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ROBUSTNESS OF MIXED MODEL ANALYSES TO ESTIMATE FIXED EFFECTS FOR LITTER TRAITS IN RABBITS

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The use of mixed models to analyse experiments in animal production was discussed taking into account that, in general, the animals used in the experiments are relatives (in some cases, close relatives as full or half sibs). An example to estimate the effects of year-season, parity order and some covariates on litter size and weight traits and kindling interval was given using three sets of genetic parameters that fall within the range of parameters reported in the literature. Results obtained from such example show that it is not necessary to have very accurate values of the genetic parameters to be used in the mixed model to get the estimates of the fixed effects. This concept was proved in results of the present study since the estimates of least square means of the fixed factors (year-season, parity order) and the regression coefficients of the covariates remain unchanged with the change of the parameter set used to solve the mixed model equations. Also, results of the tests of significance are always the same for the three sets of genetic parameters. In conclusion, to get inferences about fixed effects, the mixed model methodology seems to be robust to non negligible changes in the parameters used.

Keywords: Mixed model,fixed effects, litter traits, rabbits.

The majority of the experiments in animal production, carried out with live animals, have a genetic component, no matter the principal objective for these experiments to be non-genetic. For example, if a nutritionist is interested in estimating the differences between two types of diets for litter size in a rabbit line (Quevedo *et al.*, 2005), he has to design an experiment allocating does to each diet, in a number enough to detect such significance in the expected differences. The genetic component comes of each other but have some relationship between them. Some of these does could be full sibs or half sibs; others could have other type of relationship or could be unrelated. For statistical analysis of litter size for example, a sound model describing the records, should consider all the factors affecting such trait. In such example, we have to consider at least, type of the diet, the relationship between the does and the fact that a doe can have repeated records. The factors such as type of the diet, parity order, sex, hormonal treatment, type of management and others are considered as fixed factors. Whereas, the factors related to the animals and their relationships are considered as random factors. To include these factors into the models, it is necessary to know the variance components attributable to them or some ratios of these components as the heritability or repeatability (Henderson, 1984). These models are taking into account fixed and random factors at the same time and for this reason they are called mixed models. These models should be used to analyse experiments where the animals are related. It is difficult to set up an experiment in animal production with animals completely independent, without accounting any relationship between them.

The statistical importance of considering the random effects in the model lies in that the standard errors of the least square means of different levels of a fixed factor (or their differences) depending on the variance components of the random factors involved in the model of analysis. This methodology is called Generalised Least Squares (Henderson, 1984). In the case where the random factors refer to the individuals and their relationships, the knowledge of corresponding variance components can be represented by the knowledge of some ratios as the heritability and repeatability (Falconer, 1989). Consequently, a test trying to prove that if the difference between two levels of a fixed factor to be significant, such test should take into account the random factors and the structure of the covariance between their levels.

True variance components of the random factors affecting the traits in the populations of our interest are never known and the experiments with objectives outside the field of quantitative genetics, in general have not enough size to allow the estimates of variance components or corresponding genetic parameters to be used in the analysis (Gianola *et al.,* 1986). An alternative could be the use of genetic parameters estimated in previous experiments within the same population and for the same traits (García and Baselga, 2002a), but in many cases alternative is not possible. Another possibility is that of using the genetic parameters in the middle of the range of the ones reported in the literature (García and Baselga, 2002b), expecting that the results of the tests concerning fixed effects are very robust within the range of the most common estimates of the genetic parameters. The main objective of this work will be to check the robustness of this type of analysis using litter size and weight traits as an example to detect the influences of several non-genetic factors on these traits in rabbits.

MATERIALS AND METHODS

The experimental work was conducted at the Rabbitry of Faculty of Agriculture at Moshtohor, Banha University, Egypt.

Data and breeding plan:

The experimental material included 707 litters of New Zealand White (NZW) rabbits produced by 204 does fathered by 75 sires and mothered by 97 dams.

