0.073	0.116	2	22
	Max	2	22	0.053	0.073	0.116	3	33

a: multiplied by the number of groups; b: multiplied by the number of repetitions

Table 3. Minimum and maximum sample size results of Design 3

Number of groups	Number of repetitions	The number of subject/animal per groups Min	The number of subject/animal per groups Max	Total sample size[a] Min	Total sample size[a] Max	Total sample size (Sacrificed required)[b] Min	Total sample size (Sacrificed required)[b] Max
2	2	4	6	8	12	16	24
	3	3	4	6	8	18	24
	4	3	3	6	6	24	24
	5	2	3	4	6	20	30
	≥ 6	2	2	4	4	-	-
3	2	3	4	9	12	18	24
	3	3	3	9	9	27	27
	≥ 4	2	2	6	6	-	-
4	2	3	3	12	12	24	24
	≥3	2	2	8	8	-	-
5	2	2	3	10	15	20	30
	≥3	2	2	10	10	-	-
≥6	≥2	2	2	-	-	-	-

a: sample size multiplied by the number of groups; b: the number of repetitions multiplied by the total sample size

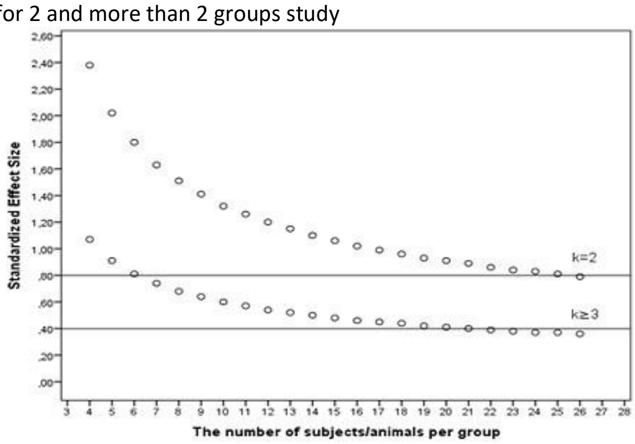
Table 4. Minimum and maximum sample size results of Design 4

Number of repetitions for $Factor_1$	Number of repetitions for $Factor_2$	Total sample size (N) Min	Total sample size (N) Max	Total sample size (Sacrificed required)[b] Min	Total sample size (Sacrificed required)[b] Max
2	2	5	7	20	28
	3	3	5	18	30
	4	3	3	24	24
	5	3	3	30	30
	≥ 6	2	2	-	-
3	2	3	5	18	30
	3	3	3	27	27
	≥ 4	2	2	-	-
4	2	3	3	24	24
	≥ 3	2	2	-	-
5	2	3	3	30	30
	≥ 3	2	2	-	-
≥ 6	≥ 2	2	2	-	-

b: Sample size multiplied by number of repetitions $Factor_1$ and $Factor_2$

Effect sizes were calculated using the G-Power package program for cases where the minimum number of animals in a group was 4 and the maximum number of 26, a two-way hypothesis for 2 group comparisons and a test power of 0.80 and a type I error of 0.05, and the results are given in Graphic 2. Three different effect size values are present as small, intermediate and large, as determined by Cohen for different trial designs to be used in clinical studies. He determined the small, intermediate and large effect sizes for two-group comparisons as 0.20, 0.50 and 0.80, respectively, and for single factor and comparison of more than 2 groups as 0.10, 0.25 and 0.40, respectively.

Graphic 2. Variation of effect size according to different sample size for 2 and more than 2 groups study



Graphic 2 demonstrates that at least 25 animals should be present in each group when the effect size is 0.80 in a study with two groups. At least 20 animals should be present in each group with an effect size as 0.40 in a study with more than two groups. In other words, it could be stated that for two-group comparisons, in cases where the sufficient sample size in each group is a minimum of 6 or 8, the effect sizes are calculated as 1.80 or 1.51 for 0.80 power, 0.05 type I error and a two-way hypothesis. When the study is performed with more than 2 groups and the power is to be 0.80 and type I error as 0.05, these values are calculated as 0.70 or 0.80.

DISCUSSION AND CONCLUSION

For single-factor and two-factor trials, Arifin et al. (19) considered calculation steps on the basis of the Resource Equation Method for cases where repeated measures are made for only one of the factors. Taking inspiration from this study, we calculated minimum and maximum sample sizes required for single-factor and two-factor models of analysis of variance, and presented them in tables. Moreover, we have calculated statistical power using sample sizes reported in Table 1 and Cohen's effect sizes (Table 2). Two-factor designs were also considered. Minimum and maximum sample sizes are reported in tables, first for the experimental designs where a single factor is repeated, then for designs where both factors are repeated (Table 3 and 4).

