COURSE GUIDE

ANP 513
ANIMAL PRODUCTION RESEARCH TECHNIQUES

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MODULE 1

Unit 1 The Design of Animal Production Research Experiments in Different Fields of Animal Science
Unit 2 Artificial Insemination Techniques in Animal Breeding
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UNIT 1 THE DESIGN OF ANIMAL PRODUCTION RESEARCH EXPERIMENTS IN DIFFERENT FIELDS OF ANIMAL SCIENCE

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1.0 INTRODUCTION

In carrying out any experiment in animals, the experimenter/researcher should be very familiar with the animals to be used. The familiarity could be in the form of direct personal experience of the animal either through observation and handling or it could be in form of background information obtained from literature written in relevant textbooks, journals etc. It is important to familiarise yourself with the animals for experimentation for the following reasons:

(i) To measure the growth rate
(ii) To make the animals less hostile
(iii) To avoid embarrassment to the animals
(iv) To ease any delicate operations of construction, debeaking, deworning, parturition etc.

1.1 Formulation of Problems in Animal Research

Research problems are usually formulated based on some past experiences or observation about the subject matter involved. Problems can be classified broadly into two groups according to the system of reasoning used in solving the problems. On the basis of system of reasoning in solving problems, there are two types of problems.

(1) **Deductive Reasoning:** This is based on records or observations for which a general principle is obtained through a conclusion

(2) **Inductive Reasoning:** This is the opposite of deductive reasoning obtained from a general principle. For example, suppose farm animal populations increased over a period of one year by quarterly deworming them, first you would say there is a relationship between populations and deworming. All problems of this type usually start with a group of observations. At times they occur naturally while at other times, they occur under controlled conditions. When these observation are made in some systems they are referred to as a "treatments"

An experiment is the process of examining the truth of statistical hypotheses relating to some research problems. For example, we can conduct an experiment to examine the usefulness of a newly developed drug on farm animals.

1.2 Scientific Methods of Research

The scientific methods of research usually involve the following elements:

(i) **Observed facts:** Science begins with observations from which many facts are then established. While research can be broadly defined as a systemic enquiring into a subject to discover new facts or principles. The procedure adopted for research is known as scientific method.

(ii) **Experiments:** It is a research used to discover something unknown or test a hypothesis. The experiment is a critical step in scientific methods, therefore the questions it seeks to answer should be crucial to support or reject the hypothesis. It is a trial or series of trials designed to test the validity of a hypothesis.

(iii) **Hypothesis:** Hypothesis is an assumption about a population or populations which may or may not be correct. You have the null hypothesis (Ho) and the alternative hypothesis (Ha).

Null hypothesis: It is the hypothesis that is to be tested for acceptance or rejection depending on the experimental results. For instance, if we want to compare four arrival diets A, B, C and

D, we must keep an open mind when starting the experiment. We may therefore assume that his four diets will perform equally well so that can be experienced as:

H_o : $\mu_A = \mu_B = \mu_C = \mu_D$ (The four diets are equally good)

Alternative hypothesis: The alternative hypothesis is the opposite of the null hypothesis. It is determined by the question implicit in the statement of the problem. It can be expressed thus:

H_a : $\mu_A = \mu_B = \mu_C = \mu_D$ (The four diet differ in their effects)

(iv) **Results and their interpretations**

Results of experiments establish addition facts and the interpretation for this new or additional fact in the light of what is already known leads to the support or rejection of the alternative hypothesis.

(v) **Literature review:** It is an examination of the period within the area of study planned by the researcher before the experiment is carried out. This may be helpful in the type of experimental design appropriate to the problem under study or related to it.

1.3 Characteristics of a Well-Planned Experiment

The characteristics of a good or a well-planned experiment are as follows:

1. **Simplicity:** The selection of treatment and experimental materials and their arrangements should be as sample an possible and should be consistent with the objectives of the experiments
2. **Absence of bias or systematic errors:** The experiment must be planned in such a way as to ensure that the experimental materials receiving the treatment such as the animals, in the systematic way differ from those receiving another treatment so that an unbiased estimate of each of the treatment effect can be obtained.
3. **Scope:** The result and conclusion of an experiment should have a wide range of validity and conclusion which could be drawn from it. A factorial set of treatment is another way of increasing the range of validity of an experiment.

4. **Calculation of the degree of uncertainty/internal estimate of experimental/error**

   In any experiment, there is always the need to indicate the degree of uncertainty of the conclusion reached with regards to the validity. The experiment should be designed in such a way that it will be possible to calculate the probability obtaining the observed result by chance alone.

1.4 Procedures for Experimentation

In order to carry out an experiment there is the need to plan and conduct it with something in mind or consider carefully making it a success. This may in the alternative be seen as the principles of scientific enquiry through the design and execution of experiments, analysis and interpretation of results. The final state involve a consideration of the analysis of the results and preparation of complete readable, reliable and correct report of the piece of work done.

The steps to be taken to achieve this include some of the following:

(1) Identification and definition of the problem

The first of this step for a good experimental design is to state the problems to be solved or to decide what hypothesis the experiment is designed to test clearly and concisely. For example, a farmer reported to have used a piece of land for 20 years without yield reductions. One way is to find out why yields did not reduce. Any research aimed at finding out why yields did not drop over the years would be based in wrong observations. This is because it is possible that the researcher who initially made the observation did not collect enough information before jumping into conclusion.

(2) Statement of the objective(s)

Objective(s) should be written out in precise terms, if there is more than one objective, then they should be stated in order of importance. The statement of objective may be in form of questions to be answered, hypothesis to be tested or effects to be estimated. Once a problem is well defined and understood, questions can be formulated around this or the experiment can be designed in such a way that the hypothesis can be accepted or rejected on the basis of the results obtained. Once this is done, the researcher can plan his experimental procedures effectively. In stating this objective(s), the researcher should not be too vague.

(3) Proper review of literature

The literature must be adequately reviewed so as to find out whether or not attempts have been made by previous researchers to solve the problem. That is, there should be a critical analysis of the problems and objectives. The reasonableness and usefulness of the aims of the experiment should be carefully considered based on the stands of knowledge as it relates to the problems. In fact, the selection of a procedure for research depends largely on the subject matter in which the research is being conducted and of course it objectives.

(4) Selection of treatments

The success for the experiment depends to a large extent on the careful selection of treatment whose evaluation would answer the question.

(5) Hypothesis setting

For each parameter to be estimated there must be a hypothesis. For example one can set up an experiment to test the hypothesis that different relations would not affect the performance of broiler chickens. Data will be collected on different parameters and after analysis the hypothesis may be accepted or rejected.

(6) Type of data to collect

The researcher should consider the types of data to collect and how to collect them to be able to answer each of the questions used in the objective of the experiment. This is to determine whether we have the expertise, instrument, laboratories and labour to meet the requirements.

2.0 OBJECTIVES

By the end of this unit, you will be able to:

- understand the basic concepts in carrying out animal production research experiments
- get acquainted with ways of formulating problems in animal research scientific research methods
- list the characteristics of a well-planned experiment and procedures for experimentation.

3.0 MAIN CONTENT

3.1 Basic Concepts of Experimental Designs

The choice of treatment, the method of apportioning treatment to experimental unit in various patterns to suit the requirement of particular problem are commonly referred to as design of experiment. In the course of experimentations, we may wish to study the effect of changes in one variable such as application of different ration/diets and another variable whose change we wish to look at the response value, while that whose effect on the response or dependent variable we wish to study is referred to as independent variable.

The responses from the experimental unit receiving the same treatment may not be identical even under same condition and their valuation may

be assorted to the inherent differences between the experimental units for inaccuracies in the physical conduct of the experiment. Variation in responses is caused by a number of factors such non heterogeneous experimental materials, sanitation, genetic difference, climate factors, animal competition within unit etc. All these factors are referred to as extraneous factors" and when the variation in responses is caused by the extraneous factors, it is known as "experimental error". However, the observed difference between the effects of two treatments is the sum of the true difference. If the errors are small we obtain a better estimate of the true difference but if the errors are large, we obtain a poor estimate of the true difference. Thus, the experimental error is the fundamental basis for deciding whether an observed difference in due to treatments is real or just due to chance.

3.2 Basic Principles of Experimental Designs

The aim of designing an experiment is to increase the degree of precision of the experiment and to achieve this we try to reduce the experimental error. To reduce experimental error, we adopt certain techniques based on the principles of experimental design such as replication, randomization and local control.

- **Replication**

Replication is the repeated application of treatment under investigation. It is only when we repeat the application of the treatment several times that we can estimate the experimental error. Therefore, as the number of replicates increases the experimental error will be reduced. Thus, the functions of replication are: (1) to provide an estimate of experimental error. (2) to improve the degree of precision of the experiment by reducing the standard deviation of a treatment mean (3) to increase the scope of inference of the experiment by selection and appropriate use of quite valuable experimental unit and (4) to effect control of the error variation.

- **Randomisation**

Randomisation is when all the treatments have equal chance of being allocated to different experimental units. It can also be defined as the allocation of treatment to different experimental units without bias. This is because for valid conclusions about our experimental results, we should not first merely have am estimate of experimental error but it should be an unbiased estimate. Furthermore, if our conclusions are to be valid, the treatment means and differences among treatment means should be estimated without bias. It is important to note here that the actual procedure for randomisation of treatments varies according to the experimental design adopted. For instance, if you rear broiler chickens in two different housing systems with each, having a number of

experimental units and birds in each of the housing system are fed with different rations, then the experiment will not lead to a valid conclusion since the housing system may affect the performance of the birds. Therefore, our conclusions about the experimental result will only be valid when both the different rations (treatments) are subjected to all types of housing systems (environment) equally.

- **Local control**

The grouping of homogenous experimental units into block is referred to as local control of error. It is a known fact that the estimate of experimental error in based on the variation from experimental unit to experimental unit. That is, the error in an experiment is a measure of "within block" variation. This therefore means that if we group the homogenous experiment unit into blocks, the experimental error will be greatly reduced. Local control can be established by uniformity or examination of the results of actual experiment.

3.3 Analysis of Variance (ANOVA)

Analysis of variance is most frequently used in basic statistics. It stands for an arithmetic procedure for partitioning total variably in a set of observations among the possible sources of variability. For instance, in a piggery, there is usually a variation in body weight. The lack of uniformity in weight could be due to age and some other unknown factors. In analysis of variance therefore, we try o estimate what fraction of the observed variability is attributable to each of the known causes. In such estimations, some assumptions are however made to include:

(i) That the error terms are randomly independently and normally distributed
(ii) The variances of different samples are homogenous
(iii) Variance and means of different samples are not correlated
(iv) The main effect are addictive

3.4 Major Experimental Designs and Treatment Comparisons

Experimental designs are simply the methods of arranging treatments in order that their effects will be meaningfully tested. When two populations are to be tested the t-test and chi square (X^2) are adequate for use. With three or more means the t-test and chi square (X^2) become inadequate as Type I error may certainly lead to the drawing of wrong conclusion in at least one test. The best bet thereof is the analysis of variance which ensures that an appropriate error term can be used for a single F-test.

(i) Are there differences in analysis of the means in question?
(ii) Are there differences beyond what could be attributed to chance or experimental error? Our concern therefore is how to choose the best design to yield the analysis of variance table which we lead for decision making. By choosing any of the designs, two questions must be answered successfully
(i) How many factors are involved? Each factor may have quantitative of qualities component or properties
(ii) What is the nature of the experimental materials? Are the animal houses or pens uniform or are there sections of the animal houses that are different? Are the animals of same age, sex, weight or physiological state?

