Chapter 4

Estimation Theory

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A linear relationship can generally be found to fit most biological data although some transformation may be required. A simple linear model has the form

$$E(y) = a + b.x$$

A linear model can generally be used to describe data. Non-linear model could be defined as well, e.g. E(y) being a function of x^a or $\log(x)^b$ or b^x. However, linear models are usually much easier to solve (estimate parameters) and many non-linear problems can often be represented as a linear model.

All models contain a set of factors composed of three parts which additively affect the observations or records of data:

i) the equation
ii) expectations and variance covariance matrices of random variables
iii) assumptions, limitations and restrictions

Estimating Fixed Effects

Consider a general model

$$y = Xb + \varepsilon \qquad ...(1)$$

$$\text{with } E(y) = Xb \quad \text{and}$$
$$var(y) = V = var(\varepsilon) \qquad …(2).$$

We want to estimate fixed effects in b and conduct hypothesis testing about the significance of differences between the different levels of effects. Note that ε is a vector with random effects. They can be caused by several random factors (e.g. animal and residual) and the different levels may be correlated (e.g. due to repeated measurements on the same animals), e.g. var(ε) maybe equal to V = ZGZ'+R.

To find good estimators of the fixed effects parameters for a set of data, trial and error could be used. However the method of least squares, developed by Gauss in 1809 and Markoff in 1900 is commonly used for estimating these parameters of which the theorem states that

> ... under the assumption of normality and the model as described in (1) and (2) the least squares estimators b_0 and b_1 are unbiased and have minimum variance among all unbiased linear estimators.

The proof is given in several texts on linear models. Unbiasedness occurs when $E(X\beta) = Xb$ where β is an estimate of b,. However to estimate the value of these estimates, consideration needs to be given to the deviation of y_i from its expected value

$$E(y) = Xb \qquad ...(3)$$

and more importantly to the sum of the *N* squared deviations (errors) given as Q where

$$Q = (y- Xb)'(y-Xb) \qquad ...(4)$$

According to the method of least squares the *best* estimators of β_0 and β_1 are those which minimise Q.

Best - maximises the correlation between true and estimated value of effects by minimising the error variance.

Linear - the factors for which estimates are required are linear functions of the observations.

Unbiased - estimates of fixed effects and estimable functions are such that $E(b \mid b) = b$.

Deriving Estimates Using Ordinary Least Squares

The general fixed effects model in matrix for is

$$y = Xb + e \qquad ...(5)$$

where *y* is a vector of observations, X is an incidence matrix linking the independent variables to the observations, *b* is a vector of effects to be solved and *e* is a vector of error terms. For ordinary least squares (OLS), error terms are independently and identically distributed random variables with a mean of zero and a variance of σ_e^2 such that var(y) = var(e) = $I_N \sigma_e^2$ where I_N is a dispersion matrix for *n* observations. Given that E(y) = Xb,

$$Q = (y - Xb)' (y - Xb)$$

which when differentiated with respect to *b* gives

$$\delta Q / \delta b = -2(X'y + X'Xb).$$

Equating to zero gives

$$X'Xb = X'y$$

which are referred to as the *normal equations* which if the inverse of X'X exists, provides the least square estimator of *b*:

$$\hat{b} = (X'X)^{-1} X'y \qquad ...(6)$$

Thus ordinary least squares assumes that all observations are uncorrelated and have a common variance σ_e^2. If estimates are derived when this is not true then they are no longer 'best' since Q is no longer minimised.

Generalised Least Squares

For ordinary least squares, the criterion (4) weights each observation equally as the assumption is that the error terms are equally and identically distributed. However σ_e^2 may not be common to all observations. More generally, let var(e) = V and V could be diagonal (uncorrelated error but unequal variances, or not diagonal (errors could be correlated). It is very often that random terms are correlated, e.g. when having more observations on the same animal or observations among genetically related animals. In that case, we could also fit a random term (e.g. animal) and residuals could still be uncorrelated. But with the respect to estimation of fixed effect, random terms are not uncorrelated and we have to generalize var(y) = V. Estimation of fixed effect is than also more complicated, leading to the generalised least squares criterion for simple linear regression is