Doğan and Doğan (15) examined minimum and maximum sample sizes for a single factor and independent groups, as well as statistical power for these sample sizes based on Cohen's effect sizes (15). The findings of the present study parallel with Doğan and Doğan's (15) findings. Findings show that a minimum of 12 and a maximum of 21 animals in total are needed for two independent groups, and the total number of animals goes up as the number of groups increases. Statistical power was calculated for different numbers of groups in Design 1, based on their respective minimum and maximum samples sizes and Cohen's effect sizes, and for most cases, power was found to be much lower than 0.80. Statistical power exceeded 0.80 only in the case of very large effect sizes (Diagram 2). It is expected that effect sizes in animal studies are larger than the highest effect sizes in clinical trials. Because the small number of animals in each group in animal studies will cause the effect size to be quite high. This indicates that for independent group comparisons, researchers can use larger effect sizes to test their hypotheses to reach 80% power with a given sample size. In addition, an effect size value determined for animal studies is not available in the literature. According to the results obtained in our study, the effect sizes for the 2 groups are 1.2, 1.5 and 2.0 for 3 or more groups 0.5, 0.7 and 0.9 can be recommended to take as small, medium and large effect sizes, respectively.

Akbulut (17) calculated minimum and maximum sample sizes for single-factor and two-factor experimental designs, as well as statistical power values for these sample sizes based on Cohen's effect sizes. He also provides tables reporting sample size and statistical power values for different dimensions in two-factor experimental designs (17). Akbulut's (17) findings, consistent with the findings of the present study, show that in single-factor, repeated-measures experimental design (Design 2), the number of animals to be included in the study decreases significantly as the number of repetitions increases. A minimum of 11 and maximum of 21 animals were needed for two repetitions, but these figures declined, respectively, to 6 and 11 for three repetitions, and 5 and 7 for four repetitions. When the number of repetitions was eight or above, the minimum and maximum numbers of animals to be included in the study were found to be 3. In Design 3, the total number of animals to be included in the study declined as the number of repetitions within each independent group increased. However, as the number of independent groups increased, so did the total number of animals to be included in the study. For all factorial designs (Designs 3 and 4), 2 or 3 animals per subgroup were found to be sufficient. This method thus shows that the number of animals per group can be lowered when the number of groups/repetitions is larger than two. These findings indicate that the Resource Equation Method can be very useful in determining sample size in animal study.

Festing (13) examined methods for calculating sample size in animal studies, as well as the relationship between effect size and sample size. He proposed using the KISS (Keep It simple, Stupid) approach. The effect sizes are recalculated with this approach. A reference study from the relevant literature is discussed. The effect size is calculated again by using the number of animals in each group in this study, the mean and standard deviation values of the parameter of interest. According to the number of animals in each group in the study, the effect size determined by Cohen for 80% power, 0.05 type 1 error and two-tailed hypothesis is found, multiplied by the standard deviation value in the reference study, and a new effect size value is determined. Then this calculated value is divided by the mean value in the study and multiplied by 100 and the percent change value is calculated (13). As a result, in order to use this method, it is also necessary to have the mean and standard deviation values.

In animal studies, decisions regarding proper experimental design and sample size are of extreme importance both for scientific and ethical reasons. Reliability of the findings to be obtained depends on using scientific methods when deciding on the proper experimental design and the sufficient sample size for that design. When experimental studies on animals are evaluated, the preferred method for sample size is generally the power analysis method. Therefore, ethics boards, editors and journal reviewers question how the number of subjects is determined in animal experiments and whether power analysis is carried out. To calculate the sample size using the power analysis method, concepts such as the mean, standard deviation and effect size should be obtained from previously published studies or by conducting a pilot study. In cases where there is no literature information on the subject and no pilot study is planned then, as an alternative, power analysis can be performed based on the standardized sample size values predetermined by Cohen for appropriate experimental designs (12). Unlike the power method, the Resource Equation Method includes easy calculation steps without the need for mean, standard deviation, and effect size.

In addition, the effect sizes would result to be quite high in order to keep the number of animals in each group at a minimum level for 0.80 power when the sample size is calculated using power analysis method. By using the Resource Equation Method, researchers will be able to avoid such ethical problems. It can be noted that the number of animals that should be present in each group in experimental designs with more than 2 groups (Design 1), and in repeat measure experimental designs (Design 2) is decreased. Repeat measure experimental designs can be chosen to increase the power of the study without increasing the number of animal/subjects. Thus, the expected power can be increased with the same number of animals by choosing cross-over or repeated-measure experimental designs as experimental designs. That is, the power of the study can be increased by choosing repeated-measurement experimental design without increasing the number of experimental animals. As a result, studies can be conducted with fewer animals by using special experimental designs such as Change-over, Crossover, Switchback, Reversal experimental designs, and various Latin square experimental designs instead of the conventional experimental designs (13-16).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

Not applicable to this article.

