

MODULE 1

Unit 1	General Introduction to Experimental Error
Unit 2	Sampling Techniques
Unit 3	Common Experimental Designs in Animal Science
Unit 4	Practical Application of Experimental Designs in Animal Science

UNIT 1 GENERAL INTRODUCTION TO EXPERIMENTAL ERROR

CONTENTS

1.0	Introduction
2.0	Objectives
3.0	Main Content
3.1	Experimental Error
3.2	Types of Experimental Error
3.2.1	Systematic Error
3.2.2	Random Error
3.3	Accuracy and Precision
3.4	Calculating Experimental Error
4.0	Conclusion
5.0	Summary
6.0	Tutor-Marked Assignment
7.0	References/Further Reading

1.0 INTRODUCTION

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- define experimental error
- name and explain the types of experimental error
- determine error precision and accuracy.

3.0 MAIN CONTENT

3.1 Experimental Error

Experimental error or uncertainty is defined as the difference between a measured or estimated value for a quantity and its true value, and it is inherent in all measurements as seen in our introduction. Scientific experiment has errors that may occur, therefore, the knowledge of the type and degree of error likely to be present is essential if data are to be used wisely. Error analysis is when a person is comparing a measured value gotten from an experiment to a predetermined value or true value. These variations are inherent in all measurements as seen in our introduction. Scientific experiments have variations or errors that may occur during the course of their experiment. It is therefore important to know the type and degree of error likely to be present if the data are to be used wisely. Error analysis therefore is the study of difference between a single measurement the range of possible values that a measurement or result might reasonably expected to have.

3.2 Types of Experimental Error

There are two common types of error: viz are systematic and random errors.

3.2.1 Systematic Error

These are errors that arise from a flaw in the measurement scheme which is repeated each time a measurement is taken. They are variations due to an imperfection in the equipment or techniques that are used to make a measurement. Systematic errors are also called determinate errors because they can be determined and corrected by the use of a correct measuring calibration or by adjusting the measuring techniques.

Common Sources of Systematic errors are:

1. Lack of uniformity in conducting an experiment
2. Lack of correct calibration of measuring instruments
3. Poorly maintained instruments
4. Faulty reading of instrument by the user
5. Taking reading on unbalanced instrument which will result to too high or too low values
6. Unaccounted environmental effects.

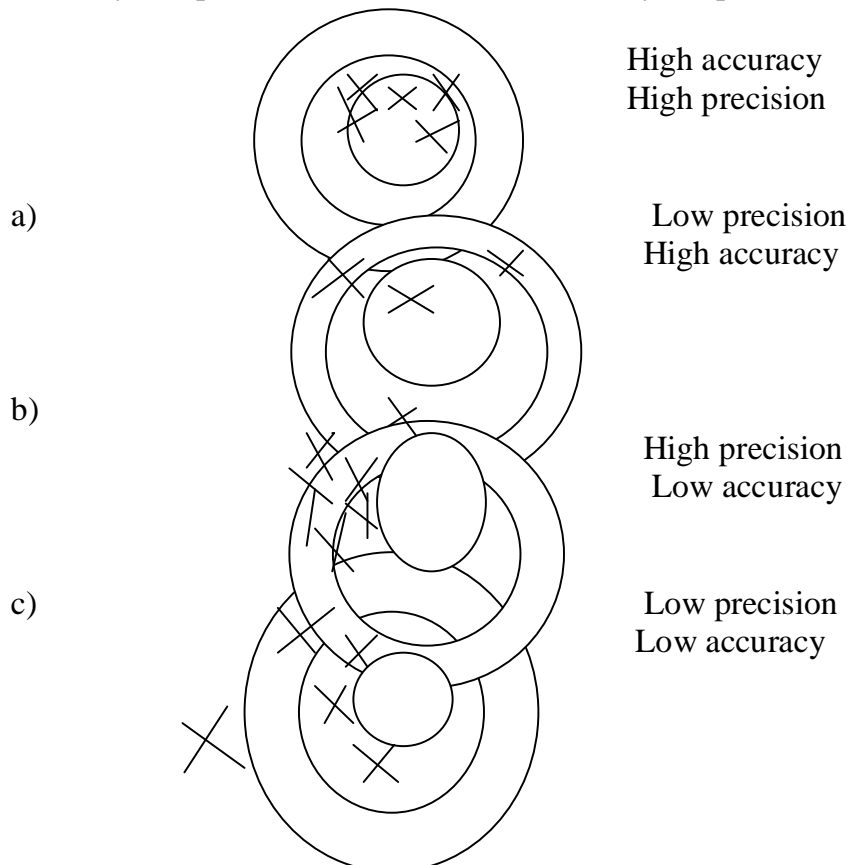
3.2.2 Random Error

These are errors that occur due to random, unpredictable phenomena. They are also known as indeterminate errors. These types of errors are directionless because they cause variations in either direction. It gives result that fluctuates below or above the accepted value. Since they are uncontrollable, a good replication of experiment may reduce the magnitude of this type of error even though it will not eradicate it totally.

