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CORRELATES OF EGGSHELL THICKNESS

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ABSTRACT

This study discussed the effects of age and genotype of birds and location of farm on eggshell thickness. The ultimate objective of the study is to determine the correlates of eggshell thickness which may be relevant to improve eggshell thickness. Secondary data on eggshell thickness collected from the Agricultural Development Programme(ADP), Umuahia were analyzed using the three-factor(fixed effect) analysis of variance. The results of the analysis showed that effects of location and genotype appeared not to be statistically significant. Age of birds was statistically significant. The two factor interactions: L X G and A X G also appeared to have significant effects on eggshell thickness. This may be attributable to the violation of the assumptions of the analysis of variance. A preliminary evaluation of the data showed that although the sample size is large, the data do not seem to have come from a normal population. The variance does not appear to be constant. Also, the data do not appear to be completely random. Therefore, a more appropriate data which meet the assumptions of the analysis of variance model should be collected in order to arrive at a more reliable conclusion.

KEY WORDS: factor, fixed effect, analysis of variance, significant, sample, population, model and random.

1.0 INTRODUCTION

Poultry farming, as an integral part of agriculture, is of utmost importance to mankind. It serves as a source of employment to people who wish to go into poultry production (Mortey et al, 1997). Poultry products such as eggs, fowls and animal droppings have various uses. The eggs and fowls are sources of animal protein. The droppings are used as manure for crop production (Banerjee, 1992). Of special interest to a poultry farmer are the eggs. This is because eggs are some of the primary poultry products. They can be eaten or sold to generate income or left to be hatched into chickens. The chickens are then reared to produce fowls.

Poultry products in general and eggs in particulars are affected by several factors. These include poultry diseases, nutrition, bird strain, bird age, management practices, water quality, housing conditions, temperature and disturbance (Robert et al, 2000). These factors affect egg quality through their influences on eggshell thickness. The eggshell plays important roles in regulating the quality of an egg. The capacity of eggshell to play its function strongly depends on its thickness. Studies have shown that thin shelled eggs are often difficult to hatch. Thus, this can lead to reduced number of chickens hatched. Again. eggshell thickness and porosity are correlated. Eggs with thick shells are more resistant to diseases. This is because thick shells have small pores which hardly allow the influx of micro organisms to the inner parts of the eggs. Storability and distribution of an egg partly depend on thickness of the egg. Eggs with a minimum thickness of 35 micrometers have been found not to break easily on transit (Anthony, 1990). Income from eggs also depends on their quality which in turn depends on their sizes, shell thickness and weights. These variables are used to grade eggs for different uses. Katie(1992) attributed differences in sizes off eggs to the various ages of the layers. She emphasized that eggs laid by hens at start are usually smaller than those laid at end of the clutch.

In recognition of these factors associated with egg production, storage and distribution, poultry farmers have adopted several strategies to ensure improved quality and quantity of eggs. These measures include good sanitation and environmental conditions, adequate feeding of hens in terms of quantity and quality and vaccination of birds. Other factors which the farmers take into consideration include age of bird at lay, location and genotype of the hen. The question here is with these precautions, has there been any improvement on eggshell thickness? This and other related questions are what this study intends to address. Furthermore, a lot of work have been done on the correlates of eggshell thickness but not much seem to have been read about the nature of the relationship between eggshell thickness in one hand and the location of farm, age of bird at lay and bird genotype on the other hand. Hence, there is need for this study. Therefore, the ultimate objective of this study is to determine the relationship between eggshell thickness in one hand and location, age and genotype of hen on the other hand which may be relevant in improving eggshell.

Specifically, the study:

i Estimated the means and standard deviations of eggshell thickness by age, genotype and location.

Ii Determined the proportion of eggs with shell thickness

Iii Estimated the proportion of eggs with shell thickness les then the valve of 35 micrometers.

Iv Determined the factors which are significantly associated with eggshell thickness.

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The results of the study may form the basis for improvement of poultry products by farmers both in quantity and or quality. Consumers may find the result useful when the need for storage arises.

2.0 METHODOLOGY

Secondary data collected from the Agriculture Development Project, Umuahia are used for this work. The variables (factors) on bases of which data were collected are age of bird at lay, location of the farm and genotype of the bird.