3.5 Methods of Experimental Designs

(a) Completely Randomised Design (CRD)

Completely randomised design is the simplest design which incorporates the principles of replication and randomisation. This design comes handy when we have one or more factors provided. The specific condition for use of this design includes:

i. When and where experimental materials are homogenous
ii. When the only source of variability apart from experimental error is the treatment applied. For instance, suppose we want to compare the laying performance of five breeds of layers in four replicates? There is no cause to suspect that various parts of the hen differ in shape or some other respect we could assume homogeneity in the animal pen. Then we proceed with the randomisation scheme characteristics of CRD

Treatment = 5 breeds of layers
Replicated = 4
Total of pens = 5 x 4 = 20
Let the breeds be A,B,C,D and E
Each breed will appear 4 times in the experiment

Table 1: Randomisation Scheme in CRD

1A	6C	11D	16A
2D	7E	12B	17E
3B	8C	13E	18B
4D	9A	14D	19C
5B	10C	15E	20A

The basic assumption here is that all the 20 pens are alike. It does not matter where any of the treatment falls.

Therefore, we are randomizing the treatment over the whole experimental area of 20 pens and this is the origin of name completely randomised design (CRD).

Data arrangement for analysis for example

Breed of layers

Replicates	**A**	**B**	**C**	**D**	**E**
1	2	3	7	1	10
2	3	6	8	2	9
3	4	4	9	1	11
4	5	5	10	3	12
Treatment totals	**14**	**18**	**34**	**7**	**42**
Treatment means	**3.5**	**4.5**	**8.5**	**1.73**	**10.5**

Hypotheses

Null Hypothesis (H_o)
$H_{o:}$ T1 = 0 (Ha: $\mu_A \neq \mu_B \neq \mu_C \neq \mu_D \neq \mu_E$). this mean treatment effect is not zero.
Liner model:
Analysis is based in $X_{ij} = \mu + T_j + E_{ij}$
Where,
X_{ij} = Value of any observation
μ = Unknown constant
T_j = Treatment effect
E_{ij} = Error term.

This means that the total variability has only two components.
Total SS Treatment SS + Error SS
(i) Grand total (GT) = $\Sigma\mu_1 + \mu_2 + \mu_3 + \mu_4 + \mu_5 + \ldots.\mu_{20}$
= Σ2+3+7+1+10+ …..12

= 115

(ii) Correction factor (CF) = $\frac{GT^2}{n}$

where, GT = Grand Total

n = total number of observations

CF = $\frac{115^2}{20}$ = 661.25

(iii) Total sum of sequences (SS) = $\Sigma\mu_1^2 + \mu_2^2 + \mu_3^2 + \mu_4^2 + \mu_5^2 + \ldots\ldots\mu_{20}^2$ + --- CF

Total SS = $\Sigma 2^2 + 3^2 + 7^2 + 1^2 + 10^2 + \ldots\ldots\ldots\ldots 12^2$ ..CF

= 895 -661.25 = 233.75

(iv) Treatment SS = $\frac{\Sigma T^2}{r}$ – CF

where, T = treatment total

r = no of replicates

CF = Correction factor

∴ Trt SS = $\frac{14^2}{4} + \frac{18^2}{4} + \frac{34^2}{4} + \frac{7^2}{4} + \frac{42^2}{4}$ – CF

= $\frac{14^2 + 18^2 + 34^2 + 7^2 + 42^2}{4}$ – CF

= $\frac{3489}{4}$ - 661.25

= 872.25 -661.25 = 211.00

(v) Error sum of squares = Total SS – Trt SS

= 233.75 – 211.00 = 22.75

Degrees of freedom (DF)

(i) Total df = rt – 1

Where r = number of replicates

t = No of treatments

∴ Total df = 4x5 -1

= 20-1 = 19

(ii) Trtt df = t-1

= 5-1 = 4

(iii) Error df = t(r-1) or (t-1)(r-1)

= 5(4-1) or (5-1)(4-1)

= 5x3 or 4

= 15

Analysis of Variance (ANOVA) Table

Sources of variance	Df	SS	Mean sum of Squares	Fcal	Ftab (5%)
Total	19	233.75			
Treatment	4	211.00	52.75	34.70	3.06
Error	15	22.75	1.52		

(iv) Mean sum of square for treatment = $\frac{\text{Treatment SS}}{\text{Treatment df}}$

$= \frac{211.00}{4} = \underline{52.75}$

MSS for Error = $\frac{\text{Error SS}}{\text{Error df}} = \frac{22.75}{15} = \underline{1.52}$

f- calculated for Treatment effect (f.cal) = $\frac{\text{T\&t MSS}}{\text{Error MSS}}$

$= \frac{52.75}{1.5} = \underline{34.70}$

F tab (4, 15) @ 5% level of probability = 3.06

F tab (4, 15) @ 1% level of probability = 4.89

Inference

Since F tab is greater the F tab, we reject the null hypothesis and accept the alternative hypothesis and accept that at least two breeds of layer differ in their performances.

Note the use of asterisks in the ANOVA table

* = significant ($P \leq 0.05$)

** = highly significant ($P \leq 0.01$)

N.S = Not significant ($P \leq 0.05$)

Advantages of CRD

(i) It is very simple classify, design and analysis

(ii) The layout is easy

(iii) Allows for maximum degree of freedom for estimates experimental error

(iv) Data can easily be analysis even when some experimental unit are missing

(v) It places no limitation on the mode of replications

Disadvantages of CRD

(i) This design is used only when the experimental material is homogenous and this condition of complete uniformity is not common in rela life. Therefore, the application of this design becomes limited.

(ii) The layer the number of treatments, the greater the number of experimental unit and consequently the greater the probability of having non-unfiorm experimental material. Therefore, this design may be suitable only for a limited or small number of treatments.

CRD with some missing data

Even through CRD takes care of missing data easily; it is advantages to have equal sample sizes for the following reasons:

(i) Ease of computations for various variances and even standard errors for least significant differences (LSD) or Duncan's Multiple Range Test (DMRT).

(ii) Minimize the possible effect of heterogeneity of population variances

(iii) Enhancing the possibility of rejecting the null hypothesis when it is false

Example 2

A researcher set out to examine the radiation in rectal temperature of sheep during the dry season months of January to April and he obtained the following data

Months				
	Jan	Feb	March	April
	11	12	17	13
	13	16	19	11
	12	14	16	11
	14	-	15	12
	11	-	15	-
T&t Total	61	42	82	47
Mean	12.2	14.0	16.0	11.8

Hypothesis

H_0: Ti = 0 (Period has no effect on rectal temperature)

H_a: Ti ≠ 0 (rectal temperature ray from month to month).

Calculation

(i) Grand total (GT) = $\Sigma\mu_1+\mu_2+\mu_3+\mu_4+----------\mu_{17}$

$= \Sigma\ 11+12+17+13+---------15$

$= \underline{232}$

(ii) Correction factor (CF) = $\underline{GT^2} = \underline{232^2}$

n 17

$= \underline{53,824} = \underline{3,166.12}$

17

(iii) Total SS = $\Sigma\mu_1^2 + \mu_2^2 + \mu_3^2 + \mu_4^2 +---------- \mu_{17}^2 ---$ CF

$= \Sigma 11^2+12^2+17^2+13^2+----------15^2$ ------CF

= 3,258 – 3,166.12 = $\underline{91.92}$

(iv) Trt SS = $\underline{\Sigma T^2}$ – CF

r

$=\Sigma\underline{61}^2 + \underline{42}^2 + \underline{82}^2 + \underline{47}^2 +$ -------CF

5 3 5 4

$=\frac{3,721}{5} + \frac{1,764}{3} + \frac{6,724}{5} + \frac{2,209}{4}$ - CF

= 744.2+588+1344.8+552.25 – 3,166.12

=3,229.25-3,166.12 = $\underline{63.15}$

(v) Error SS = Total SS – Trt SS

91.92-63.15 = $\underline{28.77}$

(vi) Total df = n -1

= 17 – 1 = $\underline{16}$

(vii) Trt df = t-1

= 4 -1 = $\underline{3}$

(viii) Trt MSS = $\frac{Trt\ SS}{Trt\ df} = \frac{63.15}{3}$ = $\underline{21.05}$

(ix) Error MSS = $\frac{Error\ SS}{Error\ df} = \frac{28.77}{13}$ = $\underline{2.21}$

(x) Trt feal = $\frac{Trt\ MSS}{Error\ MSS} = \frac{21.05}{2.21}$ = $\underline{9.53}$

ANOVA TABLE

Sources of variation	Df	SS	MSS	Feal	Ftab (5%)	Ftab (1%)
Total	16	91.92				
Treatment	3	13	21.05	9.53	3.41*	
Error	13	28.77	2.21			

(xi) **Inferences**

Since ftab >ftsb, we reject the null hypotheses and accept the alternative. This means that period significantly affected the rectal temperature of sheep.

Estimating a missing value in CRD

Data for one or more experimental unit may be missing due to some reasons. The most appropriate thing to do under such situation is to repeat the experiment. However, repeating an experiment often involve a waste of scarce resources with regards to time, money and energy. To some the situation a dummy value could be calculated to replace the missing value. A dummy value is calculated when only one value is missing and it is one in such areas that the mean value is left unchanged. That is it is assumed to evutain no error. Therefore, when we calculate a dummy value, we lose one degree of freedom for the experimental error that is we subtract one for the error degree of freedom.

Example 3: In the CRD analysis Table below, an observation is missing in treatment B and replicate 3. Calculate the dummy value for it.

Treatments				
Replicates	A	B	C	D
1	6	7	4	10
2	10	8	5	11
3	21	-	6	12
4	42	21	7	13
5	10	10	8	14

Dummy value = $\frac{7+8+21+10}{4} = 11.5 = \frac{46}{4} = \underline{11.5}$

Note that the expected error df= (rt-1)-(t-1)

= (5x4-1) – (4-1)

= (20 – 1) – (4-1)

19 – 3= 16

After calculating the dummy error df= 16-1=15

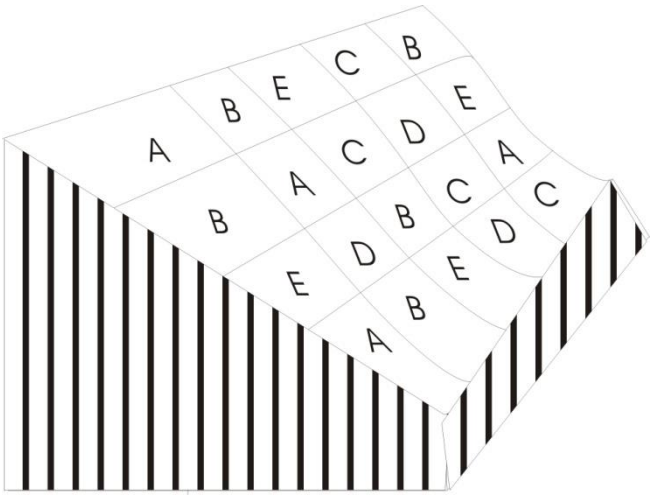
(b) Randomised Complete Block Design (RCB) or Two-Way Classification

In the CRD, total sum of squares consist sometimes of another source of variability other than those that could be identified. When such a factor is identified and can be grouped RCB comes handy. For such a factor, the form or direction of the variability must be known before any meaningful grouping can be done to qualify the magnitude of the variability. Suppose it is designed to examine the effects a litter size of feeding five levels of groundnut cake (GNC) to pigs aged between one and five year the difference in age groups may affect the response to the test diets. This is therefore another source of variability which must be carefully handled. Therefore, one cannot randomize the treatment without regards to the age of the pigs. In another example, a researcher

may discover that the annual house is located in a slope and the sloping may affect the performance of the animals located in the pens along the slope. The sloping of the house constitutes another source of variability. We therefore categorise the pens into different sections across the slope and this constitutes blocking. Each of the sections represents a replication. Every treatment must be given the chance to appear on each block to avoid big that is randomisation in restricted to the block hence we have randomised complete block design. However, other factors can constitute blocking such as growth chambers, animal pens, incubators etc.