Common sources of random error are problems in estimating a quantity that lies between the graduations of instruments and in ability to read an instrument because of fluctuations during the measurements.

3.3 Accuracy and Precision

Accuracy measures how close a measured value is to the true or accepted value. it is sometimes not possible to determine how accurate a measurement is because sometimes the true value for a physical quantity may not be known. Precision on the other hand measures how closely two or more measurements agree with one another. It measures the closeness that exists between two or more measurements taken on a replicated analysis. Precision is sometimes refers to a 'repeatability' or 'reproducibility' because its measure is highly reproducible and tend to give values which are very close to each other. The differences between accuracy and precision can be best described by the picture below:



3.4 Calculating Experimental Error

When reporting the result of an experiment, the result should describe the accuracy and precision of the experimental measurement. Common ways of reporting accuracy and precision are given below.

1. **Percent Error:** This measures the accuracy of a measurement by the difference between a measured value (E) and a true value (A) and is denoted by the formula

$$\% \text{ Error} = \frac{E-A}{A} \times 100$$

Where E= Experimental Value or measured value. A= Accepted or true value.

2. **Percent Difference:** This is applied when comparing two experimental quantities, E₁ and E₂ neither of which can be considered the correct value. It is expressed as the absolute value of the difference over the mean times 100

$$\% \text{ Difference} = \frac{|E_1 - E_2|}{\frac{E_1 + E_2}{2}} \times 100$$

4.0 CONCLUSION

As much as possible reducing experimental error in researches or experiments is paramount to having a less error in our experiments and calculations of percent error and percent difference helps one estimate the accuracy of experiment.

5.0 SUMMARY

In this unit you learnt what experimental error is, the two major types of experimental errors which are the systematic and random experimental error, you also learnt the difference between precision and accuracy of a measurement as well as how to calculate experimental error. It is obvious from this unit that there is no perfectly done experiment; errors in every experiment occur due to some causes that are beyond the control of the experimenter. Therefore before starting an experiment, careful observation of experimental materials and instruments are needed for reduction of error.

6.0 TUTOR- MARKED ASSIGNMENT

1. What is experimental Error?
2. List and explain the types of experimental error
3. Differentiate between accuracy and precision of experimental error.
4. State how to measure experimental error

7.0 REFERENCES/ FURTHER READING

John, R. T. (1997). *An Introduction to Error Analysis; The Study of Uncertainties in Physical Measurements*. (2nd ed.). University Science Books.

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UNIT 2 SAMPLING THEORY

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Characteristics of a good sample
 - 3.2 Developing a good sampling plan
 - 3.3 Various sampling techniques
 - 3.3.1 Representative Sampling
 - 3.3.2 Probability Sampling
 - 3.3.3 Sample Frame
 - 3.3.4 Random Number Sampling
 - 3.3.5 Simple Random Sampling
 - 3.3.6 Systematic Random Sampling
 - 3.3.7 Stratified Sampling
 - 3.4 Types of sample
 - 3.4.1 Composite Sample
 - 3.4.2 Integrated Samples
 - 3.4.3 Grab Samples
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Readings

1.0 INTRODUCTION

Sampling is the backbone of analysis. Since it is not possible to analyse the whole population, sampling in the population provides a glimpse of what a population look like. Sampling is a procedure where a portion of a data is taken from a large set of data. The result gotten from a sample can be used to make an inference into the population. Inappropriate sampling will give a wrong and unmeaningful result that will lead to a wrong conclusion or decision making. This unit therefore will teach you how to take a good sample and how to process it as well as give you the different types and techniques of sampling.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- give characteristics of a good sample
- state and explain the types of sampling
- explain the various methods of sampling.

3.0 MAIN CONTENT

3.1 Characteristics of a Good Sample

Since sampling involves the proper collection or gathering of a laboratory size material which is a true representative of the whole analytical material, sampling remains vital to the success of the whole analytical exercise. A good sample should possess the following characteristics.

1. It must possess the features of its population from where it was sampled or collected
2. Sample collected from the population must maintain the nature of its population throughout the analysis.
3. For readability to be ascertained, the number of sample must be large.

3.2 Developing a Good Sampling Plan

To develop a good sampling plan, one should consider the following:

1. The objective of the analysis or the experiment
2. The consequences of wrong decision
3. The accuracy and precision required to obtain data that make a correct and reliable decision
4. The level of homogeneity of the data
5. The sampling size: the number of sample to be collected must be large enough to be a true representative of the population
6. How the sample will be collected or gathered
7. The storage condition of the sample must be favourable for it to maintain its original nature
8. The choice of appropriate separation method to be used for processing the sample and
9. The size of the final sample required for the analysis.