The animals were housed in a windowed insulated rabbitry. The breeding animals were kept individually in wired cages of Californian type. Cage of each doe provided with a metal nest box for kindling and nursing its progeny during the suckling period. All the flock was kept under the same managerial and environmental conditions.

Rabbits were fed with a commercial pelleted feed (minimum of 18% crude protein and 14% crude fiber). Clean fresh water was available freely to the rabbits through automatic waterers. Urine and feaces dropped from the cages were cleaned every day in the morning.

According to Khalil (1993), at the beginning of the breeding season (during October), the females were divided at random into groups ranging from three to five does. For each group of does, a service buck was assigned at random with the only restriction of not being a close relative (avoiding full-sib, half-sib and parent-offspring matings). Each doe was transferred to the buck's cage to be mated. Does were palpated 15 days post mating to detect pregnancy, those which failed to conceive were returned to the same mating buck to be remated.

Litters were weaned at the age of 35 days after birth. Young does were added to the herd as needed to replace those lost by death or culling.

Statistical analysis

The data were analyzed applying the procedure of generalized least squares (Henderson, 1984) and using a software written by the authors in APL programming language, under the following models and sets of genetic parameters shown in Table 1:

1st)Litter size at birth and litter size at weaning:

$$Y_{ijkldm} = \mu + YS_i + P_j + S_k + D_l + I_d + e_{ijkldm} \quad \text{....... (Model 1)}$$

Where:

Y_{ijkldm} is the record of ijkldmth litter.

μ Stands for the overall mean.

Table (1): Three sets of genetic parameters used to solve the mixed model equations.

Traits	Set I		Set II		Set III	
	h^2	r	h^2	r	h^2	r
Kindling interval	0.05	0.15	0.10	0.20	0.20	0.30
Litter size at birth	0.10	0.15	0.15	0.20	0.20	0.25
Litter weight at birth	0.10	0.20	0.15	0.25	0.20	0.30
Litter size at weaning	0.10	0.15	0.15	0.20	0.20	0.25
Litter weight at weaning	0.10	0.20	0.15	0.25	0.20	0.30

h^2 = Heritability r = Repeatability

YS_i is the fixed effect of i^{th} year-season (every three consecutive months are one year-season, beginning in December of the previous year).

P_j represents the fixed effect of j^{th} class of parity order (class 1 is for parity 1; class 2 for parity 2; class 3 for parities 3, 4 & 5; class 4 for parities 6, 7 & 8; and class 5 for parities after 8).

S_k stands for the random effect due to k^{th} sire of does (genetic effect).

D_l stands for the random effect due to l^{th} dam of does (genetic effect).

I_d represents the random effect due to d^{th} doe and includes mendelian sampling and permanent environmental effects.

e_{ijkldm} is the random error.

The ratios of the error variance, to the variances of the other random effects are needed to run the analyses, and these ratios depend on the heritability (h^2) and repeatability (r) of the traits.

In order to show that the inferences about the fixed effects are robust, we have used three different sets for h^2 and r [($h^2 = 0.10$, $r = 0.15$); ($h^2 = 0.15$, $r = 0.20$) and ($h^2 = 0.20$, $r = 0.25$), Table (1)] that were in the range of values found in the literature (Khalil *et al*., 1989; Utrillas *et al*., 1991; Ferraz *et al.*, 1992; Blasco, 1996).

2nd) Litter weight at birth and litter weight at weaning:

$$Y_{ijldm} = \mu + YS_i + P_j + b\,X_{ijldm} + B_l + I_d + e_{ijldm} \quad \text{....... (Model 2)}$$

Where:

X_{ijldm} & b stand for covariate of litter size at birth or at weaning and b is the corresponding regression coefficient.

B_l is the random effect of l^{th} buck (genetic effect) mated to the

I_d includes the random genetic and permanent environmental effects due to d^{th} doe.