$$Q_G = Q = (y - X\boldsymbol{b})'V^{-1}(y - X\boldsymbol{b})$$

Minimising Q_G with respect to $\boldsymbol{b}_0$ and $\boldsymbol{b}_1$ leads to the appropriate normal equations of

$$(X'V^{-1}X)\,\boldsymbol{b} = X'V^{-1}Y$$

Determining a generalised inverse for $X'V^{-1}X$ gives the least square estimates as

$$\boldsymbol{b} = (X'V^{-1}X)^{-}X'V^{-1}Y \qquad \text{...(7)}$$

which is a general equation for Best Linear Unbiased Estimates of fixed effects model in any linear model. This will be further discussed under mixed models and we first assume we have generally V, with often simply $V = I\sigma_e^2$.

Estimability

Because a generalized inverse of $X'V^{-1}X$ is used there are a large (infinite) number of possible solutions to b. However, any solution vector can be used to compute *estimable* functions of b. An estimable function has the same numeric value, i.e. is unique, for any of the possible solution vectors. The following functions are always estimable:

- Any linear function of y is estimable
- Any linear function of E(y) is estimable
- K'b is estimable if K' = TX for some T, i.e. T is a linear combination of rows in X.
- Q'b is estimable if $Q'(X'V^{-1}X)^{-}X'V^{-1}X = Q'$

Example:

$$\begin{bmatrix} 6 & 4 & 2 \\ 4 & 4 & 0 \\ 4 & 0 & 2 \end{bmatrix} \begin{bmatrix} m \\ a_1 \\ a_2 \end{bmatrix} = \begin{bmatrix} 120 \\ 82 \\ 38 \end{bmatrix}$$

has many possible solutions, e.g. b' = [0 20.5 19] or [20 +0.5 -1]. (Verify this) However, the function $m + a_1$ *is* equal to 20.5 for all possible solutions. Also the difference $a_1 - a_2$ is always equal to 1.5. Only estimable functions have a meaning in a statistical analysis because they are unique.

Statistical packages usually give a set of solutions that is based on a constraint. Constraints enforce unique solutions for b, but because the constraints are arbitrary, the solutions are arbitrary as well. Constraints can be enforced by manipulation the X matrix such that it becomes non-singular, i.e. linear combinations of the columns should not be able to result in another linear combination of columns. The example data set 2 from the previous Chapter illustrates estimability and uniqueness of solutions. In brief again:

We can find solutions by setting a restriction:

1) put the general mean to zero
2) put one of the years to zero
3) put the sum of the year effects to zero

NB: The option you choose is arbitrary, it does effect the estimates of estimable functions, e.g. , the estimate of the year difference!
Summarizing the different options for X, and the resulting solutions:

General mean zero		First year zero (b2000=0)		Last year zero (b2002=0)		Sum of years to zero (b2000+ b2001 + b2002=0)	
X	$\hat{b}$	X	$\hat{b}$	X	$\hat{b}$	X	$\hat{b}$
1 0 0	302.5	1 0 0	302.5	1 1 0	330	1 1 0	313
1 0 0	306.5	1 0 0	4.0	1 1 0	-27.5	1 1 0	-10.5
0 1 0	330	1 1 0	+27.5	1 0 1	-23.5	1 0 1	-6.5
0 1 0		1 1 0		1 0 1		1 0 1	
0 1 0		1 1 0		1 0 1		1 0 1	
0 1 0		1 1 0		1 0 1		1 0 1	
0 0 1		1 0 1		1 0 0		1 -1-1	
μ = 0		μ = 302.5		μ = 330		μ = 313	
2000 = 302.5		2000 = 0		2000 = -27.5		2000 = -10.5	
2001 = 306.5		2001 = +4		2001 = -23.5		2001 = -6.5	
2002 = 330		2002 = +27.5		2002 = 0		2002 = 17	

We see from the different restrictions that the important parameters (the actual year differences) are always the same. In fact, with only one fixed effect in the model, these year differences can be estimated from the raw means for each year.