All these plans listed above depend on the knowledge or expertise of the sampler the nature of the population or site from which the sample is taken and level of precision and sensitivity of the required outcome.

3.3 Various Sampling Techniques

The various sampling techniques employed in analysis are:

3.3.1 Representative Sampling

This is a subset of population that accurately reflects the members of the entrance population of interest. A representative sample should be unbiased, indication of what the population is like

3.3.2 Probability Sampling

Is any form of sampling that utilises some form of random selection of analytical materials. In other to have a random selection method. One must set up some process or procedures that ensure that the different units in a population have equal probabilities of being chosen. There are some table of random sampling that are used for random sampling to ensure that each member of the population have the same chance of being selected.

The most recent use of random numbers is done through some coded forms of number in the computer to form the bases for randomisation.

3.3.3 Sample Frame

Sampling frame lists all the elements of the population that are of interest, for example if we want analyses a particular element in the population, we will need to know the identity of all the elements that are of interest to us to be able to draw our element of interest.

3.3.4 Random Number Sampling

This is a number determined totally by chance with no predictable relationship to any other number. A random number table is used for such sampling. A random number table is a list of numbers composed of digits that are arranged in such a way that each digit has no predictable relationship to the digits that preceded it or to the digit that follows it.

3.3.5 Simple Random Sampling

The simple random numbers has the following properties;

1. The entire population consist of N number
2. The sample consist of n number
3. All possible samples with n number have equal possibility of being chosen.

Simple random sampling is simple to explain as compared to others; this is because it is the best way to select a sample. It is reasonable enough to generalize the result of the sampling back to the entire population.

3.3.6 Systematic Random Sampling

Systematic sampling is a form of one stage cluster sampling that is used in place of simple random sampling. To achieve that the following steps are important:

- (i) The number of the units in the population must be from one to ten (1-10)
- (ii) Decide on the sample size that you need
- (iii) Give consideration to the interval size $K=N/m$
- (iv) Randomly select an integer between 1-K
- (v) Then take every K^{th} unit. Let assume we want to select from a population that has 100 samples and that we want to take a sample of $n=20$.

To use systematic sampling, the population must be listed in a random order. The sampling fraction would be $f=20/100 =20\%$.

3.3.7 Stratified Sampling

Stratified sampling involves the division of heterogeneous population into varying smaller groups known as strata. The strata are formed on the bases of their homogeneity. A random sample from each stratum is taken in a number proportional to the stratum's size when compared to the population. These forms of strata are then pooled to form a random sample.

Advantages of Stratified sampling

1. It can provide greater precision than a simple random sample.
2. It often requires a smaller sample which saves wastages.
3. It can guard against an 'unrepresentative' sample.

3.4 Types of Sample

Here are some of the samples that are collected for analysis.

3.4.1 Composite Sample

Composite sample give a representative sampling of heterogeneous trays in which the composition of the analytic of interest may vary over a period of time and space. These kinds of samples can be sequential or flow proportional. Sequential samples are collected using a continues and unchanged equipment while flow. Proportional composite samples are collected at a rate proportional to flow.

3.4.2 Integrated Samples

These are samples that are collected at different time but in a simultaneous form. The result desired from these samples can be best provided by analysing matures of such samples collected at different points.

3.4.3 Grap Samples

These are discrete samples that are collected at a specific spot in a place over a limited period of time. Grap sample are best collected when it known that their source are affected by time.

4.0 CONCLUSION

Sampling still remains the backbone of any reasonable analysis, because if the sampling is not done the right way, no matter how good your instruments are you may not get a concrete result that will be used for drawing a meaningful conclusion. Good sampling gives good result while bad sampling always leads to bad and results filled with errors.

5.0 SUMMARY

In this unit, we have learnt about

- Definition of sampling
- Characteristics of a good sample
- The various sampling techniques
- The types of samples.

6.0 TUTOR- MARKED ASSIGNMENT

1. Justify the statement; sampling is the backbone of analysis
2. List out the characteristics of a good sample
3. Mention and explain the various sampling techniques
4. List the types of samples

7.0 REFERENCES / FURTHER READING

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UNIT 3 COMMON EXPERIMENTAL DESIGN IN ANIMAL SCIENCE

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Experimental Design
 - 3.2 Randomisation
 - 3.3 Replication
 - 3.4 Control
 - 3.5 Common Designs in Animal Science Consist of
 - 3.5.1 Completely Randomised Designs (CRD)
 - 3.5.2 Randomised Complete Block Design
 - 3.5.3 Factorial Design
 - 3.5.4 Split Block Design or Nested Design
 - 3.6 Latin Square Designs
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Readings

1.0 INTRODUCTION

Experiment is defined as a systematic enquiry into a subject matter with a view of discovering new facts or refuting results obtained from previous researches. The various step involved in experimentation are referred to as scientific methods which include

1. Hypotheses formulation
2. Planning an experiment to test the hypotheses
3. Data collection
4. Data analysis and result interpretation

2.0 OBJECTIVES

At the end of this unit, students should be able to;

- define an experiment
- give the various ways/steps involved in experimentation
- define experimental design

- give steps taken in the design of an experiment
- give and explain the types of experimental designs in animal science
- give examples of types of experiments designs in animal science.