The other terms in this model are as defined previously in model 1.

The three sets of parameters used were ($h^2 = 0.10$, $r = 0.20$), ($h^2 = 0.15$, $r = 0.25$) and ($h^2 = 0.20$, $r = 0.30$) that were within the range of values cited in the literature (Afifi *et al.*, 1992; Ferraz *et al.*, 1992; Krogmeier *et al.*, 1994; Rastogi *et al.*, 2000).

3rd) Kindling interval:

$$Y_{ijldm} = \mu + YS_i + P_j + b\, X_{ijkldm} + S_k + D_l + I_d + e_{ijlldm} \quad \text{....... (Model 3)}$$

All the terms of model 3 have the same meaning as in the first and second models, but X_{ijkldm} stands for litter size at birth corresponds to the previous parity as a covariate, and b for the corresponding regression coefficient, with the peculiarity than the litter size at birth as a covariate corresponds to the previous parity.

The three sets of parameters considered were ($h^2 = 0.05$, $r = 0.15$), ($h^2 = 0.10$, $r = 0.20$) and ($h^2 = 0.20$, $r = 0.30$) that were within the range of values cited by Baselga and Blasco (1989) and Baselga *et al.* (2003).

The generalized least squares means and their standard errors were estimated for the fixed effects and the regression coefficient of the covariates. F-test was used to detect the statistical significance differences among levels of the fixed effects and to test if the regression coefficients are significantly different from zero or not.

RESULTS AND DISCUSSION

The estimates of the effects of the fixed effects and covariates included in the models are shown in Tables 2, 3, 4, 5 and 6, respectively for litter size at birth, at weaning, litter weight at birth, at weaning and interval between kindlings. The tables also show the significance of the differences between the levels of each fixed factor; and the value and significance of the corresponding coefficients of regression in case of including a covariate in the model. The covariate considered for litter weight at birth was litter size at birth that had a mean of 6.75 kits per litter. The corresponding least square means for litter weight at birth, as shown in Table 4, have been computed at a constant litter size at birth (the mean of the covariate). The same pattern was applied for litter weight at weaning where the mean litter size at weaning as a covariate, was 5.06 kits per litter. For kindling interval, litter size at birth of the previous litter was used as a covariate that had a mean of 6.78 kits per litter. In order to simplify the Tables, the standard errors of the least square means have been replaced by the range in standard errors obtained; giving the minimum and the maximum values of th

Table (2): Generalized least square means (kits) and standard errors (S.E.) for effects of year-season and parity order on litter size at birth under different sets of genetic parameters.

Items	Sets of genetic parameters		
	Set I	Set II	Set III
Year-Season:			
89 – winter	6.9^{abcd}	7.0^{abcd}	7.0^{abcd}
89 – spring	7.4^{ab}	7.4^{ab}	7.4^{ab}
89 – autumn	6.4^{cd}	6.4^{cd}	6.5^{cd}
90 – winter	7.6^{a}	7.6^{a}	7.6^{a}
90 – spring	7.2^{ab}	7.3^{ab}	7.3^{ab}
90 – autumn	6.6^{cd}	6.6^{cd}	6.7^{cd}
91 – winter	7.0^{abc}	7.0^{abc}	7.1^{abc}
91 – spring	5.4^{e}	5.4^{e}	5.5^{e}
91 – autumn	5.6^{e}	5.6^{e}	5.6^{e}
92 – winter	6.6^{bcd}	6.7^{bcd}	6.7^{bcd}
92 – spring	6.2^{de}	6.2^{de}	6.2^{de}
Minimum S.E.	**0.37**	**0.37**	**0.37**
Maximum S.E.	**0.80**	**0.80**	**0.80**
Parity order:			
1st	6.6	6.6	6.6
2nd	6.5	6.5	6.5
3rd	6.6	6.6	6.6
4th	6.8	6.9	6.9
5th	6.6	6.7	6.8
Minimum S.E.	**0.18**	**0.19**	**0.19**
Maximum S.E.	**1.47**	**1.45**	**1.44**

Means with no common superscripts for each effect are significantly different ($P \leq .05$).