Least Square Means

In most scientific articles we find estimates of “Least Square Means”. As we have seen, the model term μ is itself not an estimable function and has no unique solution. The overall least square mean estimator can be given by $k'\hat{b}$, e.g. k = [1 0.33 0.33 0.33 0.25 0.25 0.25 0.25]. Note that this not an estimate of μ, but of μ plus an average of all levels of effect A and an average of all levels of effect B (for example). It would be only equal t μ is the ‘sum to zero’ constraint is applied to all effects. The LS mean for level 1 of effect A would be obtained by using [1 1 0 0   0.25 0.25 0.25 0.25], including μ, that particular effect a_a and an average of other effects. In general, the LSM of a level of some factor is an estimate of μ plus that factor level plus the levels of all factors in which it is nested plus the average of all levels of other factors with which it is cross classified, plus regressions at average values of the independent variables.

Connectedness

A lack of connectedness among subclasses of fixed effects in a model can have serious consequences on estimability. If all subclasses of the fixed effects are full, i.e. contain at least one observation, then the data are completely connected and there are no problems with estimability. However, when several subclasses are empty the subclasses are not connected and some functions of b may not be estimable.

Connectedness can be evaluated by making tables of one fixed effect vs. another fixed effect and write the number of observations.

For example:

Year \ sex	Male	Female
2000	1	1
2001	2	2
2002	1	0

Although not all subclasses are filled, the data is connected. It would not if the Male in the 2002 would be castrated such that we would have 3 sex classes, as in that case there would be a disconnected subset.

Year \ sex	Male	Steer	Female
2000	1	0	1
2001	2	0	2
2002	0	1	0

If there is disconnectedness in the data, the statistical programs will generally simply give no, or a zero solution to the effect associated with the disconnected subclass (i.e. no solution for year 2002 and Steer). Sometimes certain effects are *nested* within other effects. For example, herd 1 has only date from 2000 and 2001 whereas herd 2 has only data from 2002 and 2003. In that case the herd effect can not be estimated when years are fitted. When undertaking data analysis, it is important to understand such aspects of the design. For example, one could find out (e.g. with *awk)* how many year effects are in the data as well as how many year*herd combinations there are. If this is equal we know that one effect must be nested within the other.

Confounding

The best design to estimate parameters is a balanced design. There is an estimation problem if the data is disconnected. For example, in the last Table we can not distinguish between the effect of year 2002 and the effect of steers. However, in many cases the data is not balanced, but also not disconnected. Hence, there is a certain degree of confounding. Look at the following examples 3 and 4 and decide whether or not the fixed effects are significant.

Exmp3.dat

```
2000  Male    316
2000  Female  314
2000  Male    312
2000  Male    324
2001  Female  311
2001  Male    312
2001  Female  293
2001  Female  304
```

	male	female
2000	3	1
2001	1	3

```
model statement: weight ~ mu con(sex) con(year)
```

Output: exmp3.asr

```
6 con(year)    1       2.06       2.06   5.806     [DF F_i F_a SED]
5 con(sex)     1       4.36       1.19   5.806     [DF F_i F_a SED]
```

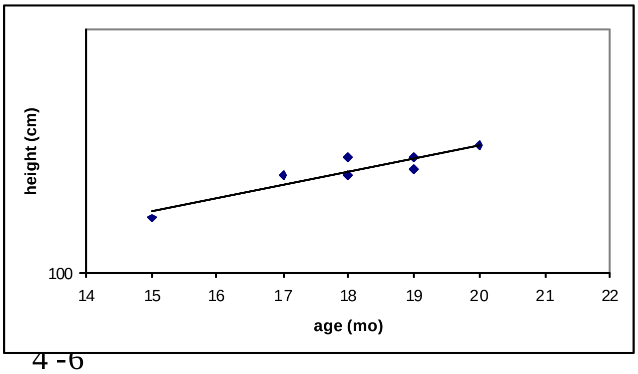
```
model statement: weight ~ mu con(year) con(sex)
```

Output: exmp3.asr

```
6 con(sex)     1       1.19       1.19   5.806     [DF F_i F_a SED]
5 con(year)    1       5.23       2.06   5.806     [DF F_i F_a SED]
```

Exmp4.dat

```
15  109       287
17  116       298
18  119       306
18  116       303
19  117       302
19  119       312
20  121       316
21  122       324
```



```
analysis of test data 4 LM course
  age
  height
  weight
exmp4.dat
weight ~ mu height age
```