REFERENCES

1. Ankaralı H, Ankaralı S. (2019). Experimental Designs and Sample Sizes for Increased Efficiency in Animal Experimentation. Anatol Clin. 24(3): 248-258.
2. Balkan A, Balkan M. (2013). Legal Requirements Involving Ethics in Animal Research, Laboratory Standardization, and Animal Care. Turk Toraks Derg. 14 (Suplement 2): 6-9.
3. Kilkenny C, Parsons N, Kadyszewski E, et al. (2009). Survey of The Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals. PLoS One. 4(11): e7824.
4. Aske KC, Waugh CA. (2017). Expanding The 3R Principles: More Rigor and Transparency in Research Using Animals. EMBO Reports. 18(9): 1490-1492.
5. Flecknell P. (2002). Replacement, Reduction and Refinement. Altex. 19:73-78.
6. Bolarinwa OA. (2020). Sample Size Estimation for Health and Social Science Researchers: The Principles and Considerations for Different Study Designs. Niger Postgrad Med J. 27: 67-75.
7. Lwanga SK, Lemeshow S. (1991). Sample Size Determination in Health Studies: A Practical Manual. World Health Organization; Geneva.
8. Serdar CC, Cihan M, Yücel D, Serdar MA. (2021). Sample Size, Power and Effect Size Revisited: Simplified and Practical Approaches in Pre-Clinical, Clinical and Laboratory Studies. Biochem Med (Zagreb). 31(1): 27-53.
9. Stevenson MA. (2021). Sample Size Estimation in Veterinary Epidemiologic Research. Front Vet Sci. 7: 539573.
10. Charan J, Kantharia ND. (2013). How to Calculate Sample Size in Animal Studies? J Pharmacol Pharmacother. 4(4): 303-306.
11. Altman DG. (1982). How large a sample? In: Gore SM AD (editor). Statistics in Practice I. UK: British Medical Association, London.
12. Cohen J. (1988). Statistical Power Analysis for The Behavioral Sciences. 2 nd Ed. Lawrence Erlbaum Associates, New York.
13. Festing MFW. (2018). On Determining Sample Size in Experiments Involving Laboratory Animals. Lab Anim. 52(4): 341-350.
14. Cangür Ş, Ankaralı H, Ankaralı S. (2013). Special Experimental Designs Using Laboratory Animals and Sample Size: Review. Turkiye Klinikleri J Biostat. 5(2): 75-88.
15. Doğan I, Doğan N. (2020). Estimation of Sample Size with Resource Equation Method in Experimental Animal Studies. Turkiye Klinikleri J Biostat. 12(2): 211-217.
16. Ilyas MN, Adzim MKR, Simbak NB, Atif AB. (2017). Sample Size Calculation for Animal Studies Using Degree of Freedom (E); An Easy and Statistically Defined Approach for Metabolomics and Genetic Research. Curr Trends Biomedical Eng&Biosci. 10(2): 555785.
17. Akbulut Ö. (2023). Hayvan Deneylerinde Örneklem Büyüklüğünün Kaynak Eşitlik Yöntemi ile Belirlenmesi ve Güç Analizi. KSÜ Tıp Fak Der. 18(2): 117-125.
18. Mead R, Gilmour SG, Mead A. (2012). Statistical Principles for The Design of Experiments: Applications to Real Experiments. Cambridge University Press, Cambridge.
19. Arifin WN, Zahruddin WM. (2017). Sample Size Calculation in Animal Studies Using Resource Equation Approach. Malays J Med Sci. 24(5): 101-105.
20. Festing MFW. (2010). The UFAW Handbook On the Care and Management of Laboratory and Other Research Animals. In: The Design of Animal Experiments. Kirkwood J, Hubrecht RC (eds). pp.23-36. A John Wiley& Sons Ltd, United Kingdom.
21. Festing MFW, Altman DG. (2002). Guidelines for The Design and Statistical Analysis of Experiments Using Laboratory Animals. ILAR J. 43(4): 244-258.
22. Hau JH, Schapiro SJ. (2014). Handbook of Laboratory Animal Science. Volume: III, Animal Models. CRC press. New York.
23. Gürbüz F, Başpınar E, Çamdeviren H, Keskin S. (2003). Tekrarlanan Ölçümlü Deneme Düzenlerinin Analizi. Yüzüncü Yıl Üniversitesi Matbaası. Van.

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