These standard errors are shown with one digit more than the corresponding least square means to get a more precise idea of their changes with using different sets of genetic parameters.

In this paper, we do not discuss the values of the effects and their signification, compared with similar results for the same traits and raising conditions because the main objective of this work is different. In general, we can say that the results here are not different from the ones obtained in

Table (3): Generalized least square means (kits) and standard errors (S.E.) for effects of year-season and parity order on litter size at weaning under different sets of genetic parameters.

Items	Sets of genetic parameters		
	Set I	**Set II**	**Set III**
Year-Season:			
89 – winter	5.4^{a}	5.3^{a}	5.3^{a}
89 – spring	3.8^{bcd}	3.8^{bcd}	3.7^{bcd}
89 – autumn	3.6^{bcd}	3.6^{bcd}	3.6^{bcd}
90 – winter	3.0^{d}	3.0^{d}	3.0^{d}
90 – spring	4.1^{abc}	4.1^{abc}	4.1^{abc}
90 – autumn	4.3^{ab}	4.3^{ab}	4.3^{ab}
91 – winter	3.6^{bcd}	3.7^{bcd}	3.7^{bcd}
91 – spring	2.0^{d}	2.1^{d}	2.1^{d}
91 – autumn	3.8^{bcd}	3.8^{bcd}	3.8^{bcd}
92 – winter	4.4^{ab}	4.5^{ab}	4.5^{ab}
92 – spring	3.3^{cd}	3.4^{cd}	3.4^{cd}
Minimum S.E.	**0.46**	**0.46**	**0.47**
Maximum S.E.	**1.00**	**1.00**	**1.00**
Parity order:			
1st	4.4^{a}	4.5^{a}	4.5^{a}
2nd	3.5^{b}	3.6^{b}	3.6^{b}
3rd	3.9^{b}	3.8^{b}	3.8^{b}
4th	4.5^{a}	4.5^{a}	4.5^{a}
5th	2.5^{c}	2.5^{c}	2.5^{c}
Minimum S.E.	**0.22**	**0.23**	**0.24**
Maximum S.E.	**1.83**	**1.82**	**1.81**

Means with no common superscripts for each effect are significantly different ($P \leq .05$).

Concerning the main objective of this work, that is to check the robustness of the mixed model analysis when different genetic parameters, within the common range reported in the literature, the results obtained here are also completely in favour of the robustness. For litter size traits, the the changes in the estimated least squares constants when changing the set of parameters are almost negligible and the pattern of significance for differences among means always remains unchanged (Tables 2 & 3). The same occurs for litter weight traits since least square means and coefficients of regression remain unchanged with the change of

Table (4): Generalized least square means (grams) and standard errors (S.E.) for effects of year-season, parity order and litter size at birth (grams per kit, as a covariate) on litter weight at birth under different sets of genetic parameters.

Items	Sets of genetic parameters		
	Set I	Set II	Set III
Year-Season:			
89 – winter	336^{f}	334^{f}	333^{f}
89 – spring	465^{bc}	464^{bc}	463^{bc}
89 – autumn	513^{a}	512^{a}	511^{a}
90 – winter	490^{b}	490^{b}	490^{b}
90 – spring	399^{e}	399^{e}	400^{e}
90 – autumn	451^{cd}	451^{cd}	451^{cd}
91 – winter	441^{cd}	441^{cd}	441^{cd}
91 – spring	430^{cde}	431^{cde}	432^{cde}
91 – autumn	455^{cd}	454^{cd}	453^{cd}
92 – winter	427^{de}	427^{de}	426^{de}
92 – spring	396^{e}	397^{e}	397^{e}
Minimum S.E.	**13.2**	**13.2**	**13.2**
Maximum S.E.	**29.1**	**29.0**	**28.8**
Parity order:			
1^{st}	463^{a}	463^{a}	464^{a}
2^{nd}	435^{b}	435^{b}	435^{b}
3^{rd}	441^{b}	440^{b}	440^{b}
4^{th}	436^{b}	435^{b}	435^{b}
5^{th}	409^{c}	408^{c}	407^{c}
Minimum S.E.	**6.4**	**6.5**	**6.7**
Maximum S.E.	**53.0**	**52.4**	**51.8**
Litter size at birth	54.9*±1.4	55.0*±1.4	55.0*±1.4