The data were analyzed using analysis of variance technique. The three factor fixed effect model with interaction, according to Edward (1972), is of the form

$$X_{ijkl} = \mu + \alpha_i + \beta_j + \lambda_{ij} + \gamma_k + \lambda_{ik} + \lambda_{jk} + \lambda_{ijk} + \ell_{ijkl} \quad ..(2.1)$$

Where,

X_{ijkl} is the eggshell thickness of the egg involving ith location of farm, jth age and kth genotype of bird.

μ is the grand mean

α_i is the main effect of the ith location on eggshell thickness, i =1,2,3.

β_j is the main effect of the jth age of birdon eggshell thickness, j = 1,2,3.

λ_{ij} is the interaction between ith location and jth age of bird.

γ_k is the main effect of kth genotype, k = 1,2,3,4.

λ_{ik} is the interaction between ith location and kth genotype.

λ_{jk} is the interaction between the jth age and kth genotype.

λ_{ijk} is the three factor interaction involving ith location, jth age and kth genotype.

ℓ_{ijkl} Is the error associated with X_{ijkl}.

For this fixed effect model, the following restrictions are placed on the parameters:

$$\sum_{ij} \lambda_{ij} = \sum_{ik} \lambda_{ik} = \sum_{jk} \lambda_{jk} = \sum_{ijk} \lambda_{ijk} \quad ...(2.3)$$

In using this model, it is assumed that:

(i) The error terms ℓ_{ijkl} are normally distributed with zero mean and constant variance σ^2.

(ii) The X_{ijkl} is assumed to be a linear additive function of ℓ_{ijkl} and other fixed factors.

Therefore, X_{ijkl} is normally distributed with mean μ_x and variance σ^2. Where

$$\mu_x = \alpha_i + \beta_j + \lambda_{ij} + \gamma_k + \lambda_{ik} + \lambda_{jk} + \lambda_{ijk} \quad ..(2.4)$$

The estimates of the parameters in equation 2.4 are contained in appendix I.

2.1: INTERACTION AND ITS EFFECTS

When the ANOVA indicates the presence of interaction, it is important to determine whether interactions actually exist or there may be some other explanations for the occurrence of the interaction in the data.

Sometimes, an investigator may believe that there is no interaction, yet the data point to sizeable interactions. Such unexpected interactions may be caused by a problem in the data e.g.

(i) the presence of an outliers
(ii) an erroneous response
(iii) Lack of randomness in the selection of samples or application of treatments
(iv) An appreciable time effect
(v) Some other uncontrolled variable may be affecting the observations.
(vi) The dependent variable may have been measured on in appropriate scale.

When the main effects are very large, unexpected interactions may also occur.

However, this disappears if the investigator lessens the differences among levels of a treatment making the main effect less pronounced. Such unexpected interaction may be an indication that some of the assumptions of the model being used have been violated. When important interactions result from the dependent variable being measured on an inappropriate scale the magnitude of such interaction can be reduced or eliminated by a simple transformation of the dependent variable, so that they become unimportant. Such interactions are called transformable (removable) interactions. Interactions that cannot be so removed by a simple transformation are called non- transformable (non-removable) interactions.

It is possible that two factors interact, yet the main effect for one or both factors are zero. This sometimes may be the result of interactions in opposite directions that balance out over one or both factors. Thus, there would be definite factors effect, but these would not be disclosed by the factor level means.

2.2: METHOD OF MULTIPLE COMPARISON

When the ANOVA table indicates that the treatment effect is significant, the next step is to determine which of the treatments that is significantly different from the other. Several statistical methods have been developed for this purpose. However, for the purpose of this work, the least significant difference method(LSD) has been adopted.

It is one of the most commonly used methods perhaps because it is simple to compute and interprete. Under the null hypothesis that the treatment effects are the same, the LSD is given by Montegomery (1976) as

$$LSD = t_{\alpha/2,(N-A)}\sqrt{\frac{2MSE}{n}} \quad ....(2.5)$$

where α = significance level, N – A = error degree of freedom and n = number of replications. The statistic in equation (2.5) is only used for balanced designs. On the other hand, if an unbalanced design is considered, the LSD for comparing two treatment means is given by

$$LSD = t_{\alpha/2,(N-A)}\sqrt{2MSE\left(\frac{1}{n_i} + \frac{1}{n_j}\right)} \quad ....(2.6)$$