Output: exmp4.asr

```
  1 age        1        3.03        3.03 [DF F_inc F_all]
  2 height     1       70.50        1.67 [DF F_inc F_all]
```

```
analysis of test data 4 LM course
  age
  height
  weight
exmp4.dat
weight ~ mu age height
```

Output: exmp4.asr

```
  2 height      1        1.67        1.67 [DF F_inc F_all]
  1 age         1       71.87        3.03 [DF F_inc F_all]
```

The conclusion is that an inappropriate design does not allow you to make clear inferences about the different fixed effects. This might be ok if fixed effects are just ‘nuisance parameters, e.g. when you are mainly interested in genetic parameters of EBVs, and fixed effects need to be corrected for. However, even in those cases, inadequate designs make estimates of fixed effects not very accurate. In example 3, the sex difference is estimated based on one comparison in each year (what is the female in 2000 happened to be a good one?) Inaccurate fixed effect estimates do affect accuracy of genetic parameter estimates as well.

Analysis of an example data set

Calf ID	**Age of Dam (yr)**	**Breed**	**Growth Rate (kg/day)**
1	2	AN	2.10
2	3	AN	2.15
3	4	AN	2.20
4	5+	HE	2.35
5	5+	HE	2.33
6	2	HE	2.22
7	3	HE	2.25
8	3	HE	2.27
9	4	SM	2.50
10	5+	SM	2.60
11	2	SM	2.40
12	2	SM	2.45

An appropriate model to describe this data would be a two-way cross classified model without interaction:

$$y_{ijk} = b_0 + b_i + b_j + e_{ijk}$$

where

y_{ijk} is an observation on the growth rate of calves
b_0 is the overall mean
b_i is an effect due to the age of dam of the calf (i = 1,…4)
b_j is an effect due to the breed of the calf (j=1,….3)
e_{ijk} is the residual for each observation

The model written in matrix notation is

$$y = Xb + e$$

The assumptions of the model are

- there are no breed by age of dam interactions
- all other effects were the same for all calves, eg. diet, age, cg
- errors terms are independent and random variables identically distributed around a mean of 0 and a variance of σ_e^2.

The expectation of y is

$$E(y) = Xb$$

and the variance of y is

$$V(y) = I\,\sigma_e^2$$

where

$$Xb = \begin{bmatrix} 1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & 1 & 0 \\ 1 & 1 & 0 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 1 & 0 & 0 & 1 \\ 1 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \beta_0 \\ \beta_{11} \\ \beta_{12} \\ \beta_{13} \\ \beta_{14} \\ \beta_{21} \\ \beta_{22} \\ \beta_{23} \end{bmatrix}$$

Normal Equations

The normal equations for GLS are

$$(X'V^{-1}X)b = X'V^{-1}y$$

however as $V(y) = I\,\sigma_e^2$ then

$$\sigma_e^{-2}\,(X'X)b = \sigma_e^{-2}\,X'y$$

and the GLS equations reduce to those of OLS equations, ie.

$$(X'X)b = X'y$$

which in expanded matrix form is

$$\begin{bmatrix} 12 & 4 & 3 & 2 & 3 & 3 & 5 & 4 \\ 4 & 4 & 0 & 0 & 0 & 1 & 1 & 2 \\ 3 & 0 & 3 & 0 & 0 & 1 & 2 & 0 \\ 2 & 0 & 0 & 2 & 0 & 1 & 0 & 1 \\ 3 & 0 & 0 & 0 & 3 & 0 & 2 & 1 \\ 3 & 1 & 1 & 1 & 0 & 3 & 0 & 0 \\ 5 & 1 & 2 & 0 & 2 & 0 & 5 & 0 \\ 4 & 2 & 0 & 1 & 1 & 0 & 0 & 4 \end{bmatrix} \begin{bmatrix} b_0 \\ b_{11} \\ b_{12} \\ b_{13} \\ b_{14} \\ b_{21} \\ b_{22} \\ b_{23} \end{bmatrix} = \begin{bmatrix} 27.82 \\ 9.17 \\ 6.67 \\ 4.70 \\ 7.28 \\ 6.45 \\ 11.42 \\ 9.95 \end{bmatrix}$$