3.0 MAIN CONTENT

3.1 Experimental Design

Refers to all preliminary steps taken to ensure appropriate data collection, data analysis, interpretation and valid inference obtained from an experiment. These steps include;

1. Problems definition
2. Statement of objectives
3. Selection of treatment
4. Selection of experimental materials
5. Selection of experimental design
6. Selection of experimental units and replication
7. Control of effect of adjacent unit
8. Conducting the experiment and analysing data and interpreting results.

3.2 Randomisation

This involves the assignment of treatments into experimental unit such that each unit is given equal chance to be assigned to treatment. It provides an unbiased estimate of treatment means and experimental error. Just like random sampling, the aim of randomisation is to reduce the possibility of bias judgment.

This refers to the assignment of similar experimental units together in a group. It helps in checking variations among treatment groups and removing experimental error.

3.3 Replication

This means the appearance of a treatment in an experiment more than once. It is important because it helps in estimating error and also provides a more precise measure of treatment effect.

3.4 Control

These are neutral and untreated experimental units, which are allowed for certain restriction on randomisation. This is done to control experimental error

3.5 Common Designs in Animal Science Consist of

1. Single factor design
2. Multiple factor design

A single factor design is a design that have only one independent factor or variable of treatment in which a factor is manipulated at various levels.

Multiple factor design is a design that has more than one independent factor or variable of treatment in which more than one factor are manipulated at various levels.

3.5.1 Completely Randomised Designs (CRD)

CRDs are the simplest form of designs in which all the experimental materials are homogenous except the treatment or the factor that is needed to be examined. In CRD, treatment is assigned to experimental units completely at random or by chance. Each experimental unit has equal chance of receiving a treatment. Such experimental units could be plots, animals or soil samples. For example if we have a number of treatments say $n_1, n_2, n_3, \dots, n_n$, and we have experimental units say $p_1, p_2, p_3, \dots, p_n$, each of the experimental unit has equal chance of being treated with each of the treatments.

Advantages of CRD includes

1. It is flexible, because one can have as much number of treatments and that any number of treatment can be as the available number of experimental units
2. Statistical analysis is simple even if there are unequal number of experimental unit per treatments can be investigated
3. The number of degree of freedom is large for estimating experimental error.
4. When data is missing, the analysis remains simple.

Disadvantages of CRD

1. There may be less of precision if the experimental units are not homogenous
2. It is less efficient than other designs

3. It can fit large number of treatment because large treatment amount of experimental material will be.

3.5.2 Randomised Complete Block Design

A randomised complete block design is used when experimental units can be grouped in blocks according to some defined sources of variability before assigning treatments to them. Blocks are groups that are used to explain another part of variability, but the test of their difference is usually not of primary interest. The number of experimental units in each block is equal to the number of treatments, and each treatment is randomly assigned to one experimental unit in each block. The precision of the experiment is increased because variation between blocks removed in the analysis and the possibility of detecting treatment effects is increased. The characteristics of randomised complete designs are:

1. Experimental units are divided to a treatment and blocks. Each treatment appears in each block only once
2. The treatments are assigned to units in each block randomly.

This design is balanced, each experimental unit is grouped according to blocks and treatments, and there is the same number of blocks for each treatment. Data obtained from this design are analysed with a two way ANOVA, because two ways of groupings, block and treatment are defined.

Advantages of Randomised Complete Block Design include;

1. It helps in the reduction of experimental error
2. It gives a more satisfactory precision
3. Random error is kept as small as possible because experimental unit does not contribute to the observed treatment variability
4. It does not allow for restriction of number of treatment or blocks.

Disadvantages

1. Large error term results due to the facts that large treatment number makes it impossible to have homogenous units within each block

3.5.3 Factorial Design

A factorial experiment has two or more sets of treatments that are analysed at the same time. Recall that treatment denotes particular levels of an independent categorical variable, often called a factor. Therefore, if two or more factors are examined in an experiment, it is a factorial experiment. A characteristic of a factorial experiment is that all combinations of factor levels are tested. The effect of a factor alone is called a main effect. The

effect of different factors acting together is called an interaction. The experimental design is completely randomised. Combination of factors is randomly applied to experimental units. Consider an experiment to test the effect of protein content and type of feed on milk yield of dairy cows. The first factor is protein content and the second is type of feed. Protein content is defined in three levels, and two types of feed are used. Each cow in the experiment receives one of the six proteins and feed combinations. This experiment is called a 3x2 factorial experiment, because three levels of the first factor and two levels of the second factor are defined. An objective could be to determine if cow's response to different protein levels is different with different feeds. The characteristics of a factorial experiment is particularly useful when little is known about factors and all combinations have to be analysed in order to conclude which combination is best. There can be two, three or more factors in an experiment. Accordingly, factorial experiments are defined by numbers of factors in the experiment.