where n_i = number of replications of ith treatment.
n_j = number of replications of jth treatment and $n_i \neq n_j$. Since the variance is assumed to be constant, the mean square error (MSE) is usually taken to be the estimate of the common
variance. Therefore, the error degree of freedom is taken as the degree of freedom of the LSD. Consequently, the difference between each pair of treatment $\left[\bar{y}_i, \bar{y}_j\right]$, is considered to be significant if

$$\left|\bar{y}_i - \bar{y}_j\right| \rangle LSD.$$

The least significance difference has only been found appropriate for comparing a pair of treatments. If more than two treatments are involved, the LSD is then used to compare adjacent treatment means when they are arranged in order of magnitude. This kind of comparism may result in a danger of judging certain comparisms significant when they are not significant at the chosen level of significance (Little et al, 1966).

3.0 ESTIMATES AND TEST OF SIGNIFICANCE OF FACTOR EFFECTS

The estimate and test of significance of the effect of the factors on the eggshell thickness are considered in this section. The factors considered are location of poultry farm (L), age of birds at Lay (A) and genotype of birds (G). Estimates of the mean and standard deviation of the eggshell thickness according to these characteristics are given in table 3.1

As table 3.1 shows, the mean shell thickness of all eggs considered is about 0.41mm with a standard deviation of about 0.038mm. According to the location, mean eggshell thickness ranged from about 0.408mm in location 2 to about 0.411mm in location 3. According
to the age of bird at lay, eggshell thickness decreased from about 0.419m among birds aged one month to about 0.405mm among those aged 4 months. With respect to genotype, eggshell thickness appears highest(0.412) among birds of LC X GL and least 0.408 among birds of GL X LC genotype. The SPSS package is used to obtain these estimates and other results in this section. Based on the aforementioned results, it is evident that eggshell thickness varies with respect to age, genotype and location. However, how statistically significant these effects are yet to be determined. To determined the effects that are statistical significant, the ANOVA table (Table 3.2) is used.

3.1: RESULT AND DISCUSSION

TABLE 3.1: ESTIMATES OF MEAN AND STANDARD DEVIATION OF EGGSHELL THICKNESS BY FACTOR(LOCATION, AGE AND GENOTYPE).

Factor	Mean	Standard deviation	No of cases
Location			
1	0.410	0.037	800
2	0.408	0.038	800
3	0.411	0.038	800
Age (in month)			
1	0.419	0.036	600
2	0.412	0.036	600
3	0.410	0.036	600
4	0.405	0.036	600
Genotype			
LCXLC	0.409	0.040	600
LCXGL	0.412	0.034	600
GLXLC	0.408	0.041	600
GLXGL	0.410	0.035	600
ALL	0.410	0.038	2400

TABLE 3.2: ANOVA TABLE FOR THE EGGSHELL THICKNESS DATA

Source of variation	Df	SS	Ms	FCal	P.value
Constant	1	403.21	403.21	3152249.1	0.000**
Location	2	0.003	0.0014	1.074	0.342
Age	3	0.020	0.0066	5.153	0.001**
LXA	6	0.008	0.0014	1.062	0.383
Genotype(G)	3	0.006	0.0019	1.486	0.216
LXG	6	0.023	0.0038	2.968	0.007**
AXG	9	0.306	0.0341	26.625	0.000**
LXAXG	18	0.037	0.0020	1.588	0.055
Error	2352	3.008	0.0013	-	
Total	2400	406.618	-	-	

The effect of age appears significant at 0.05 significance level. This is consistent with established result (Juliet et al 2000). The effects of location as well as genotype is not significantly different from zero.

However, the interaction effects between location and genotype and that between age and genotype are significant. Thus, the mean effects of genotype on eggshell thickness vary from one location to another. The mean effects of genotype of hen on eggshell thickness also differ with age of hen.

The three factor interaction is not significant. This implies that though the mean effects of genotype on eggshell thickness vary at different location, the degree and pattern of variation remain the same at different ages of hen. Again, though the mean effects of genotype on eggshell thickness may vary with age of hen, the degrees of these variations are not significantly different at different locations. In other words, when studying the effects of age, genotype and location together on eggshell thickness in the same experiment, the factor combinations should be made specific to each genotype. The interaction effect between location and age on eggshell thickness is not significant. This implies that the mean effects of age at different locations on eggshell thickness are the same and mean effects of location is the same for different ages.