Means with no common superscripts for each effect are significantly different (P<.05).

**= Value significantly different of zero (P≤0.05).*

Table (5): Generalized least square means (grams) and standard errors (S.E.) for effects of year-season, parity order and litter size at weaning (grams per kit, as a covariate) on litter weight at weaning under different sets of genetic parameters.

Items	Sets of genetic parameters		
	Set I	Set II	Set III
Year-Season:			
89 – winter	2816^{bc}	2823^{bc}	2827^{bc}
89 – spring	2492^{cd}	2498^{cd}	2502^{cd}
89 – autumn	3248^{a}	3248^{a}	3246^{a}
90 – winter	2899^{b}	2903^{b}	2907^{b}
90 – spring	2711^{bc}	2714^{bc}	2717^{bc}
90 – autumn	3302^{a}	3309^{a}	3316^{a}
91 – winter	2719^{bc}	2724^{bc}	2728^{bc}
91 – spring	2564^{c}	2599^{c}	2630^{c}
91 – autumn	2619^{c}	2620^{c}	2620^{c}
92 – winter	2496^{cd}	2502^{cd}	2506^{cd}
92 – spring	2324^{d}	2334^{d}	2343^{d}
Minimum S.E.	**110.6**	**110.9**	**111.4**
Maximum S.E.	**377.2**	**378.4**	**379.1**
Parity order:			
1^{st}	2716	2718	2720
2^{nd}	2705	2706	2706
3^{rd}	2735	2738	2741
4^{th}	2638	2643	2646
5^{th}	2928	2957	2979
Minimum S.E.	**61.3**	**62.0**	**62.8**
Maximum S.E.	**435.1**	**432.4**	**429.7**
Litter size at weaning	450*±13.9	450*±14.0	450*±14.0

Means with no common superscripts for each effect are significantly different ($P \leq .05$).

**= Value significantly different of zero ($P \leq 0.05$).*

Table (6): Generalized least square means (days) and standard errors (S.E.) for effects of year-season, parity order and litter size at birth of previous parity (days per kit, as a covariate) on kindling interval under different sets of genetic parameters.

Items	Sets of genetic parameters		
	Set I	Set II	Set III
Year-Season:			
89 – spring	31.4^{a}	31.5^{a}	31.8^{a}
89 – autumn	40.4^{bc}	40.5^{bc}	40.7^{bc}
90 – winter	44.1^{c}	44.2^{c}	44.3^{c}
90 – spring	30.8^{a}	30.6^{a}	30.3^{a}
90 – autumn	43.3^{c}	43.1^{c}	42.8^{c}
91 – winter	34.1^{ab}	34.0^{ab}	33.8^{ab}
91 – spring	35.3^{abc}	35.0^{abc}	34.5^{abc}
91 – autumn	63.6^{d}	63.8^{d}	63.9^{d}
92 – winter	45.8^{c}	45.6^{c}	45.1^{c}
92 – spring	34.2^{ab}	33.7^{ab}	32.5^{ab}
Minimum S.E.	**3.81**	**3.84**	**3.91**
Maximum S.E.	**7.80**	**7.86**	**7.92**
Parity order:			
2nd	41.7	41.7	41.5
3rd	42.0	41.8	41.4
4th	42.0	42.0	42.0
5th	35.6	35.3	35.0
Minimum S.E.	**1.52**	**1.61**	**1.78**
Maximum S.E.	**12.93**	**12.91**	**12.78**
Litter size at birth	-0.13±0.39	-0.14±0.39	-0.17±0.40

Means with no common superscripts for each effect are significantly different ($P \leq .05$).