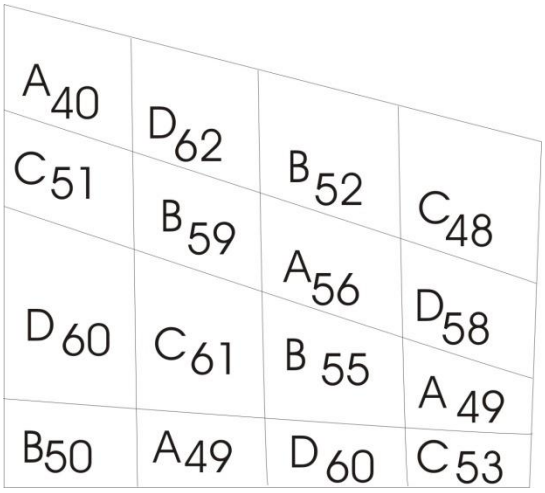
(c) Randomisation scheme for RCBD

Suppose, five treatments, A, B, C, D, E were fed to broiler chickens in a poultry house located on a slope we could randomise the treatment an shown below.



Example 1:

If an animal scientist sets out to investigate the effect of four plant protein sources on the weight again of broiler chicken reared in a housing system located on a slope for eight weeks and obtained the data below. Test whether the treatment (plant produce sources) and sloping of the house significantly affected the weight of the birds.



Data arrangement for analysis

Treatments

Blocks	**A**	**B**	**C**	**D**	**Block Total**	**Mean (B)**
I	40	52	48	60	200	50
II	49	55	51	62	216	54
III	56	59	53	60	228	57
IV	49	50	61	58	218	54.5
Trt Total	193	216	213	240		
Means (x)	48.3	54.0	53.3	60.0		

Hypotheses

For Treatment effect

H_0: $T_i = 0$ ($\mu A = \mu B = \mu C = \mu D$)

H_a: $T_i \neq 0$ ($\mu A \neq \mu B \neq \mu C \neq \mu D$)

For Block

H_0: $B_i = 0$ (No difference among the blocks)

H_a: $T_i \neq 0$ (at least, one block differs)

Linear Model

$Xij = \mu + T_i + B_i + E_{ij}$

Where,

μ = unknown constant
Ti = treatment effect
Bi = Block effect
Eij = Error term
Xij = Value of any observation

Calculations

(i) Grand total (GT) = $\Sigma\mu_1+\mu_2+\mu_3+\mu_4+----------\mu_{16}$
$= \Sigma 40+52+48+6+60+-------58$
$= \underline{\underline{862}}$

(ii) Correction Factor (CF) = $\frac{GT^2}{n}$
$= \frac{862^2}{16} = \frac{743044}{6} = \underline{\underline{46440.25}}$

(iii) Total SS = Σx^2 --- CF
$= \Sigma\mu_1^2+\mu_2^2+\mu_3^2+\mu_4^2+ ----------\mu_{16}^2 -------CF$
$= \Sigma 40^2+52^2+48^2+60^2+ ----------58^2 ----CF$
$= 47024 - 46440.25 = \underline{\underline{583.75}}$

(iv) Trt SS = $\frac{\Sigma T^2}{r} - CF$
$= \Sigma\frac{t_1^2 + t_2^2 + t_3^2 + t_4^2}{r} - CF$
$= \Sigma\frac{193^2+216^2+213^2+240^2}{4} - CF$
$= \frac{37249+46656+45369+57600}{4} - CF$
$= \frac{186874}{4} - 46440.25$
$= 46718.5 - 46440.25 = \underline{\underline{278.25}}$

(v) Block SS = $\frac{\Sigma B^2}{r} - CF$
$= \Sigma\frac{b_1^2+ b_2^2+ b_3^2+ b_4^2}{r} - CF$
$= \Sigma\frac{200^2+216^2+228^2+218^2}{4} - CF$
$= \frac{40000+46656+51984+57524}{4} - CF$
$= \frac{186184}{4} - 46440.25$
$= 46541 - 46440.25 = \underline{\underline{100.75}}$

(vi) Error SS = Total SS – Block SS
$= 583.75-27825 - 100.75 = \underline{\underline{204.75}}$

(vii) Degrees of freedom (Df)

i. Total df = rt – 1
$= 4x4 - 1$
$= 16 - 1 = \underline{\underline{15}}$

ii. Trt df = t – 1
$= 4 - 1 = \underline{\underline{3}}$

iii. Block df = r – 1

= 4 – 1 = 3

iv. Error df = (rt – 1)-(t-1)(r-1) or (r-1)(t-1)
= (4x4 – 1)-(4-1)-(4-1) or (4-1)(4-1)
= (16-1) – (4-1) – (4-1) or (3)(3) = 9
= 15 -3 – 3 = 9

Analysis of Variance (ANOVA) Table

Sources of variation	Df	SS	MSS	Feal	Ftab (5%)	1%
Total	15	583.75				
Treatment	3	278.25	92.75	4.08	3.21*	3.86*
Block	3	100.75	35.58	1.48	3.21^{NS}	3.86^{NS}
Error	9	204.74	22.75			

Treatment Mean sum of squares (MSS) = $\frac{\text{Trt SS}}{\text{Trt df}}$

$= \frac{278.25}{3} = 92.75$

Block MSS = $\frac{\text{Block SS}}{\text{Block df}} = \frac{100.75}{3} = 35.58$

Error MSS = $\frac{Error\ SS}{\text{Error df}} = \frac{204.74}{9} = 22.75$

Treatment fcal = $\frac{\text{Trt MSS}}{\text{Error MSS}} = \frac{92.75}{22.75} = 4.08$

Block fcal = $\frac{\text{Block MSS}}{\text{Error MSS}} = \frac{35.58}{22.75} = 1.48$

Inferences

(i) For Treatment: Since Fcal > Ftab we reject the null hypothesis and accept the alternative hypothesis. This means that different plant, protein, sources, significant affected the weight of broiler chickens

(ii) For Block: Since Fcal < Ftab, we accept the null hypothesis and reject the alternative hypothesis this mean that sloping did not significantly affect the weight gain of broiler chickens.

Advantages of RCBD

i. When blocking is effective RCB increases precision
ii. It allows the use of any member of treatment and replications within the limit of the experimental material
iii. Missing data can be easily handled just like as in CRD
iv. Analysis relatively easy
v. Block means cash be used to estimate block effect.
vi. Error variance like in CRD can be easily sub divided according to the treatment levels.

Disadvantages of RCBD

i. It is suitable for a limited member of treatments. With large member of treatment, a block many no longer be homogenous

ii. When blocking is with effective, the loss of block degree of freedom (r-1) for estimating error mean square will reduce the efficiency of the experiment

Missing observation in RCBD

When one observation is missing in RCB based experiment a dummy value can be calculated just as we did in CRD and adjustment made in error degree of freedom

Dummy value (x) = $\frac{tT+bB}{(t-1)(b-1)}$ - S

Where, X = dummy

t = Number of treatments

T = Sum of observations for same treatment as missing term.

b = Number of blocks

B = Sum of items in same block a missing term

S = Sum of all observations

Example 2: Estimate the missing value form the table below

	Blocks			
Treatment	I	II	III	IV
A	10	14	5	1
B	11	15	2	2
C	12	-	4	3
D	13	10	6	4

Dummy (x) = $4\frac{(19)+4(39)}{(4-1)(4-1)} - 112$

$= \frac{76+156}{(3)(3)} - 112$

$= \frac{120}{9} = \underline{13.33}$

Note that since the dummy value has been calculated, you subtract 1 from the expected error df of 9

∴ Error df = 9 – 1 = $\underline{8}$

(d) Latin square design

In Latin square design, two major source of variability can be identified other the treatment and error. Suppose a researcher intends to assess the performance of pigs fed diet of varying levels of protein, he many discover that the construction of the house is such that it slopes from one side towards another. Besides, the pigs available may also be of different weight categories. In this instance, one can predict variability in two directions apart for variability due to the varying levels of protein. These two additional sources of availability must be taken care of by blocking and should be due in two directions (rows and columns). The purpose of this double grouping is to eliminate the errors cell difference among rows and columns. Therefore Latin square design provides a greater opportunity than RCBD for reduction of errors by skillful planning. The key to this planning is restriction of randomization of treatment in two directions (rows and columns). The rows and columns refer to criteria of classification with just physical directions. Another important feature of the design in that the number of treatment must be square to the number of replications.

Randomisation scheme in Latin square design
In Latin square design, each treatment appears only once in each error and each column. Therefore, for easy layout, one way randomised treatments within the just row and just column. The rest can be easily fixed up in such a way that no treatment appears twice in a given row or column. We can therefore low a 3x3, 4x4 5x5 etc Latin square. For example, 4x4 and 5x5

A	B	C	D
B	C	D	A
C	D	A	B
D	A	B	C

4 X 4 = 16

A	B	C	D	E
B	C	D	E	A
C	D	E	A	B
D	E	A	B	C
E	A	B	C	D

5 X 5 = 25

Example 1: A researcher set out to examine the effect of feeding four vegetable protein sources to pigs at four stages of frequency under four housing system. The parameter used for assessment was the little size. For the data below test whether vegetable protein sources, stages of frequency and housing system was effect on the lither size of pigs

	Stages of pregnancy (Months)			
Housing system	0	1	2	3
A	GNC(2)	SBM(8)	CSM(1)	RSM(12)
B	CSM(2)	RSM(6)	SBM(9)	GNC(3)
C	SBM(10)	GNC(4)	RSM(5)	CSM(3)
D	RSMG(5)	CSM(0)	GNC(5)	SBM(10)

Let 4 stages of pregnancy = Columns
4 housing system = Rows
4 vegetable protein sources = treatment (GNC, SBM, CSM, CSM RSM)
Figure = observation (litter size)
Hypothesis

(i) Treatment effect

H_0 : Ti = (μGNC = μSBM = μCSM = μRSM)
H_a : Ti ≠ (μGNC ≠ μSBM≠ μCSM≠ μRSM)

(ii) Column effect

H_0: Ci = 0 (Stages of pregnancy did not affect litter size)
H_a: Ci ≠ 0 (litter size very according to stage of pregnancy)

(iii) Row effect

H_0: Ri = 0 (Housing system had no effect in litter size)
Ha: Ri ≠ 0 (Housing system had effect on letter size)
LINEAR MODEL
Xij : $\mu + C_i + R= + T= + E_{ij}$