Advantages of Factorial Experiments include

1. It allows for assessment of more than one animal at a time
2. It is efficient and comprehensive because it allows for the test of treatment effect as well as possible interaction.

3.5.4 Split Block Design or Nested Design

The split-plot design is applicable when the effects of two factors are organised in the following manner. Experimental material is divided into several main units, to which the levels of the first factor are randomly assigned. Further, each of the main unit is again divided into sub-units to which the levels of the second factor are randomly assigned. The split plot design plan can include combinations of completely randomised design, randomised block designs, or Latin square designs, which can be applied either on the plots or subplots

3.6 Latin Square Designs

The major feature of Latin Square design is its capacity to simultaneously handle two known sources of variation among experimental units. It employs the use of rows and column. It treats the sources of two independent blocking instead of only one as in CRD and CRBD. This is achieved by ensuring that every treatment occurs only once each of the row and column block. The two basic restrictions of Latin square are that the number of rows must be equal to the number of columns and equal number of treatment and that no one treatment must appear twice in the same row

or column. This procedure makes it possible to estimate variation among rows block as well as among column blocks and easily eliminate them from the experimental error.

Latin square design can be applied in trial which the experimental plots are arranged in a straight line and in lab trials with replications over time. Such that variations among units conducted the same time and those over time constitute the two main sources of variations. It can also be applied in animal science feed trail.

Advantages of Latin Square Designs includes

1. It controls more variations than CRD and CRBD because of the use of row and columns
2. That can be analysed simply
3. Analysis can be done even when some plots are missing

Disadvantages

1. Number of treatment is limited to the number of replicates which in most cases does not reach 10
2. If less treatment are employed the degree of freedom for controlling random variations will be large and that of error will be small
3. It is difficult to evaluate interaction between row and columns or treatments vice-verse.

4.0 CONCLUSION

Experimental designs in animal science are done to ensure appropriate data collection, data analysis interpretation and valid reference with the aim of reducing experimental error in an experiment.

5.0 SUMMARY

The essences of experimental designs are to appropriately collect data that will be analysed to give a meaningful and understanding conclusion. Some of the measures taken to reduce experimental error are randomisation, replication and control. Also, common designs in animal science the randomised complete block design, factorial design, the split block design among others.

6.0 TUTOR- MARKED ASSIGNMENT

1. Define experiment and give the steps involved in experimentation.
2. Briefly explain the following;
 - a. Blocking
 - b. Randomisation
 - c. Replication and

- d. Control
- 3. Explain the various steps in experimentation
- 4. Give advantages and disadvantages of CRD

7.0 REFERENCES / FURTHER READING

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UNIT 4 PRACTICAL APPLICATION OF COMMON DESIGNS IN ANIMAL EXPERIMENTS

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1
 - 3.2 Analysis of Variance for Rcbd
 - 3.3 Latin Square Design
 - 3.4 Nested Design/Split Plot Design
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

In the last unit, we looked at various designs of experiments in animal science such as.

- 1. Complete Randomised Design CRD
- 2. The Complete Randomised Block Design (RCBD)
- 3. Latin Square
- 4. Split Plot Design or Nested Design

In this unit therefore, we will look at how these designs are practically applied in animal science and the steps in calculating if they are significance.

2.0 OBJECTIVES

At the end this unit, students should be able to;

- comfortably use calculations in CRD
- calculate analysis of variance (ANOVA) in RCBD
- calculate ANOVA from data in Latin square
- solve calculations (ANOVA) from data in nested design.

3.0 MAIN CONTENT

3.1 As stated before in unit 3,CRD is a design in which all the treatment except materials are homogenous except the treatments that are allocated in this design, variation is measured only in one direction. Generally, the ANOVA table for CRD is as shown below;

Source of variation	DF degree of freedom	SS sum of squares	Mean squares (MS) = $\frac{SS}{Df}$	Calculated F	Tabulated F
Treatment	t – 1	SS _R	MS _T		
Error	n – t	SS _{error}	MS _E	$\frac{MST}{MSE}$	
Total	n – 1	SS _{Total}			

Consider the data collected on 20 goats randomly assigned to 4 treatments and the effect the treatments is measured.