(Tables 4 & 5). For kindling interval, the same trend was observed (Table 6).

Concerning the standard errors of the least square means, there is a general trend indicating that standard errors increased very slightly with the increase of values of heritability and repeatability; except the maximum ... to the 5th parity. These maximum standard errors are due to that the number of records in 5^{th} parity was the smallest. In this concern, it is not easy in general to predict the change of the standard errors of the estimates of the fixed effects because they are depending on the dispersion parameters of the random effects, the relationship between levels of such random effects and the balance of association between levels of different factors included in the model, either fixed or random (Henderson, 1984).

Conclusively, in the majority of the experiments in animal production, the animals used are relatives and the way to consider this circumstance is to use mixed models including random factors related to the effects of the animals and taking into account the relationship among the animals. The use of mixed models requires knowing previously some genetic parameters, such as the heritability, repeatability of the traits or others. In this paper, we have shown that it is not necessary to use very accurate estimates of genetic parameters in order to get the inferences about the least square means of different fixed effects and to detect the statistical inferences for differences among levels of these effects.

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قوة التحليل بالموديل الأحصائى المختلط لتقدير التأثيرات الثابتة لصفات البطن فى الأرانب

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إستخدام الموديل الأحصائى المختلط فى التحليل الأحصائى لتجارب الأنتاج الحيوانى تمت مناقشته مع الأخذ فى الأعتبار أنه، بصفه عامة، الحيوانات المستخدمة فى التجارب تكون بينها صلة قرابة (فى بعض الأحيان، قرابة قوية مثل الأخوات الأشقاء أو أنصاف الأشقاء). تم أستخدام أحد الأمثلة لتقدير التأثيرات الثابتة لكل من سنة- موسم الميلاد، رقم الولادة، بعض علاقات الأنحدار على صفات حجم البطن (عند الميلاد وعند الفطام) ووزن البطن (عند الميلاد وعند الفطام) والفترة بين الولادة الحالية والسابقة فى الأرانب، بأستخدام ثلاثة قيم للمعاير الوراثية (المكافىء الوراثى، المعامل التكرارى)، لكل صفة من الصفات المدروسة، تقع فى مدى التقديرات المسجلة فى النشرات العلمية.

أظهرت النتائج المتحصل عليها من هذا المثال أنه ليس من الضرورى أن تكون هناك قيم دقيقة جداً للتقديرات الوراثية للصفة لأستعمالها فى الموديل الأحصائى المختلط للحصول على تقدير للتأثيرات الثابتة. هذا المفهوم أو التصور تم إثباته فى نتائج الدراسة الحاليه حيث أن المتوسطات المقدرة للعوامل الثابتة التأثير (سنة- موسم الميلاد، رقم الولادة) ومعاملات الإنحدار ظلت لاتتغير بتغير قيمة المعايير الوراثية المستعملة لحل معادلة الموديل الأحصائى المختلط. أيضاً نتائج إختبارات المعنوية للتأثيرات الثابتة كانت دائماً واحدة ولم تتغير بتغير قيم المعايير الوراثية المستعملة فى الموديل الأحصائى المختلط.

الخلاصة أنه فى غالبية تجارب الأنتاج الحيوانى فأن الحيوانات المستعملة تكون أقارب والحل لأعتبار هذة الظروف هو إستعمال الموديلات الأحصائية المختلطه المتضمنة العوامل العشوائية المتعلقة بتأثيرات الحيوانات والأخذة فى الأعتبار علاقات القرابة بينهم.