Where, μ = unknown constant
C_i = Column effect
R_i = row effect
T_i = treatment effect
E_{ij} = Error term
X_{ij} = value of any observation

Analysis

(1) Arrangement of data for rows and columns totals

Stages of pregnancy (Months)					
Housing systems	0	1	2	3	Row Total
A	2	8	1	12	23
B	2	6	9	3	20
C	10	4	5	3	22
D	15	0	5	10	30
Column Total	29	18	20	28	95

(2) Treatment Table: All treatments arranged in row

Treatments				
Rows	GNC	SBM	CSM	RSM
I	2	8	1	12
II	3	9	2	6
III	4	10	3	5
IV	5	10	0	15
Trt Total	14	37	6	38
Trt Means (X)	3.50	9.25	1.50	9.50

(3) Calculations

(I) $= \Sigma\mu1 + \mu2 + \mu3 + \mu4$ --- X16

$= \Sigma 2+8+1+12+$ ------10 = 95

(II) CF = $\frac{GT2}{n} = \frac{GT2}{n} = \frac{952}{16} = \frac{9025}{16}$ = 278.94

(III) Total SS = $\Sigma\mu_1^2 + \mu_2^2 + \mu_3^2 + \mu_4^2 + 2x$---- μ_{16}^2 ---- CF

$= \Sigma 2^2+8^2+1^2+12^2+$ ------ 10^2 – CF

843 – 564.06 = 278.94

(IV) Column SS = $\frac{\Sigma C_1^2 + C_2^2 + C_3^2 + C_4^2 +}{2}$ ---- CF

$= \frac{\Sigma 29^2+18^2+20^2+28^2}{4}$ --- CF

$= \frac{2349}{4}$ – 564.06

578.25 – 564.06 = 23.19

(V) Row SS = $\frac{\Sigma R_1^2 + R_2^2 + R_3^2 + R_4^2}{r}$ - CF

$= \frac{\Sigma 23^2+20^2+22^2+30^2}{4}$ --- CF

$$= \frac{2313}{4} - 564.06$$

$= 578.25 - 564.06 = \underline{\underline{14.19}}$

(VI) Trt SS $= \frac{\Sigma t_1^2 + t_2^2 + t_3^2 + t_4^2}{r} - CF$

$= \frac{\Sigma 14^2+37^2+6^2+38^2}{4} --- CF$

$= \frac{3045}{4} - 564.06$

$= 761.25 - 564.06 = \underline{\underline{197.19}}$

(VII) Error SS = Total SS – Trt SS – Column SS – Row SS
= 278.94 – 197.19 – 23.19 – 14.19 = $\underline{\underline{44.37}}$

(VIII) Degree of freedom for Latin square
Total df = k^2-1 (since mode of treatments, rows and columns are equal)

Treatment df = K – 1
Column df = K-1
Row df = k-1
Error df = K^2-1 – (K-1) - (K-1) - (K-1) = (K-1) (K-2)

Therefore,
Total df = K^2-1 = 4^2-1 = 16-1 = $\underline{15}$
Trt df = K-1 = 4-1 = $\underline{3}$
Column df = K-1 = 4-1 = $\underline{3}$
Row df =K-1 = 4-1 = $\underline{3}$
Error df = (K^2-1) - (K-1) - (K-1) - (K-1) - (K-1)(K-2)
= 4^2-1 – (4-1) - (4-1) - (4-1) or (4-1) (4-2)
= 16-1 – 3- 3 -3 or (3)(2) = $\underline{6}$
= 15 – 9 = $\underline{6}$
ANOVA Table

Sources of variation	Df	SS	MSS	FEAL	Ftab(%)
Total	15	278.94			
Treatment	3	197.19	65.73	8.88	4.76*
Column	3	23.19	7.73	1.05	4.76^{NS}
Row	3	14.19	4.73	0.64	4.76^{NS}
Error	6	44.39	7.40		

(i) Trt MSS = $\frac{\text{Trt SS}}{\text{Trt df}} = \frac{197.19}{3} = \underline{65.73}$

(ii) Column MSS = $\frac{\text{Column SS}}{} = \frac{23.19}{} = 7.73$

Column df 3

(iii) Row MSS = $\frac{\text{Row SS}}{\text{Row Df}} = \frac{14.19}{3} = \underline{4.73}$

(iv) Error MSS = $\frac{\text{Error SS}}{\text{Error Df}} = \frac{44.39}{6} = \underline{7.40}$

(v) Trt Feal = $\frac{\text{Trt MSS}}{\text{Trt MSS}} = \frac{65.73.39}{7.40} = \underline{8.88}$

(vi) Column Feal = $\frac{\text{Column MSS}}{\text{ErrorMSS}} = \frac{7.73}{7.40} = \underline{1.05}$

(vii) Row Feal = $\frac{\text{Row MSS}}{\text{ErrorMSS}} = \frac{4.73}{7.40} = \underline{0.64}$

Inferences

(i) Treatment effect: since feal > T-tab we reject the null hypothesis and accept the alternative hypothesis. This means that litter size was affected by the vegetable protein

(ii) Row effect: Feal < Ftab and therefore accept H_0 and reject H_a which means that housing system had no effect in litter size.

(iii) Column effect: Feal < Ftab, we accept H_0 and reject H_a. This means that litter size did not very due to pregnancy stages

Advantages of Latin square design

(i) It allows for controlling two sources of variability in the experimental material.

(ii) When the double group in effective, if makes for precise estimates for experimental error.

(iii) When well used it written the scope of inference were than RCBD

Disadvantages of Latin square design

(i) When there are many treatments, the size of experiment become unnecessarily large.

(ii) As the block size increase. The experimental error per unit treatment unlikely to increase.

(iii) With few treatment,. The squares are small and provide just few degree of freedoms for estimating experimental error.

(iv) Where heterogeneity of various in suspected the error cannot be easily subdivided as in the case of RCBD.

Missing observation for Latin Square

If one observation is missing in a latin square experimental, its driving can be easily estimated using the formula below

$X = \frac{K(R+C+I)}{(k-1)(k-2)} - 2S$

Where, K = No of rows/columns/treatments

R = Sum of observation in the same row as driving

C = Sum of observation in the same column driving

T = Sum of items in the same treatment as missing value.

S = Sum of all observations available

X = Dummy.

Comparison of means pair wise

When the, F – test for any testable, effect in the ANOVA table indicate significant differences, it suggest that at least one pair of the means in question must be different. This implies that whatever differences exist is too big to be attributed to experimental error. Several methods exist for comparing all possible pairs of means when significant difference exists. Such methods are:

(i) Leash significant difference (LSD)
(ii) Duncan's multiple range Test (DMRT)
(iii) Bayesian Modification of LSD (BLSD)
(iv) Turkey's Honest Significant difference ((HSD)
(v) Scheffe's method
(vi) Student Newman – Keul's Test
(vii) Dunnett's Test

However the two most commonly used methods in Agricultural and Biological Sciences are LSD and DMRT.

(1) **Least Significant Difference (LSD)**

The LSD was formulated and later modified by R.A. Fisher. The modified by R.A. Fisher. The modification stipulates that it should be used why when the F – test indicates significance. LSD is usually regarded as a valid test criterion for planed comparison of paired means. It is a student t-test that uses a pooled error variance. The general formula for calculating LSD is:

$$\text{LSD} = t\frac{\propto}{2}(\text{error df})\frac{\sqrt{2\text{EMs}}}{r}$$

Where, $\propto$ = significant level

r = No of replicates/observation per treatment/total

t = t-test table
error df = Error degree of freedom
EMS = Error Mean Square

Example 1: In a CRD experiment with six treatment, four replicates and error mena square of 11.42, the following mean values are obtained:

Means		A	B	C	D	E	F
	10.5	6.5	11.9	12.2	16.0	10.7	

Calculate and use the LSD to compare the means at $\propto$- risk of 0.05 and error df of 18.

PROCEDURE:

$LSD = t\ \frac{\propto}{2}\ (\text{error df})\ \frac{\sqrt{2EMs}}{r}$

Where,

$t = \frac{\propto}{2} = 0.025$

error df = 18
EMS 11.42
r = 4

$LSD = 0.025\ (18)\ \frac{\sqrt{2x11.42}}{4}$

$= 2.101\ \frac{\sqrt{22.848}}{4}$

$= 2.101\ \frac{\sqrt{5.712}}{}$

$\therefore$ LSD = 2.101x2.390 = <u>5.02</u>

Step 1: Arrange the means in a decreasing order omitting the lowest horizontally and in an increasing order vertically, omitting the highest.

		E	**D**	**C**	**F**	**A**	**B**
		16.0	12.2	11.9	10.7	10.5	6.2
B	6.2	9.8	6.0	5.7	4.5	4.3	0
A	10.5	5.5	1.7	1.4	0.2	0	
F	10.7	5.3	1.5	1.2	0		
C	11.9	4.1	0.3	0			
D	12.2	3.8	0				
E	16.0	0					

Step 2: Subtracts the vertical for the horizontally figures to get a matrix of difference

Step 3: line up the means in a decreasing order and compare the matrix of values with the LSD value = <u>5.02</u>

E D C F A B

16.0^{a}	12.2^{ab} 6.2^{c}	11.9^{ab}	10.5^{bc}	10.5^{bc}	

Take the column under E and check whether nay value in less than or bigger/larger/greater than 5.02. in the column, E is greater than B. A and F in comparing with the LSD but less than C and D this means that means E is significant different from means B, A and F but not different from C and D. we therefore allocate letter 'a' on superscript to E but because C and D values are less than LSD they show the same superscript 'a' with E to indicates their similarities. When we do the same that to D, we notice that D in larger than B but not different from A, F and C, we therefore allocate 'b' to D, A F and C. repeat the process for C, F and A.

Step 4: Rearrange the means in alphabetical order

A	B	C	D	E	F
16.0^{a}	12.2^{ab} 10.7^{bc}	11.9^{ab}	10.5^{bc}	6.2^{c}	

Note than means with varying superscripts differ significantly @ 5%

Advantage of LSD

(i) It is relatively simple, to compute
(ii) It has only a single value for comparison
(iii) It is have sensitive to small difference than most other procedure
(iv) When used in conjunction with preliminary F-test, it has much to recommend it use.

Disadvantages of LSD

(i) It is often abused and used for carrying all possible mease even without the f –test showing significant difference

2. Duncan's Multiple Range Test (DMRT)

Duncan purposely developed this test to reduce experiment wise error rate. If there is no tree difference among a set of means, DMRT is less likely to declare a difference than LSD.

Using the same example for LSD, the means are:

A	B	C	D	E	F
10.5	6.2	11.9	12.2	16.0	10.7

Procedure

Step 1: Arrange the means in ascending order with the least first

B	6.2
A	10.5
F	10.7
C	11.9
D	12.2
E	16.0

Step 2: Calculate the standard error (S.E) using the formula below

$$S.E = \frac{\sqrt{EMS}}{r}$$

Where,

r = number of replicates = 4

EMS = error mean square = 11.42

$$\therefore SE = \frac{\sqrt{11.42}}{4} = \underline{1.699}$$

Step 3: Obtain the significant standardized ranges the statistical table using 2 0.05 and error df of 18 and multiply each with the standard error (S.E) to obtain the least significant Ranges (LSR) using the formula below.