First thing to do is to set up a null and the alternate hypothesis. Null hypothesis (H) = assumes the unchanged state

Ho = means of all the data are the same for the diff diet: $y_1 = y_2 = y_3 = y_4$

Alternative hypothesis (Ha) = means treatments are not the same: $y_1, \neq y_2 \neq y_3 \neq y_4$

The module for this design as follows

$$Y_{ij} = U + T_i + e_{ij}$$

Where: y_{ij} = any observation, U = overall mean, T_i = effect of treatment and e_{ij} = random error.

Assuming, the 4 treatment are applied to these goats as show or the table below:

Trt1	Trt2	Trt3	Trt4	Total
40	39	44	55	178
44	52	40	35	171
37	45	50	56	188
50	38	43	44	175
Total 213	209	227	239	Total = 888

Means of treatments

Means = $\frac{203}{5}, \frac{209}{5}, \frac{227}{5}, \frac{239}{5} = 42.6, 41.8, 45.4, 47.8$

Step 1

Calculate the correction factor (CF) = $\frac{(\text{Grandtotal})}{\text{total of observation}} = \frac{(888)}{20} = \frac{(788,544)}{20}$

Cf = 39427.20

Step 2

Calculate the sum of square total (SS_{Tot}) = Crude sum of squares- CF

SS_{tot} = $40^2 + 39^2 + 44^2 + \dots + 49^2 - 39427.20$
 = $40196 - 39,427.20 = \underline{768.8}$

Step 3

Calculate the SS treatment

SS_{tret} = $(\frac{\text{crudesumofsquaretreatment}}{\text{No.ofanimalspertreatment}}) - \text{CF}$
 = $\frac{3122+2092+2272+2392}{5} - \frac{\text{CF}}{4} = \frac{197,700}{4} - \text{Cf} = 39540 - 3942.20 = \underline{112.8}$

Step 4 calculate the SSerror

SS_{Error} = SS_{total} – S_{tret} = 768.8 – 112.8 = 656

Step 5 Construct an ANOVA table and input the values

Sources of variation (SOV)	Degree of freedom (DF)	Sum of squares (SS)	Mean square (MS)	F _{calculated}	F _{tabulated}
Treatment	t – 1 = 41 – 3	112.8	37.6		
Error	t(r – 1) = 4(5 – 1) = 16	656	41.00	0.917 NS	3.24
Total	n-1 = 20–1 =19	768.8			

From the table above

MS is gotten by dividing $\frac{\text{sum of squares}}{\text{degree of freedom}}$ or each class.

That for MS, = $\frac{\text{SS}_{tret}}{\text{df}} = \frac{112.8}{3} = 37.6$

MS_{Error} = $\frac{\text{SS}_{Error}}{\text{df}} = \frac{656}{16} = 41$

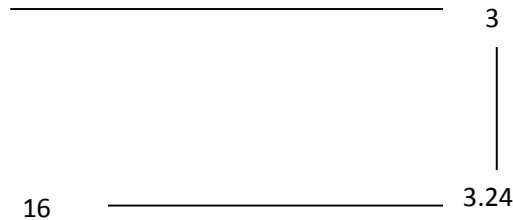
F_{cal} value is calculated by dividing MS_{tret} by MS_{Error}

= $\frac{\text{MS}_{tret}}{\text{MS}_{Error}} = \frac{37.6}{41} = 0.917$

For F tabulated, an F distribution table is used for this example; we will check the value of f at 5% (0.05) at degree of freedom.

df₁ = degree of freedom for treatment which is on the horizontal line of the table.

df_2 = degree of freedom of error which is checked on the vertical axis f
 tabulate $df(3)/df_{16}$.



Compare the calculate F value with the tabulated f value and decide on the level of significance of the difference among the group using the following rules:

1. If the calculated F value is greater than tabulated F value at 5%, the treatment is said to be significant. Such a result is indicated by asterisk (*). If it is significant at 1%, it is said to be highly significant and it is indicated by (**) on the calculated f-value.
2. If the calculated value is smaller than or equal to the tabulated f-value at 5% level of significance, the treatment difference is said to be non-significant, such result is indicted by placing NS on the calculated F-value. For our example above, the tabulated value 3.2 is greater than the calculated value 0.917. This means that effect of treatment is not significant; we will therefore; accept the null hypothesis that said that treatment means are the same.

3.2 Analysis of Variance for Rcbd

As discussed in unit 3 in the RCBD, the main feature is the use of blocking of equal size, each of which contain all the treatments. The model for RCBD is given bellow;

$Y_{jk} = U + T_i + B_j + e_{ijk}$ where; Y_{jk} = any observation, U = overall mean, T_i = effect of treatment

B_j = effect of blocking and e_{ijk} = random error

The ANOVA for RCBD,

Source of variation	Degree of freedom	Sum of squares	(MS = $\frac{SS}{Df}$ = Calculated F)	Tabulated F
Replication	$r - 1$	SS_R	MS_R	
Treatment	$t - 1$	SS_t	MS_t	$\frac{MST}{MSE}$
Error	$(r - 1)(t - 1)$	SS_{error}	MS_E	
Total	$rt - 1$	SS_{Total}		

Example 1

Consider a data collected from RCBD experiment in which a diet was fed to animals and the following data was obtained.