Obtaining Solutions

X'X is a positive semi-definite matrix with a rank of 6. The dependencies are that columns 2, 3, 4 and 5 and then columns 6, 7 and 8 both sum to give column 1 and thus two constraints on the solution are needed. Letting b_0 and b_{11} be then set to zero, a generalised inverse of X'X is equal to $(X'X)^-$ which we will call G.

$$G = \begin{bmatrix} 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 \\ 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 \\ 0.000 & 0.000 & 0.714 & 0.225 & 0.322 & -0.313 & -0.414 & -0.137 \\ 0.000 & 0.000 & 0.225 & 0.793 & 0.194 & -0.339 & -0.167 & -0.247 \\ 0.000 & 0.000 & 0.322 & 0.194 & 0.670 & -0.172 & -0.396 & -0.216 \\ 0.000 & 0.000 & -0.313 & -0.339 & -0.172 & 0.551 & 0.194 & 0.128 \\ 0.000 & 0.000 & -0.414 & -0.167 & -0.396 & 0.194 & 0.524 & 0.141 \\ 0.000 & 0.000 & -0.137 & -0.247 & -0.216 & 0.128 & 0.141 & 0.366 \end{bmatrix}$$

for which the corresponding solution vector is $\hat{b} = (X'X)^{-}X'y = GX'y$;

$$\begin{bmatrix} \hat{b}_0 \\ \hat{b}_{11} \\ \hat{b}_{12} \\ \hat{b}_{13} \\ \hat{b}_{14} \\ \hat{b}_{21} \\ \hat{b}_{22} \\ \hat{b}_{23} \end{bmatrix} = \begin{bmatrix} 0.000 \\ 0.000 \\ 0.052 \\ 0.082 \\ 0.147 \\ 2.105 \\ 2.204 \\ 2.430 \end{bmatrix}$$

However G above is one of several generalised inverses for X'X and thus there are several possible solution vectors. In fact there are an infinite number of possible solution vectors which are given by the formula,

$b^o = (X'X)^{-}X'y + (I - (X'X)^{-}X'X)z$ where z is an arbitrary vector of constants.

Estimable Functions

By computing the expected value of the solution vector, the *functions* of true parameters that have been estimated by a particular generalised inverse can be determined. These solutions are estimable because *the solution vector is a linear function of y which is always estimable*.

Estimable functions are unique regardless of the solution vector. Consider the function $\hat{b}_{12} - \hat{b}_{11}$ (this function is obtained by multiplying the third row of the matrix of estimable function by b), which can be more generally written as

$$k'\hat{b} = (0\ \ -1\ \ 1\ \ 0\ \ 0\ \ 0\ \ 0\ \ 0)\hat{b} = \hat{b}_{12} - \hat{b}_{11}$$

If another solution vector is used, the same value will be produced for the same function. Thus one quick way to determine if a function is estimable is to multiply it by b and b^o; if the results differ then that function is not estimable. A further method to determine if a function is estimable is to check if

$$k_i'(X'X)^{-}X'X = k_j'$$

Variance of Estimable Functions

The variance of an estimable function is given as

$$\begin{aligned} V(k'\hat{b}) &= k'V(\hat{b})k \\ &= k'V((GX'y)k \\ &= k'GX'V(y)XG'k \\ &= k'(X'X)^{-}X'X(X'X)^{-\prime}k\,\sigma_e^2 \end{aligned}$$

and since $k'(X'X)^{-}X'X = k'$, if k is estimable

$$= k'(X'X)^{-}k\,\sigma_e^2$$

So when k' = (0 -1 1 0 0 0 0 0) and $k'\hat{b}$ = 0.052 then $V(k'\hat{b})$ =

$$\begin{pmatrix}0 & -1 & 1 & 0 & 0 & 0 & 0 & 0\end{pmatrix}\begin{bmatrix} 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 \\ 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 & 0.000 \\ 0.000 & 0.000 & 0.714 & 0.225 & 0.322 & -0.313 & -0.414 & -0.137 \\ 0.000 & 0.000 & 0.225 & 0.793 & 0.194 & -0.339 & -0.167 & -0.247 \\ 0.000 & 0.000 & 0.322 & 0.194 & 0.670 & -0.172 & -0.396 & -0.216 \\ 0.000 & 0.000 & -0.313 & -0.339 & -0.172 & 0.551 & 0.194 & 0.128 \\ 0.000 & 0.000 & -0.414 & -0.167 & -0.396 & 0.194 & 0.524 & 0.141 \\ 0.000 & 0.000 & -0.137 & -0.247 & -0.216 & 0.128 & 0.141 & 0.366 \end{bmatrix}\begin{bmatrix}0 \\ -1 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0\end{bmatrix}\sigma_e^2$$