$LSR_1 = r \propto (pedf) \times S.E$

Where,

$\propto$ = level of significance

p = relative position in the array

edf = error degree of freedom

r = Duncan's Multiple Range test table

$LSR_2 = 0.05(2,18) \times 1.699$

$= 2.97 \times 1.699 = \underline{5.05}$

$LSR_3 = 0.05(3,18) \times 1.699$

$= 3.12 \times 1.699 = \underline{5.30}$

$LSR_4 = 0.05(4,18) \times 1.699$

$= 3.21 \times 1.699 = \underline{5.45}$

$LSR_5 = 0.05(5,18) \times 1.699$

$= 3.27 \times 1.699 = \underline{5.56}$

$LSR_6 = 0.05(6,18) \times 1.699$

$= 3.32 \times 1.699 = \underline{5.64}$

Step 4: To compare the means, calculate the range between means by subtracting the least means you're the largest repeat the process with the next least mean until you obtained range of values for between the

means in question. Then compare the ranges between mean with LSR stating form the highest LSR to the lowest.

E	-	B	=	16.0	-	6.20	=	9.8	> 5.64
E	-	A	=	16.0	-	10.5	=	5.5	< 5.56
E	-	F	=	16.0	-	10.7	=	5.3	< 5.45
E	-	C	=	16.0	-	11.9	=	4.1	< 5.30
E	-	D	=	16.0	-	12.2	=	3.8	< 5.05
D	-	B	=	12.2	-	6.2	=	6.0	> 5.56
D	-	A	=	12.2	-	10.5	=	1.7	< 5.45
D	-	F	=	12.2	-	10.7	=	1.5	< 5.30
D	-	C	=	12.2	-	11.9	=	0.3	> 5.05
C	-	B	=	11.9	-	6.2	=	5.7	> 5.45
C	-	A	=	11.9	-	10.5	=	1.4	< 5.30
C	-	F	=	11.9	-	10.7	=	1.2	< 5.05
F	-	B	=	10.7	-	6.2	=	4.5	< 5.30
F	-	A	=	10.7	-	10.5	=	0.2	< 5.05
A	-	B	=	10.5	-	6.2	=	4.3	< 5.05

The compare E with any of the other means, the LSR we expect is 5.64. when we look at the ranges between E and other means, E in greater than only B but less than A, F, C and D. this means that E in only significantly different four B but not different from A, F, C and D. therefore, you allocate, superscript 'a' to E, A, F, C and D to indicate similarities between the means.

E	D	C	F	A	B
16.0^{a}	12.2^{a}	11.9^{a}	10.7^{ab}	10.5^{ab}	6.2^{bc}

Step 5: Rearrange the means in an alphabetical order

A	B	C	D	E	F
10.5^{ab}	6.2^{b}	11.9^{a}	12.2^{ab}	16.0^{a}	
	10.7^{ab}				

Note that means with varying superscript different significant ($p < o.05$). The result of this comparison is different from that of LSD.

Advantages of DMRT

(i) It is an good on LSD in pair wise comparison but less likely to lead to Type I error when there is no time differences in a set of many lessons

(ii) Takes into account the number of treatments

(iii) F-test does not have to be significant before one can proceed.

Disadvantages of DMRT

(i) Very cumbersome to calculate when the means are many.
(ii) It has different values to be considered

Sampling/nested classification

In certain experiments, more than one observation may be made for each experimental unit. For example, an animal scientist many decide to take three different blood samples from an arrival in one experimental unit and analyses them differently rather than analysing one bulky sample. In other words, a single sample may be subdivided into sub samples so that for each experimental unit, more than one observation in recorded.

Example 1: An annual scientist set out to compare the total serum protein level of broiler chicken feed with Groundout Care (GNC) processed through three different methods. At the end of the feeding trial, he collected three blood samples from each experimental that and each treatment was replicated four times. The data obtained are presented in the table below.

	Blood	Blocks			
Treatments	Samples	I	II	III	IV
	1	1.2	1.0	1.8	1.6
A	2	1.5	1.4	1.9	1.8
	3	1.6	1.7	1.6	1.6
	1	2.0	1.9	2.0	2.5
B	2	1.9	2.4	3.0	2.4
	3	2.5	2.5	2.5	3.0
	1	3.0	3.1	3.1	3.3
C	2	2.5	3.6	3.2	3.2
	3	3.4	3.2	3.3	3.5

Step 1: Rearrange the data to suit statistical analysis.

	Blood	**Blocks**				
Treatments	**Sample**	**I**	**II**	**III**	**IV**	**Trt Total**
A	1 2 3	1.2 1.5 1.6	1.0 1.4 1.7	1.8 1.9 1.6	1.6 1.8 1.6	
Trt Sub Total		**4.3**	**4.1**	**5.7**	**5.0**	**19.1**
B	1 2 3	2.0 1.9 2.5	1.9 2.4 2.5	2.0 3.0 2.5	2.5 2.4 3.0	

Trt Sub Total		**6.4**	**6.8**	**7.5**	**7.9**	**28.6**
C	1 2 3	3.0 2.5 2.4	3.1 3.6 3.2	3.1 3.2 3.3	3.3 3.2 3.5	
Trt Sub Total		**8.9**	**9.9**	**9.6**	**10.0**	**38.4**
Block Total		**19.6**	**20.8**	**22.8**	**22.9**	

Computation

1. Components f variability
   Total SS = Trt SS + Block SS + Ex Tal error SS + Sampling SS
2. Linear Model
   Xijk = μ + Ti + Ej + Eij + dijk
   Where, μ = unknown constant (population mean for all treatments)
   Ti = Treatment effect
   Ej = Block effect
   Eij = Experimental error effect
   Dijk = Sampling error

Calculations

1. Grand total = Σμ + μ2 + μ3 + μ4 + …..μ36
   GT = Σ 1.2 + 1.0 + 1.8 + 1.6 + ……3.5 = 86.1
2. Correction factor = $\frac{GT2}{n} = \frac{86.12}{36} = \frac{}{36}$ = 205.92
3. Total SS = $\Sigma\mu_1^2 + \mu_2^2 + \mu_3^2 + \mu_4^2 +$ ----------$x_{36}^2 - CF$
   = $\Sigma 1.2^2 + 1.0^2 + 1.8^2 + 1.6^2 +$ -----3.5^2 ----CF
   = 224.79 – 205.92 = 18.87
4. Trt SS = $\frac{\Sigma t_1^2 + t_2^2 + t_3^2}{3b}$ + ----- CF

Where S = No of sample = 3
b = No of blocks = 4

$= \frac{\Sigma 19.1^2 + 28.6 + 38.4^2}{3 \times 4}$ + ---- CF

$= \frac{2657.33}{12}$ – CF

= 221.44 – 205.92 =15.52

5. Block SS = $\frac{\Sigma b_1^2 + b_2^2 + b_3^2 + b_4^2 +}{st}$ ---- CF

where, S = No of Sample = 3
t = No of Treatments = 3

$$\Sigma = \frac{19.6^2+20.8^2+22.8^2+22.9^2+}{3x3} \text{ -- CF}$$

$$= \frac{1861.05}{9} - CF$$

$$= 206.78 - 205.92 = \underline{0.86}$$

6. Exptal error SS = $\frac{\Sigma a_1^2 + a_2^2 + a_3^2 + a_4^2 + \text{---- a12}}{r}$ – CF Trt SS - Block SS
   where, S = Sub treatment Total
   S = No of samples = 3
   $= \Sigma 4.3^2+4.1^2+5.7^2+5.0^2+$ ---- 10.02 – CF – Trt SS – Block – SS
   $= \frac{668.03}{3} - 205.92 - 15.52 - 0.86$
   222.68 – 205.92 – 15.52 0.86 = 0.38
7. Sampling Error SS = Total SS – Trt – Block SS – Exptal Error is
   = 18.87 – 15.52 -0.86 – 0.38 =2.11
8. Degree of freedom for Nested Classification
   (i) Total df = rts - 1
   Where, r = No of blocks = 4
   t = No of treatments = 3
   s = No of samples = 3
   ∴ Total df = 4x3x3 – 1
   = 36 – 1 = 35
   (ii) Trt df = t – 1
   = 3 -1 = 2
   (iii) Block df = r – 1
   = u -1 = 3
   (iv) Exptal error df = (r-1)(t-1)
   = (4-1)(3-1)
   = (3)(2) = 6
   (v) Sampling error df = rt (S-1)
   = 4x3 (3-1)
   =12x2 = 24

ANOVA table for nested classification

sources **of variation**	**Df**	**SS**	**MSS**	**Feal**	**Ftab (5%)**
Total	35	18.87			
Treatment	2	15.52	7.76	129.33	5.14
Black	3	0.86	0.29	4.83	4.76
Exptal error	6	0.38	0.06	0.67	2.51
Sampling error	24	2.11	0.09		

(i) Trt MSS = Trt SS = $\frac{\text{Trt SS}}{\text{Trt df}} = \frac{15.52}{2} = \underline{\underline{7.76}}$

(ii) Block MSS = $\frac{\text{Block SS}}{\text{Block df}} = \frac{0.86}{3} = \underline{\underline{0.29}}$

(iii) Exptal error MSS = $\frac{\text{Exptal Error SS}}{\text{Exptal error df}} = \frac{0.38}{6} = \underline{\underline{0.06}}$

(iv) Sampling error MSS = $\frac{\text{sampling error SS}}{\text{sampling error df}}$

$= \frac{2.11}{24} = \underline{\underline{0.09}}$

(v) Trt Feal = $\frac{\text{Trt MSS}}{\text{Exptal error MSS}} = \frac{7.76}{0.06} = \underline{\underline{129.3}}$

(vi) Block feal = $\frac{\text{Block MSS}}{\text{Exptal error}} = \frac{0.29}{0.06} = \underline{\underline{4.83}}$

(vii) Exptal error Feal = $\frac{Exptal\ error\ MSS}{Sampling\ error\ MSS} = \frac{0.06}{0.09} = \underline{\underline{0.67}}$

Factorial experiments

Our previous discussions have been based on situations where only one treatment is involved. However, in Biological and Agricultural researches, at least two treatments are usually involved. For instance, we can talk of the effects of feeding different pigs protein levels to different breeds of pigs. An experiment which has its different qualitative or quantitative components is a factorial Experiment. In such a case, an experimental unit or plot receives a combination of treatment and not just a single treatment. A factor in a type of treatment usually denoted with capital letter A,B,C,D etc. Suppose was have four protein levels and four breeds of chicken.