Rep	Diet				total
	t1	t2	t3	t4	
1	4	5	9	5	23
2	6	4	12	7	29
3	5	6	9	8	28
Total	15	15	30	20	80
Mean	5	5	10	6.67	

Run an ANOVA for the result

Calculate the correction factor = $\frac{(\sum x)^2}{n} = \frac{(80)^2}{12} = 533.33$

$SS_{total} = CSS - CF$
 $= 4^2 + 5^2 + 9^2 + \dots + 8^2 - CF$
 $= 598 - 533.33 = 64.67$

$SS_t = \frac{CSS_{Treatment}}{replication} - CF = \frac{152+152+302+202 - CF}{3} = \frac{1750}{3} - CF = 50.0$

$SS_{rep} = \sum \frac{y_j^2}{t} = \frac{232+292+282 - CF}{4}$
 $\frac{2154}{4} - Cf = 538.5 - 533.33 = 5.17$

$SS_{error} = SS_{total} - (SS_{trt} + SS_{rep}) = 64.67 - (50.00 + 5.17) = SS_{error} = 9.5$

Construct the ANOVA table as shown bellow

SOV	Df	SS	Ms	Fcal	F 0.05	F 0.01
Treatment	t-1 = 4-1 = 3	50.00	16.67	10.55	4.76	9.78
Rep	r-1 = 3-1 = 2	5.17	2.59			
Error	(r-1)(t-1) = 6	9.50	1.58			
Total	n-1 = 11					

Compare the F tabulated with the F-calculated, check at 0.05 and 0.01 at df 3 and 6. Since the tabulated at both F0.05 and F0.01, is less than F-cal, report at 0.01 two means that the effect is said to be highly significant and is represented by (**) (two asterisk).

3.3 Latin Square Design

As discussed in the last unit, Latin square employ the control of error using row and column randomisation in this unit takes care of treatment being equal to the No of row and columns. In Latin square no one treatment must appear twice. The design can be 3x3, 4x4 or 5x5 e.g.

Example: The following data was obtained from an experiment gotten from a Latin Square design. Run its ANOVA and make conclusion.

Row

	III	I →	V	II	IV	Total
5	C7	D6	B6	E12	B7	38
2	EII	C7	E10	B9	A4	41
4	D7	E8	C6	D3	E9	33
1	B8	A5	D7	A5	C6	31
3	A2	B10	A3	C4	D7	26
Total	35	36	32	33	33	169

$$CF = \left(\frac{(GT)^2}{n} \right) = \frac{169}{25} = \frac{28561}{25} = 1142.44$$

$$SS_T = 7^2 + 6^2 + 6^2 + \dots + 7^2 - CF$$

$$= 1297 - 1142.44 = 154.56$$

$$SS_{row} = \frac{CSS \text{ Row} - CF}{\text{column}} = \frac{382+412+332+312+262 - CF}{5}$$

$$= \frac{5851}{5} - 1142.44$$

$$1170.2 - 1142.44 = 27.76$$

$$SS_{\text{column}} = \frac{\text{CSS c-CF } 352+362+322+332 +332-\text{CF}}{\text{row} \quad 5}$$

$$\frac{5723}{5} - 1142.44$$

$$= 1144.6 - 1142.44 = 2.16$$

SStret = sum all the values attached to a particular Alphabet and square the answers

$$A = 2 + 5 + 3 + 5 + 4 = 19$$

$$B = 8 + 10 + 6 + 9 + 7 = 40$$

$$C = 7 + 7 + 6 + 4 + 6 = 30$$

$$D = 7 + 6 + 7 + 3 + 7 = 30$$

$$E = 11 + 8 + 10 + 12 + 9 = 50$$

$$SS_{\text{trt}} = \frac{192+402+302+302+402 -\text{CF}}{5} = \frac{6261}{5} - \text{CF}$$

$$1252.2 - 1142.44 = 109.76$$

$$SS_{\text{error}} = SS_{\text{total}} - (SS_{\text{r}} + SS_{\text{c}} + SS_{\text{trt}}) = 154.56 - (27.76 + 2.16 + 109.76)$$

$$= 14.88$$

ANOVA Table

SOV	Df	SS	MS	Fcal
Row	r-1 = 5-1 = 4	27.76	6.94	
Column	C-1 = 5-1 = 4	2.16	0.54	
Tret	t-1 = 5-1 = 4	109.76	27.44	22.31
Error	(r-1)(c-2) = 4x3 = 12	14.88	1.23	
Total	n-1 = 25-1 = 24			

$$F_{\text{cal}} = \frac{MS_{\text{TRET}}}{MS_{\text{ERROR}}} = \frac{27.44}{1.23} = \underline{22.31}$$

Since the Ftab at both 0.05 and 0.01 at df 4:12 are less than the calculated F (22.31), the result can be said to be highly significant.