3.2: COMPARISON OF MEAN EGGSHELL THICKNESS BY AGE

The LSD is calculated using equation 2.5 as shown below.

$$LSD = t_{0.025}^{2352} \times \sqrt{\frac{2 \times 0.013}{600}} = 0.0041$$

Here, $\alpha = 0.05$.

The table 3.3 below contains the difference, between each pair of eggshell means with respect to age.

TABLE 3.3: DIFFERENCE BETWEEN EACH PAIR OF MEAN EGGSHELL THICKNESS BY AGE

Means in descending order		Mean in ascending order			
		$\overline{X_1}$	$\overline{X_2}$	$\overline{X_3}$	$\overline{X_4}$
		0.405	0.410	0.412	0.419
$\overline{X_4}$	0.419	0.014*	0.009*	0.007*	
$\overline{X_3}$	0.412	0.007*	0.002		
$\overline{X_2}$	0.410	0.005*			
$\overline{X_1}$	0.405				

The differences marked asterisk in table 3.3 above are significantly greater than the LSD value of 0.0041. Thus, mean shell thickness of eggs laid by birds in the first month is significantly greater than others. There seems to be no difference between the means of eggshell thickness of eggs laid by birds in the second and third months. However, they are significantly greater than the mean shell thickness of eggs laid by birds in the fourth month.

3.3: DATA EVALUATION

In this context, the data are subjected to test for the validity of the assumptions underlying the use of analysis of variance. These tests are easily carried out by using the tables of values (some of which are descriptive statistical measures).

TABLE 3.4: DESCRIPTIVE STATISTICS FOR THE ORIGINAL DATA

Statistic	Value
Mean	0.00003
Skewness	-0.4500
Kurtosis	1.2663
Median	0.0020

It is very clear from the table above that the original data are not normally distributed. This is because for the given data to be normally distributed, its mean, median and mode must be equal. Again the coefficients of skewness and kurtosis should not be significantly different from 3 and 0 respectively.

Another important condition required for any given data to be fit for the ANOVA technique is that of constant variance or homosccdasticity. A simple test for constant variance can be carried out by finding out if there is any relationship between means and standard deviations of the data when arranged according to factors e.g Age, location and Genotype. These arrangements are as follows:

TABLE 3.5: MEANS AND STANDARD DEVIATIONS OF EGGSHELL THICKNESS BY LOCATION

Location	Mean	Standard deviation
1	0.937	14.835
2	1.841	23.394
3	2.500	29.5700

TABLE 3.6: MEANS AND STANDARD DEVIATIONS OF EGGSHELL THICKNESS BY GENOTYPE

Genotype	Mean	Standard deviation
1	2.5300	30.0400
2	0.4127	0.0359
3	1.0330	15.2520
4	3.0600	32.4400

TABLE 3.7: MEANS AND STANDARD DEVIATIONS OF EGGSHELL THICKNESS BY AGE

Age	Mean	Standard deviation
1	0.937	14.835
2	1.841	23.394
3	2.500	29.57

It is certain that means and standard deviations are related in each of the classifications of eggshell thickness by location, genotype and age as show in table 3.5 to 3.7 above. Thus the constant variance assumption in not met in the data. From the foregoing, it is imperative to say that the violation of the basic assumptions of ANOVA in the data may be one of the factors responsible for significant interaction effects(LXG and AXG) when the main effects (L and G)
are not significant in the table 3.2 above.

4.0: SUMMARY

This study examined the influence of age of birds at lay, genotype and location on the eggshell thickness. The ultimate objective of the work is to determine the extent to which the effect of these factors can be used to improve the quality of the eggshell thickness. The relevance of the study lies in the fact that improved eggshell thickness enhances the quality, storability and portability of eggs.

Secondary data on eggshell thickness collected from Abia State Agricultural Development program was analyzed using the three factor analysis of variance for crossed classification. The result of the analysis show that eggshell thickness appears to be significantly influenced by age of bird, interactions between location and genotype and between age and genotype at 5% level of significance. However, the effects of location and genotype on eggshell thickness appeared not to be significant. Consequently, the significant effect of age on eggshell thickness is line with results of the previous work (Juliet et al 2000). The nature of this relationship is such that as flock age increases, eggshell thickness decreases following increase in egg size and reduced calcium production. However, the significance of interaction effects when the main effects are not significant may be attributed to the failure of the data to meet some or all the assumptions of ANOVA models.