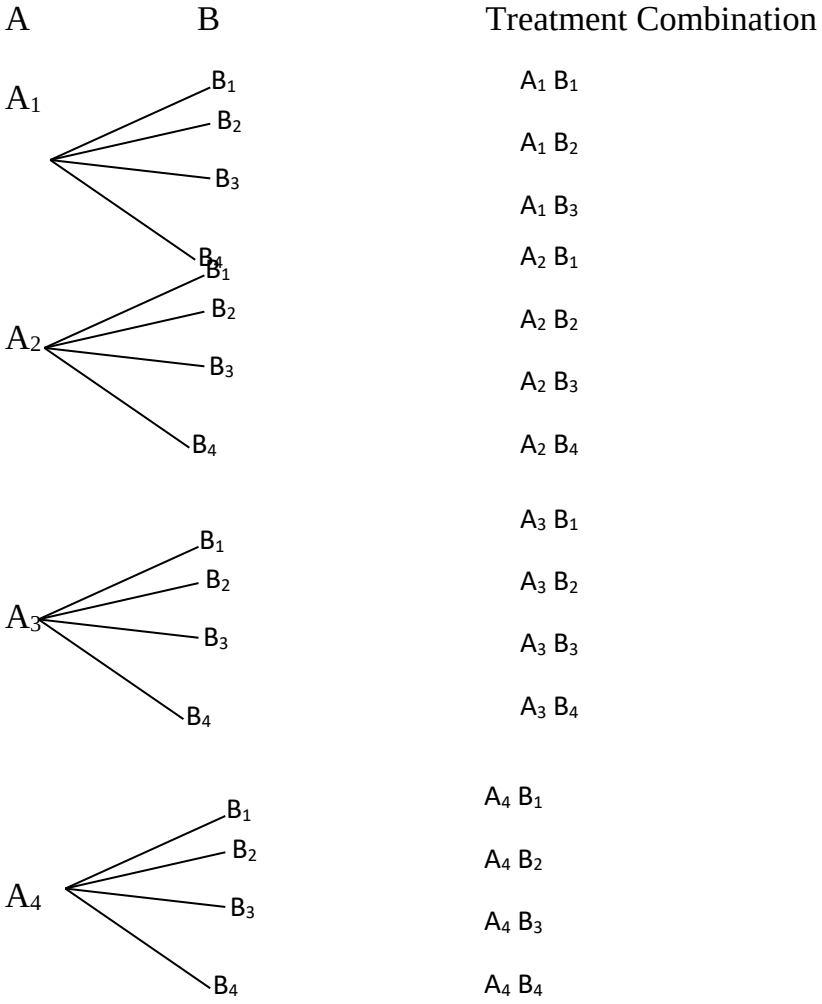
Let 4 levels of protein = 0,10,15 and 20
4 breeds of chickens = Rose Anak, Hubbard and Hacko
Factor A = Breeds of chickens (A_1,A_2,A_3 and B_4)

Levels of a factor – The levels of factor are different qualitative or quantitative forms of a factor. Figure like 0,1,2,3,4 etc are used to denote different levels of a factor and such figures are attended to shall or capital letters. For example,
Factor A: Levels a_1,a_2,a_3,a_4,
Factor B: Levels b_1,b_2,b_3,b_4,
Factor A: Levels A_1,A_2,A_3,A_4,
Factor B: Levels B_1,B_2,B_3,B_4,

Treatment combinations

Let us take the example of four breeds of chicken (factor A) and four levels of protein (factor B) in this experiment, each breed of chicken must receive each of the protein levels. So, the possible treatment combinations are illustrated below.

Factors



You therefore have 16 treatments combination which you randomize in the experimental units.

Randomisation scheme

Treatment combinations are randomised according to the design into which the factual in filled. For instance, a 2x2 factorial litted into a RCB design, the treatment combinations bowed be A_1, B_1, A_1, B_2, A_2, B_1, A_2, B_2. Theses treatment combination will be randomised within each block.

Blocks				
I	$A_1, B_1,$	$A_2, B_2,$	$A_1, B_2,$	$A_2, B_1,$
II	$A_2 , B_1,$	$A_1, B_2,$	$A_1 , B_1,$	$A_2 , B_2,$
III	$A_2, B_2,$	$A_1, B_1,$	$A_2, B_1,$	$A_1, B_2,$

Naming of factorial experiment

Factorials are named according to the number of factors and levels of factors involved. For instance, 2x2 factorial has two factor, each with two levels, 3x4 factorial is a two factorial, sue with three levels and the other with four levels.

Example 1: An experiment was conducted to determine the effect of calcium (C_a) andphosphorous (p) on the body weight of growing turkeys up to eight weeks. The design was randomised complete block (RCB) with three replications of each treatment combination. There were 3 levels of C_a (0, 1.0, and 2.0%) and 4 levels of P (0, 0.5, 1.0, 1.5%). Analyse the data in the table below and test whether the main effects and interaction are significant.

Treatments		**Blocks**		
A	B	I	II	III
	1	1	2	3
	2	2	4	4
	3	5	6	5
	4	7	8	6
	1	1	3	2
	2	4	6	5
	3	7	5	7
	4	8	9	8
	1	2	3	3
	2	4	3	4
	3	1	2	3
	4	2	1	2

Analysis

Step 1: Data arrangement of a two factor factorial analysis to include Trt and block totals as well as treatment combinations. Prepare a treatment table to include A totals and B totals.

Step 2: Computations

1. Grand total = $\Sigma\mu_1 + \mu_2 + \mu_3 +$ --------- μ_{36}
   = $\Sigma 1 + 2 + 3 +$ ---------- 2 = 148

2. Correction factor (CF) = $\frac{GT^2}{n} = \frac{148^2}{36} = \frac{21{,}904}{36} = 608.44$

3. Total Ss = $\Sigma\mu_1^2, + \mu_2^2, + \mu_3^2, +$ ---------- x36 -------CF
   = $\Sigma\mu_1^2, +2^2 + 3^2 +$ ----------2^2 --- CF
   = 794 – 608.44 = 185.56

Treatments			**Blocks**		**Trt**	**Treatment**
A	B	I	II	III	Totals	Combinations
	1	1	2	3	6	A_1B_1
	2	2	4	4	10	A_1B_2
	3	5	6	5	16	A_1B_3
	4	7	8	6	21	A_1B_4
	1	1	3	2	6	A_2B_1
	2	4	6	5	15	A_2B_2
	3	7	5	7	19	A_2B_3
	4	8	9	8	25	A_3B_4
	1	2	3	3	8	A_3B_1
	2	4	3	4	11	A_3B_2
	3	1	2	3	6	A_3B_3
	4	2	1	2	5	A_3B_4
Block Total		**44**	**52**	**52**	**148**	

Table of Treatment totals

		B			
A	1	2	3	4	**A Totals**
1	6	10	16	21	53
2	6	15	19	25	65
3	8	11	6	5	30
B Totals	**20**	**36**	**41**	**51**	

4. Block SS = $\frac{\Sigma B_1^2 + B_2^2 + B_3^2}{ab}$ ------CF

where, B = Block totals
a = levels of A
b = levels of B

$= \Sigma \frac{44^2 + 5_2^2 + 5_2^2}{3x4}$ -----CF

$= \frac{7,344}{12}$ -----608.44

612 – 608.44 = 3.56

5. Trt SS = $\Sigma \frac{T_1^2 + T_2^2 + T_3^2 + T_4^2 + \text{------} T_{12}^2}{R} = CF$

where, T = Trt totals
r =No of blocks = 3

$= \Sigma \frac{6^2 + 10^2 + 16^2 + 21^2 + \text{---------} 5^2}{3}$ ----CF

$= \frac{2326}{3} - 608.44$

= 775.33 – 608.44 = 166.89

6. Error SS = Total SS –Trt SS – Block SS
= 185.56 – 166.89 – 3.56 = 15.11

7. Sum of Square of A (SS_A) = $\Sigma \frac{A_1^2 + A_2^2 + A_3^2}{rb}$

Where, A = A totals
r = No of blocks
b = Levels of B

$= \Sigma \frac{53^2 + 65^2 + 30^2 +}{3x4}$----CF

$= \frac{7,934}{12} - 608.44$

= 661.17 -608.44 = 5373

8. Sum of square of B (SSB) = $\Sigma \frac{B_1^2 + B_2^2 + B_3^2 + B_4^2}{ra} = CF$

where, B = B Totals
r = No of blocks
a = Levels of A

$= \Sigma \frac{20^2 + 36^2 + 41^2 + 51^2}{3x3}$---- CF

$= \frac{5,978}{9} - 608.44$

= 664.22 – 608.44 = 55.78

9. SS_{AB} = TrT SS – (SS_A + SS_B)
= 166.89 – (52.73 + 55.78)
= 166.89 – 108.51 = 58.38

10. Degrees of freedom

(i) Total df = rab – 1
Where r = No of blocks
a = levels of A
b = Levels of B
∴ Total df = 3x3x4 – 1
= 36 -1 = 35

(ii) Block df = r -1
= 3-1 = 2

(iii) A df = a -1
= 3-1 = 2

(iv) B df = b -1
= 3-1 = 2

(v) AB df = (a-1)(b-1)
= (3-1)(4-1)
= (2)(3) = 6

Exptal Error df = (ab-1)(r-1)
= (3x4-1)(3-1)
= (11)(2) = 22

ANOVA Table

Sources of Variation	Df	SS	MS9.73	Feal	Ftab(5%)
Total	35	18.56			
Blocks	2	3.56	1.78	2.59	5.72^{NS}
A	2	52.73	26.37	38.22	5.72*
B	3	55.78	18.59	26.94	4.82*
AB	6	58.38	9.73	14.10	3.76*
Error	22	15.11	0.69		

(i) Block MSS = $\frac{\text{Block SS}}{\text{Block df}} = \frac{3.56}{2}$ =1.78

(ii) A MSS = $\frac{\text{A SS}}{\text{A df}} = \frac{52.73}{2}$ =26.37

(iii) BMSS = $\frac{\text{B SS}}{\text{B df}} = \frac{55.78}{3} = \underline{18.59}$

(iv) AB MSS = $\frac{\text{AB SS}}{\text{AB df}} = \frac{58.38}{6} = \underline{9.73}$

(v) Error MSS = $\frac{\text{Error SS}}{\text{Error df}} = \frac{15.11}{22} = \underline{0.69}$

(vi) Block feal = $\frac{\text{Block MSS}}{\text{Error MSS}} = \frac{1.78}{0.69} = \underline{2.59}$

(vii) A feal = $\frac{\text{A MSS}}{\text{Error MSS}} = \frac{26.37}{0.69} = \underline{38.22}$

(viii) B feal = $\frac{\text{B MSS}}{\text{Error MSS}} = \frac{18.59}{0.69} = \underline{26.94}$

(ix) AB feal = $\frac{\text{AB MSS}}{\text{Error MSS}} = \frac{9.73}{0.69} = \underline{14.10}$

Interpretation

The first thing to do in interpreting a factorial experiment is to look at the interactions of the ANOVA table whether it is significant. When an interaction is significant, there is no need interpreting the main effect of the factors concerned. Interpreting the main effect is only useful when the interaction is not significant.

4.0 CONCLUSION

The use of appropriate statistical tool is important in animal production experiments. This allows the experiment to come up proper deductions and conclusions thus avoiding errors in judgment.

5.0 SUMMARY

In this unit the characteristics of a well planed experiment were expressed. The various experimental designs to include CRD, RCBD and Latin Square Design were discussed. Pair wise comparisons. Worked examples were also provided for better understanding by the students.

6.0 TUTOR-MARKED ASSIGNMENT

1. Briefly describe the different steps involved in a research process.
2. Distinguish between research method and methodology.
3. What do you mean by research? Explain its significance in modern times?

7.0 REFERENCES/FURTHER READING

Kothari, C. R. & Garg, Guarav (2014). *Research Methodology Methods and Techniques.* (3rd ed.). New Age International Publishers.