$$= 0.714\,\sigma_e^2$$

Similarly if a number of estimable functions are to be considered then

$$K' = \begin{bmatrix} 0 & -1 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \end{bmatrix} \text{ then } \quad K'\hat{b} = \begin{bmatrix} 0.082 \\ 0.147 \\ 2.105 \end{bmatrix}$$

and

$$V(K'\hat{b}) = K'(X'X)^{-}K\,\sigma_e^2 = \begin{bmatrix} 0.793 & 0.194 & -0.339 \\ 0.194 & 0.670 & -0.172 \\ -0.339 & -0.172 & 0.552 \end{bmatrix}\sigma_e^2$$

The standard errors of the estimable functions are obtained as the square root of the variances of the estimable functions located on the diagonals above.
In ASREML you can use '*contrast*' to test hypothesis.

Least Square Means

Least square means (LSM) are commonly used in scientific articles as they relate directly to the actual measurements of data and are thus readily understood. However least square means are not equal to the actual raw means but are estimable functions and as such are, of course, unique. In fact LSMs are simply estimators of the marginal means of different classes or subclasses that would be expected in a balanced design, for example

	sex 1	**sex 2**	**LSM (**Year**)**
Year 1	11.0	9.0	10.0
Year 2	16.0	12.0	14.0
LSM (sex**)**	13.5	10.5	12.0

Here the LSM for sex 1 corrected for year effects is 13.5 or alternatively the LSM for Year 1 is 10.0 corrected for sex effects. In the same way least square means can be derived for the different sub-classes of each effect in the previous data corrected for all other effects through the use of regression coefficients. For instance the least square means for the different levels of the effect of age of dam would be

Age of Dam	**L.S. Mean**
2	2.247
3	2.299
4	2.329
5+	2.394

These are not simply $\hat{b}_0 + \hat{b}_{1i}$, especially in this case as this is not an estimable function. Instead these means are estimating $\hat{b}_0 + \hat{b}_{1i} + 1/3(\hat{b}_{21} + \hat{b}_{22} + \hat{b}_{23})$. Alternatively the least square means for the different levels of breed effects are

Breed	**L.S. Mean**
Angus	2.176
Hereford	2.275
Simmental	2.501

which are estimating $\hat{b}_0 + 1/4(\hat{b}_{11} + \hat{b}_{12} + \hat{b}_{13} + \hat{b}_{14}) + \hat{b}_{2i}$.

Exercises for linear models

1 Revision questions

Write a fixed effect model for two independent variables
Give the expectation of the dependant variable (1st moment)
Give the variance of the dependant variable (2nd moment)
State the assumption of the model

2 Regression Model:

We have measured the litter size of a group of sows, and are interested in some effects on this trait, in particular the effect of the age of the sow, and the effect of fat depth at insemination.

single regression:

y = litter size pigs [7 8 9 8 9 10 9 10 11 12]
x = sow weight at insemination (kg) [100 110 120 125 125 130 130 145 150 160]

multiple regression

y = as before,
x1 = as x before
x2 = fat depth at insemination (mm) [20 30 25 40 25 30 35 40 35 35]

Estimate regression coefficients for linear regression models
(You may try also 2^{nd} order regression if you like)

3 Regression and class effects

Consider the following data where fat depth was measured on bulls in two feeding regimes. The bulls were measured at different ages.

Fat Depth (mm)	Feeding Regime	Age at measuring (Mo)
20	Intensive	10
20	Int	14
19	Int	15
24	Int	16
24	Int	17
25	Int	20
26	Int	20
19	Extensive	17
19	Ext	19
21	Ext	21
20	Ext	23

1) Estimate the effect of age on Fat Depth without consideration of feeding regime
2) Estimate the same effect with consideration of feeding regime