In view of these results, it is recommended that for purposes of storability and portability, farmers should separate eggs laid by birds at early ages at lay. This is important since thicker eggshell from younger birds prevents easy cracks during transit (Anthony, 1990). It also helps in preserving the inner content of the egg.
Furthermore, future data collection should be conduct in such a way that the data will meet the assumptions of the model

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APPENDIX I: ESTIMATES OF THE PARAMETERS IN THE THREE FACTOR FIXED EFFECT MODEL

The estimates of the parameters by least squares method are given by

$$\hat{\mu} = \overline{X}_{....} \quad \dots(1)$$

$$\hat{\alpha}_i = \overline{X}_{i...} - \overline{X}_{....} \quad \dots(2)$$

$$\hat{\beta}_j = \overline{X}_{.j..} - \overline{X}_{....} \quad \dots(3)$$

$$\hat{\lambda}_{ij} = \overline{X}_{ij..} - \overline{X}_{i...} - \overline{X}_{.j..} + \overline{X}_{....} \quad \dots(4)$$

$$\hat{\gamma}_k = \overline{X}_{..k.} - \overline{X}_{....} \quad \dots(5)$$

$$\hat{\lambda}_{ik} = \overline{X}_{i.k.} - \overline{X}_{i...} - \overline{X}_{i.k.} + \overline{X}_{....} \quad \dots(6)$$

$$\hat{\lambda}_{jk} = \overline{X}_{.jk.} - \overline{X}_{.j..} - \overline{X}_{..k.} + \overline{X}_{....} \quad \dots(7)$$

$$\hat{\lambda}_{ijk} = \overline{X}_{ijk} - \left(\overline{X}_{ij..} + \overline{X}_{i.k.} + \overline{X}_{.jk.} + \overline{X}_{i...} + \overline{X}_{.j..} + \overline{X}_{..k.}\right) + \overline{X}_{....} \quad \dots(8)$$

$$\hat{\ell}_{ijkl} = \overline{X}_{ijkl} - \overline{X}_{ijk.} \quad \dots(9)$$

Where,

$$\overline{X}_{....} = \frac{X_{....}}{N} = \frac{\sum X_{ijkl}}{\gamma \times L \times A \times G} \quad \dots(10)$$

$$\overline{X}_{i...} = \frac{X_{i...}}{\gamma \times A \times G} = \frac{\sum_{jkl}^{\gamma,A,G} X_{ijkl}}{\gamma \times A \times G} \quad \dots(11)$$

$$\overline{X}_{.j..} = \frac{X_{.j..}}{\gamma \times G \times L} = \frac{\sum_{ikl}^{\gamma,G,L} X_{ijkl}}{\gamma \times G \times L} \quad \dots(12)$$

Hence,

$$\hat{X}_{ijkl} - \hat{\mu} = \hat{\alpha}_i + \hat{\beta}_j + \hat{\lambda}_{ij} + \hat{\gamma}_k + \hat{\lambda}_{ik} + \hat{\lambda}_{jk} + \hat{\lambda}_{ijk} + \hat{\ell}_{ijkl} \quad \dots(13)$$

Or

$$\begin{aligned}\hat{X}_{ijkl} - \hat{X}_{....} = & \left(\overline{X}_{i..} - \overline{X}_{....}\right) + \left(\overline{X}_{.j..} - \overline{X}_{....}\right) + \left(\overline{X}_{ij..} - \overline{X}_{i...} - \overline{X}_{.j..} + \overline{X}_{....}\right) \\ & + \left(\overline{X}_{..k.} - \overline{X}_{....}\right) + \left(\overline{X}_{i.k.} - \overline{X}_{i...} - \overline{X}_{..k.} + \overline{X}_{....}\right) \\ & + \left(\overline{X}_{.jk.} - \overline{X}_{.j..} - \overline{X}_{..k.} + \overline{X}_{....}\right) \\ & + \left(\overline{X}_{ijk.} - \left(\overline{X}_{ij..} + \overline{X}_{i.k.} + \overline{X}_{.jk.} + \overline{X}_{i...} + \overline{X}_{.j..} + \overline{X}_{..k.}\right) + \overline{X}_{....}\right) \\ & + \left(\overline{X}_{ijkl} - \overline{X}_{ijk.}\right) \quad \dots(14)\end{aligned}$$