UNIT 2 ARTIFICIAL INSEMINATION TECHNIQUES IN ANIMAL BREEDING

CONTENTS

1.0 Introduction
2.0 Objectives
3.0 Main Content
    3.1 Definition of Artificial Insemination
    3.2 Advantages of Artificial Insemination
    3.3 Disadvantages of Artificial Insemination
    3.4 Symptoms of Heat in Farm Animals
    3.5 Artificial Insemination in Cattle
    3.6 Artificial Insemination in Swine
    3.7 Artificial Insemination in Chickens
4.0 Conclusion
5.0 Summary
6.0 Tutor-Marked Assignment
7.0 References/Further Reading

1.0 INTRODUCTION

Improvement in the efficiency of production is virtually the main objective in animal breeding. Artificial Insemination is one of the established practices available for use in the improvement of farm animals. It is used in every species of domestic animals. However, this practice has been adopted particularly by the dairy and beef industry all over the world. The process of artificial insemination involves semen collection dilution and insemination of the farm animal.

2.0 OBJECTIVES

By the end of this unit, you will be able to:

- describe the various techniques in performing artificial insemination in some farm animals.

3.0 MAIN CONTENT

3.1 Definition of Artificial Insemination

Artificial Insemination is the technique in which semen with living sperms is collected from a male and introduced into the female reproductive tract of same species at the proper time with the aid of instruments to produce pregnancy.

3.2 Advantages of Artificial Insemination

- With natural breeding resulting in the deposition of more semen than needed as well as physical stress to the male, artificial insemination uses diluted semen hence provides more doses. This allows for more females to be made pregnant at the same time.
- Increases the potential for genetic improvement.
- Reduces the possibility of disease transmission.
- Increases the safety of both the animals and the farmers.

3.3 Disadvantages of Artificial Insemination

- Laborious in terms of detecting females on heat compared to instinctive detection by male animals.
- **Increasing** the number of offspring per male is advantageous only if the best males can be accurately determined.
- Decreases genetic variability in a population by increasing the offspring from the same male.

3.4 Symptoms of Heat

- Animals are seen to be excited by way of restlessness and nervousness.
- Feed intake is reduced.
- They are seen trying to lick and smell other animals.
- The animals in heat are observed to stand still when males try to mount them that are standing heat.
- They also seen trying to mount other animals.
- Frequent urination is observed.
- Clear mucous discharge from swollen vulva is also noticed.
- The tail is observed to be a raised position.

3.5 Artificial Insemination in Cattle

In cattle, semen is collected from the bull mostly through the use of an artificial vagina. Bulls are taught to serve or ejaculate into this instrument while it is held by an operator alongside the flank of a teaser cow. To prepare the artificial vagina for use, a non-spermicidal lubricant should be used to lubricate the entrance of the casing. The inner lining is then wrapped with thin rubber tubing with an opening through which warm water is introduced. The temperature of the water which should range between 40-45^{o}C, is critical in stimulating ejaculation.

Following the collection of the semen from the bull, it is diluted or extended as soon as possible. The most common semen extender is skim

milk or homogenised milk to which 10% glycerol is added. Glycerol is added to the extended semen after cooling to a temperature of 5ºC. This semen then packaged in glass ampoules 0.5-1ml of extended semen.
The recto-vaginal method is mostly used in insemination procedure in cattle. In carrying out this procedure, the external genitalia is thoroughly cleaned. Thereafter, one gloved hand is introduced into the rectum and used to grasp the cervix. The artificial insemination pipette is then introduced through the vulva and vagina to the external os of the cervix. With slight pressure and manipulation of the cervix on the pipette it gets to the uterus where the semen is slowly deposited. The optimum time to inseminate a cow is during the last half of standing oesrus and not later than 6 hours after oestrus.

3.6 Artificial Insemination in Swine

Semen is collected from a boar while it mounts a sow in heat or a dummy so that it has been trained to mount. With gloved hands the tip of the penis is held firmly. This stimulates ejaculation. The semen is then collected by directing the tip of the penis into a 500ml thermos bottle at 35ºC. Collection of semen from boars should not be sooner than every 3 days. Collected semen should be handled carefully to avoid bacterial contamination and sudden drop or repeated changes in temperature. Boar semen can be stored for up to 30 hours at 7ºC in egg yolk-glucose-sodium bicarbonate solution. On the other hand, it can be stored in heat treated milk. Sows ovulate from about 40 hours from onset of standing heat. However, in view of the fact that commencement of heat in sows is often 12-24 hours before it is usually observed, sows should be inseminated both at 12 and 24 hours following when standing heat is first observed. At insemination, a pipette is inserted into the cervix and then slowly from an attached syringe semen is forced into the uterus.

3.7 Artificial Insemination in Chicken

Semen is collected from chickens by stimulating the cock to protrude its copulatory organ. This is done by massaging the underside of the abdomen and the back over the testes. This is quickly followed by pushing the tail forward with one hand while at the same time the thumb and forefinger are used to milk semen from the ducts of the organ. The semen is usually collected with an aspirator. To inseminate the hen, pressure is applied to the abdomen around the vent. This action everts the cloaca thus causing the oviduct to protrude. This then allows for the syringe to be inserted about an inch into the oviduct and the semen delivered. To maintain fertility at a high level, hens should be inseminated at intervals of five to seven days.

4.0 CONCLUSION

This lecture has been able to highlight the advantages and disadvantages of artificial insemination as well as the techniques involved in the insemination of some farm animals.

5.0 SUMMARY

In this unit we have learnt the techniques involved in the collection and insemination in cattle, swine and chickens. An overview of the advantages and disadvantages of artificial insemination were also addressed in this lecture.

6.0 TUTOR-MARKED ASSIGNMENT

1. Define Artificial Insemination.
2. Highlight the advantages and disadvantages of artificial insemination.
3. What are the symptoms of heat?
4. Briefly describe the process of insemination in cattle, chicken or swine.

7.0 REFERENCES/FURTHER READING

Hafez, B & Hafez, E. S. E. (2000). *Reproduction in Farm Animals*. (7th ed.). USA:New York Lippincott Williams and Wilkens.

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UNIT 3 DIGESTIBILITY TRIALS – *IN VIVO* AND *IN VITRO*

CONTENTS

1.0 Introduction
2.0 Objectives
3.0 Main Content
  3.1 *In vivo* Digestibility Trials
  3.2 *In vitro* Digestibility Trials
4.0 Conclusion
5.0 Summary
6.0 Tutor-Marked Assignment
7.0 References/Further Reading

1.0 INTRODUCTION

Digestibility in animal science is the measure of how much nutrition a feed provided an animal is absorbed through the intestines into the blood stream. In other words, it is the difference between the nutrients in the feed the animal is offered and that in what the animal excretes. Digestibility provides an important measure of feeds nutritional value and quality. Generally, as the quality of ingredients in the feed increases so also the digestibility and nutrient availability.

2.0 OBJECTIVE

By the end of this unit, you will be able to:

- understand how digestibility trials can be conducted *in vivo* and *in vitro*.

3.0 MAIN CONTENT

3.1 *In Vivo* Digestibility Trials

These are trials that are carried out with live animals to ascertain the extent to which the crude nutrients as observed from proximate analysis of the feed ingredients have been digested and/ or possibly absorbed by the animal. Experimental animals to be used for such trials are usually of same breed, sex and about the same liveweight to reduce error in the final judgment. The test feed material is usually fed for a preliminary period of 10-14 days for ruminants. For non ruminants, this period is for 3 days. This period is for the animals to get used to know how much of the test material the voluntarily will consume.

After this preliminary period, the animals are transferred to metabolism crates. In these crates a carefully weighed amount of the test material is offered. This is followed by daily collection of faeces and urine for seven days. The crates allows for accurate separation of the faeces from the urine. The faeces collected is weighed and volume of urine recorded. These are then taken to the laboratory or stored for proximate analysis.
The difference between the proximate composition of the test material and that determined in the wastes collected is assumed to have been digested. It should be noted however that certain factors like chemical composition of the feed, the efficiency of the digestive system of the animal and processing methods affect the digestibility of nutrients.

Determination of digestion coefficients and total digestible nutrient
From the data generated above, the digestion coefficients of the nutrient contents can be calculated. As earlier observed, proximate analysis provides information on only the amount of dry matter, ether extracts (Fat), protein, fibre, mineral matter (Ash) and nitrogen free extract present in the feed sample. It fails to provide information on how much of the nutrient in the feed have been relatively utilized by the animal. Hence the need to determine the digestion coefficients of the individual nutrients arises. The digestion coefficient of a nutrient is the average percentage of the nutrient in question that has been digested in the feed.
It is determined by dividing the amount of each nutrient digested by the amount of each nutrient consumed and then multiplied by 100. The steps involved in this calculation for each nutrient are:

(i) Consumed = Kilogram of feed consumed x Percent nutrient in feed.
(ii) Excreted = Kilogram of faeces excreted x Percent nutrient in faeces.
(iii) Digested = Consumed – Excreted
(iv) Digestion Coefficient = $\frac{\text{Digested}}{\text{Consumed}} \times 100$

Following the calculation of the digestion coefficient, the digestible nutrient is calculated thus:

(v) Digestible Nutrient = Percent Nutrient in Feed x Digestion Coefficient

It should be noted that this calculations are done individually for the nutrients except for moisture and ash. The Total Digestible Nutrient (TDN) is derived by summing the values for the individual digestible nutrients using the formula:

(vi) TDN = Digestible Crude Protein + Digestible Crude Fibre + Digestible Nitrogen Free Extract + (Digestible Extract x 2.25).

The ether extract fraction is multiplied by 2.25 to compensate for the higher energy value of fat as compared to protein and carbohydrates. Digestion coefficients are not calculated for mineral matter (ash) since a large proportion of the minerals present in the faeces have been utilized and excreted into the gut.

3.2 *In Vitro* Digestibility Trials

In vitro digestibility is an anaerobic fermentation performed in the laboratory to stimulate digestion particularly in the rumen. This method of digestibility trials was first described by Tilley and Terry in 1953.
Upon acquiring rumen fluid from fistulated or cannulated animals, a buffer is added to stimulate saliva production. This fluid is then mixed with the test feed and placed in an incubator for 24, 30, 48 hours as the case may be at room temperature (39^{o}C), where it is digested by acid pepsin and the residual part is discarded as indigestible. In vitro trials are fast and accurate in producing multiple data. It is cheap and requires no animal host. However, the major constraint is availability of stable power source especially in the third world.

4.0 CONCLUSION

A digestibility trial in farm animals is of utmost relevance in providing an insight about the worth of feed materials. To date *in vivo* and *in vitro* trials methods have been very useful in this regard.

5.0 SUMMARY

In this unit, we have been acquainted with how *in vivo* and *in vitro* digestibility trials are carried out. As well we have learnt at least one method of how to calculate the digestion coefficients of nutrients and Total Digestible Nutrient (TDN) of a feed material.

6.0 TUTOR-MARKED ASSIGNMENT

1. In a digestion experiment comprising five rams, an average consumption of the experimental ration was observed to be 63g. This test ration was made up of 70% maize and 30% cassava peels. If the average weight of the animals was 28kg and the faecal waste averaged 42g, calculate the TDN for the test ration from the following data:

Nutrient	Moisture	Crude	Ether	Ash	Crude	Nitrogen Free

		Fibre	Extract		Protein	Extract
% consumed	15.3	35.8	3.4	6.0	12.5	27.0
% Excreted	30.5	25.8	1.5	18.7	5.2	18.3

2. Differentiate between *in vivo* and *in vitro* digestibility Trials.

7.0 REFERENCES/FURTHER READING

Van Soest, P. J. (1982). *Nutritional Ecology of the Ruminant*. O and B Bookks Inc. Oregon, USA.

UNIT 4 ASSAYS INVOLVING PER, NPU AND BV

CONTENTS

1.0 Introduction
2.0 Objectives
3.0 Main Content
    3.1 Protein Efficiency Ratio (PER)
    3.2 Net Protein Utilisation (NPU)
    3.3 Biological Value (BV)
4.0 Conclusion
5.0 Summary
6.0 Tutor-Marked Assignment
7.0 References/Further Reading

1.0 INTRODUCTION

Biological assays in animal production are analytical methods used to estimate potency of substances or agents by observing their effects on living animals (*in vivo*) or tissues (in vitro).

2.0 OBJECTIVES

By the end of this unit, you will be able to:

- understand and explain biological assays such as:
  Protein Efficiency Ratio (PER)
  Net Protein Utilization (NPU)
  Biological Value (BV).

3.0 MAIN CONTENT

3.1 Protein Efficiency Ratio (PER)

Protein efficiency ratio, which is a measure of protein quality, is defined as the ratio of grams of protein consumed. In other words, it is based on the weight of test subject divided by its intake of a particular food protein during a test period.

$$\text{PER} = \frac{\text{Gain in body mass (g)}}{\text{Protein intake (g)}}$$

PER has been the most widely used measure of protein quality particularly because of its simplicity.

3.2 Net Protein Utilisation (NPU)

The Net Protein Utilisation of a food is the percentage of protein contained in that food that is retained by the body after the food has been eaten. Hence, it is used to describe the value or usefulness of certain proteins in a diet. A simple equation for NPU is:

$$NPU = \frac{\text{Nitrogen Retained} \times 100}{\text{Nitrogen Intake}}$$

3.3 Biological Value (BV)

Biological value of a food is the percentage of absorbed protein from the food that is retained in the body and available for incorporation into the proteins in the body of the organism that consumed it. It is a useful value when on a diet or dieting e.g. for weight loss or muscle gain. Proteins are the major source of dietary Nitrogen hence the Equation for BV is:

$$BV = \frac{\text{Nitrogen Retained } (N_r) \times 100}{\text{Nitrogen Absorbed } (N_a)}$$

For accurate determination of BV, the test organism must only consume protein from the test diet; the test protein must contain no known sources of non-protein sources of nitrogen e.g. urea and the test diet must be of good quantity to prevent protein in the diet being used as primary energy source. Typically a BV test can last for over one week. Fasting prior to testing helps produce consistency between subjects.

4.0 CONCLUSION

The use of biological assay techniques in animal production has turned out to be vital in the assessment of the potency of proteins in feedstuffs.

5.0 SUMMARY

The use of Protein Efficiency Ratio (PER), Net Protein Utilization (NPU), Biological Value (BV) etc. in assessing protein in animal feeds has been seen to be important in the measurement of their quality. Besides, insight into the percentage protein in the feed that is retained and incorporated into the body of the animal can be determined by these bioassay techniques.

6.0 TUTOR-MARKED ASSIGNMENT

What are biological assays? Briefly explain Biological Value, Net Protein Utilisation and Protein Efficiency Ratio as biological assay analytical methods.

7.0 REFERENCES/FURTHER READING

Manz, A. Dittrich, Petra, S. (1974). *Bioanalytical Chemistry. Pamme, Nicole., lossifidis, Dimitri.* (2nd ed.). London.

UNIT 5 SEPARATION AND CHARACTERISATION OF COMPONENTS INVOLVING THE USE OF CHROMATOGRAPHY, ELECTROPHORESIS, RADIOSCOPY, CALORIMETRY AND SPECTROMETRY

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1.0 INTRODUCTION

Generally, most materials appear homogeneous whereas, they are actually a combination of substances. Separation and characterization of the components of these materials thus allow for adequate analysis hence a scientist can figure out what the compound is made up of. Separating these components can be achieved by several methods by way of: Dissolving them in appropriate liquids, subjecting them to the influence of an electric field and measuring changes in the state of a material due to heat. Others include x-ray radiography of bioassay for the study of the interaction between matter and electromagnetic radiation.

2.0 OBJECTIVES

By the end of this unit, you will be able to:

- highlight component separation methods involving:

  Chromatography
  Electrophoresis

Radioscopy
Calorimetry
Spectrophotometry.

3.0 MAIN CONTENT

3.1 Chromatography

Chromatography is an important biophysical technique used by scientists to separate, identify and purify the components of a mixture or material for qualitative and quantitative analysis. The concept of chromatography was first presented by a Russian botanist named M. S. Tswett in 1903.

3.1.1 Principles of Chromatography

Chromatography is one of the most important analytical techniques. This method is based on the principles of separation of molecular mixtures through their distribution between two phases. One of these phases is in the form of a porous bed, liquid, layer or film adsorbed on the surface of a solid support and referred to as the stationary phase while the other is a fluid or gas referred to as the mobile phase that percolates through or over the stable phase.

Separation results from repeated sorption/adsorption circles during the movement of the sample components along the stationary phase as the mobile phase continues to travel through the stable phase. The result for any chromatographic analysis is thus arrived at from the point at which the different components of the compound stop moving and separate from the other components.

3.1.2 Types of Chromatography

Although various chromatography methods have been developed to date, only four major types will be discussed here. These include Liquid Chromatography, Gas Chromatography, Thin-layer Chromatography and Paper Chromatography.

(i) Liquid chromatography

This type of chromatography is used the world over to test water for pollution in places like lakes and rivers. It is also used to analyze metal ions and organic compounds in solutions. Liquid chromatography uses liquids which may incorporate hydrophilic insoluble compounds.

(ii) Gas chromatography

Gas chromatography is a simple but highly sensitive and rapidly applied technique for the separation of very minute molecules. The stationary phase in this method is a liquid which is adsorbed unto the surface of an inert solid. Its mobile phase consists of gasses such as helium (He) and nitrogen (N_2). This method is used in airports to detect bombs and in forensics to analyze fibres on a person's body or blood found in a crime scene.

(iii) Thin-layer chromatography

The thin-layer chromatography is a solid/liquid adsorption chromatography. The stationary phase in this method is a solid absorbent substance coated on glass plates. In this method, the mobile phase travels upward through the stationary phase. The solvent travels up the thin glass or plastic plates by means of capillary action. The rate of this upward movement depends on the polarity of the material, solid phase and of the solvent. This a simple and rapid method used to check the purity of organic compounds. It is also used to detect pesticide or insecticide residues in food and in forensics to analyse dye composition of fibre.

(iv) Paper chromatography

In this method, a thick filter paper highly saturated with water make up the stationary liquid phase while the mobile phase consists of an appropriate liquid placed in a developing tank. Capillary action is used to pull the solvents up through the paper and so separate the solutes. Paper chromatography is regarded as a liquid/liquid chromatography. It is a simple and cheap method of chromatography.

3.2 Electrophoresis

Electrophoresis is a separation technique that is based on the mobility of ions in an electric field. In other words, it is the movement of charged particles in a fluid or gel under the influence of an electric charge. This is based on the fact that ions have different migration rates depending on their total charge, size and shape hence can be separated. It is used mostly for macro molecules such as proteins. Although this is a widely used technique, it is time consuming, expensive and requires skilled personnel.

3.2.1 Types of Electrophoresis and their Techniques

Broadly, electrophoresis can be divided into two types that is, Slab electrophoresis and Capillary electrophoresis.

(a) Slab electrophoresis is the classical method widely in use. However, it is slow, time consuming and bulky. Based on the principles used for separation, slab electrophoresis is further divided into three types thus: zone electrophoresis, isoelectrofocusing and immune-electrophoresis.

(b) Capillary electrophoresis is the type that takes place in a capillary tube. It is an advanced method of electrophoresis which was developed with the intent to reduce the time taken for separation and analysis. Capillary electrophoresis usually requires small samples in the range of 0.1 to 10ηl as compared to slab electrophoresis method that require samples in μl. As well, this method yields higher speed and high resolution separations. Separated components that exit from one end of the capillary are immediately analyzed at the other end of the tube by detectors.

3.3 Radioscopy

Radioscopy is the examination of objects opaque to light by means of another form of radiation, usually x-rays. It is a non-destructive means of examining an object. It provides immediate information with regards to nature, size and location. It also provides rapid check of the dimensions, configuration as well as the presence and positioning of components in a mechanism. As with conventional radiography, radioscopy is broadly applicable to any material or object such as metals, stone ceramics wood etc. Although closely related to the radiographic method, it has a lower operating cost in terms of time, manpower and material. Long term records of radioscopic image may be stored as motion- picture or video recordings or as photographs.

3.4 Calorimetry

Calorimetry is the field of science that deals with the measurement of the state of a body with respect to heat in order to examine its physical and chemical changes. The physical changes could be melting, evaporation etc and the chemical changes being burning, acid-base neutralization etc. Calorimetry is extensively applied in thermo-chemistry in calculating enthalpy, stability, heat capacity etc. Different types of calorimeters are used in calorimetry and they include:

- Adiabatic calorimeter
- Reaction calorimeter

- Bomb calorimeter
- Constant pressure calorimeter
- Differential scanning calorimeter

3.5 Spectrometry

Spectrometry is the method used to acquire a quantitative measurement of the spectrum, that is, a band of colors like in a rainbow, produced by separation of the components of light by their different degrees of reflection according to wavelength.

The study of spectrometry dates back to when Isaac Newton first discovered that focusing light through glass splits it into different colors of the rainbow. The device used for measuring wavelengths of light over a wide range of electromagnetic spectrum is referred to as a spectrophotometer. It is commonly used for spectroscopic analysis of materials. The major types of spectrometers are:

- Optical spectrometer which is used to measure the intensity of light absorption and emission over a portion of the electromagnetic spectrum used to typically identify materials. The deflection of light is produced by refraction in a prism.
- Mass spectrometer is another type of spectrometer that is used to identify the amount and type of chemicals present in a sample. A mass spectrometer produces charged particles (ions) from the chemical substances to be analysed. It then uses electric and magnetic fields to measure the mass (weight) of the charged particles. This type of spectrometer is used for all kinds of chemical analyses as with petroleum products, biological materials.

4.0 CONCLUSION

Although materials mostly appear homogenous in nature, they actually are combination substances that need to be separated and characterized for detail analysis and understanding.

5.0 SUMMARY

From this lecture it is obvious that scientists employ various methods to separate and characterise materials which allow for adequate analysis to figure out the compounds are composed of. Chromatographic separation method is important for the separation, identification and purification of compounds of mixtures or materials for quantitative and qualitative analyses. Electrophoresis is a separation technique based on the movement of ions in a fluid or gel under the influence of an electric

field. Radioscopy as a separation technique examines objects opaque to light by means of another form of radiation such as x-rays. Calorimetry measures the state of a body with respect to heat in order to examine its physical and chemical changes. The separation method spectrometry is used to acquire a quantitative measurement of the spectrum produced by the components of light by their degrees of refraction based on wavelength.

6.0 TUTOR-MARKED ASSIGNMENT

1. Define and describe the principles of component separation methods to include:
   a.) Chromatography
   b.) Electrophoresis
   c.) Radioscopy
   d.) Calorimetry
   e.) Spectrometry.

7.0 REFERENCES/FURTHER READING

Butnariu, M. (2016). *Methods of Analysis (Extraction, Seperation, Identification and Quantification) of Carotenoids from Natural Products.*

Alexander, Renee R., & Joan M. Griffiths (1993). *Basic Biochemical Methods.* (2nd ed.). New York: Wiley-Liss.