To test the hypotheses about the significance of the main effects and interaction effects, the sums of squares are calculated as follows:

$$SS_L = C_i - C \quad \dots(15)$$

$$SS_A = C_j - C \quad \dots(16)$$

$$SS_G = C_k - C \quad \dots(17)$$

$$SS_{LA} = C_{ij} - C_i - C_j + C \quad ...(18)$$

$$SS_{LG} = C_{ik} - C_i - C_k + C \quad ...(19)$$

$$SS_{AG} = C_{jk} - C_j - C_k + C \quad ...(20)$$

$$SS_{LAG} = C_{ijk} - \left(C_{ij} + C_{ik} + C_{jk}\right) + C_i + C_j + C_k - C \quad ...(21)$$

$$SS_e = C_{ijkl} - C_{ijk} \quad ...(22)$$

Where,

$$C = \frac{X^2_{....}}{LAG\gamma} \quad ...(23)$$

$$C_{ik} = \frac{\sum_{ik} X^2_{i.k.}}{A\gamma} \quad ...(24)$$

$$C_i = \frac{\sum_i X^2_{i...}}{AG\gamma} \quad 25)$$

$$C_j = \frac{\sum_j X^2_{.j..}}{LG\gamma} \quad ...(26)$$

$$C_{ij} = \frac{\sum_{ij} X^2_{ij..}}{G\gamma} \quad ...(27)$$

$$C_k = \frac{\sum_k X^2_{..k.}}{LA\gamma} \quad ...(28)$$

$$C_{jk} = \frac{\sum_{jk} X^2_{.jk.}}{L\gamma} \quad ...(29)$$

$$C_{ijk} = \frac{\sum_{ijk} X^2_{ijk.}}{\gamma} \quad ...(30)$$

and

$$C_{ijkl} = \sum_{ijkl} X^2_{ijkl} \quad ...(31)$$

If r = 1 i.e only one observation is taken per cell, the error is confounded with the interaction.The summary of the procedure is given in the ANOVA table below.

TABLE 1: ANOVA TABLE FOR THREE FACTOR FIXED EFFECT MODEL

Source of variation.	Df	Sum of squares	Mean SS	E (MS)	F
$Location(L)$	$L-1$	SS_L	MSL	$\sigma^2 + AG\gamma\theta_L^2$	MSL/MSE
$Age(A)$	$A-1$	SS_A	MSA	$\sigma^2 + LG\gamma\theta_A^2$	MSA/MSE
$L\times A$	$(L-1)(A-1)$	SS_{LA}	$MSLA$	$\sigma^2 + G\gamma\theta_{LA}^2$	$MSLA/MSE$
$Genotype(G))$	$G-1$	SS_G	MSG	$\sigma^2 + LA\gamma\theta_A^2$	MSG/MSE
$L\times G$	$(L-1)(G-1)$	SS_{LG}	$MSLG$	$\sigma^2 + A\gamma\theta_{LG}^2$	$MSLG/MSE$
$A\times G$	$(A-1)(G-1)$	SS_{AG}	$MSAG$	$\sigma^2 + L\gamma\theta_{AG}^2$	$MSAG/MSE$
$L\times A\times G$	$(L-1)(A-1)(G-1)$	SS_{LAG}	$MSLAG$	$\sigma^2 + \gamma\theta_{LAG}^2$	$MSLAG/MSE$
$Error$	$L\times A\times G(R-1)$	SS_e	MSe	σ^2	
$Total$	$LAGR-1$				

Where,

$$\theta_L^2 = \frac{\sum \alpha_i^2}{L-1} \quad \ldots(32)$$

$$\theta_A^2 = \frac{\sum \beta_j^2}{A-1} \quad \ldots(33)$$

$$\theta_G^2 = \frac{\sum \gamma_k^2}{G-1} \quad \ldots(34)$$

$$\theta_{LA}^2 = \frac{\sum \lambda_{ij}^2}{(L-1)(A-1)} \quad \ldots(35)$$

$$\theta_{LG}^2 = \frac{\sum \lambda_{ik}^2}{(L-1)(G-1)} \quad \ldots(36)$$

$$\theta_{AG}^2 = \frac{\sum \lambda_{jk}^2}{(A-1)(G-1)} \quad \ldots(37)$$

$$\theta_{LAG}^2 = \frac{\sum \lambda_{ijk}^2}{(L-1)(A-1)(G-1)} \quad \ldots(38)$$