3.4 Nested Design/Split Plot Design

The model for nested design is;

$$Y_{ijk} = U + S_i + D_j + e_{ijk}$$

Y_{ijk} = record of the k^{th} offspring of the j^{th} dam mated to the i^{th} , U = overall mean

S_i = effect of the i th sire, D_j = effect of the j th dam mated to the i th sire and e_{ijk} = random error

Example of a nested design calculation is seen in data gotten from a quail's experiment where 3 sires were mated to 4 dams, each and each dam has the following progenies as seen in the table.

Nested Design (Split-Plot Design)

	I	II	III
Z_1y_1	2	3	4 = 9
y_2	5	6	7 = 18
y_3	8	9	8 = 25
y_4	3	4	2 = 9
	18	22	21
Z_2y_1	3	4	3 = 10
y_2	2	6	7 = 15
y_3	8	8	8 = 24
y_4	9	2	3 = 14 63
	22	20	21
Z_3y_1	2	3	4 = 9
y_2	6	5	2 = 13
y_3	2	3	9 = 14
y_4	5	5	5 = 15
	15	16	20

51

Solution

Replication = Sire(main treatment) = 3 Dam (sub treatment) = 4

$$CF = \frac{(61+63+51)}{36} = \frac{(175)}{36} = 850.69$$

$$SS_T = CSS - CF$$

$$CSS = 2^2 + 3^2 + 4^2 + \dots + 5^2 = CF$$

$$= 1049 - 850.69 = 198.31$$

$$SST = \underline{198.31}$$

SSr = sum of replication

Replication I = 18+22+15 = 55

Replication II = 22+20+16 = 58

Replication III = 21+21+20 = 62

$$\text{Therefore SSr} = \frac{55^2 + 58^2 + 62^2}{12} - \text{CF}$$

$$= \frac{3025 + 3364 + 3844}{12} - 850.69 = \frac{10233}{12} - 850.69 = 852.75 - 850.69$$

SSREP = 2.06

SSz = sum of main treatment

$$\text{SSz} = \frac{61^2 + 63^2 + 51^2}{12} - 850.69$$

$$= \frac{3721 + 3969 + 2601}{12} - 850.69$$

$$= \frac{10291}{12} - 850.69$$

$$= 857.58 - 850.69 = 6.89$$

SSz = 6.89

$$\text{Error (a)} = \frac{18^2 + 22^2 + 21^2 + 22^2 + 21^2 + 15^2 + 16^2 + 20^2}{4} - (\text{CF} + \text{SSR} + \text{SSZ})$$

$$= \frac{3455}{4} - (850.69 + 2.06 + 6.89)$$

$$= 863.75 - (859.64)$$

$$= 863.75 - 859.64$$

∴ error(a) = 4.11

SSy = sum of sub-treatment: Sum of

y1 = 9 + 10 + 9 = 28

y2 = 18 + 15 + 13 = 46

y3 = 25 + 24 + 14 = 63

y4 = 9 + 14 + 15 = 38

$$\text{Therefore, SSy} = \frac{28^2 + 46^2 + 63^2 + 38^2 - \text{CF}}{9}$$

$$= \frac{784 + 2116 + 3969 + 1444}{9} - 850.69 = \frac{8313}{9} - 850.69 = 923.67 - 850.69$$

SSy = 72.98

SS interaction = sum of square of interaction

$$= \frac{92 + 182 + 252 + 92 + 102 + 152 + 242 + 142 + 92 + 132 + 142 + 152 - (\text{CF} + \text{SSmain treat} + \text{SSSub treat})}{3}$$

$$= \frac{2879}{3} - (850.69 + 6.89 + 72.98) = 959.67 - 930.56$$

SS interaction = 29.11

SSerror (b) = SST - all others

= 198.31 - (SSr + SSz + SSy + SS interaction)

= 198.31 - (2.06 + 6.89 + 72.98 + 29.11)

$$= 198.31 - 111.04$$

$$SS \text{ error (b)} = \underline{87.29}$$

ANOVA Table

Source	Df	SS	MS	Fcal	Ftab
Replication	(3-1)=2	2.06	1.03	1.002	0.05 0.01
				NS	
Main treatment	(3-1)=2	6.89	3.445	3.35	6.94,18.00
				NS	
Error (a)	(2-1)(y-1)=4	4.11	1.028		
Sub-treatment	(4-1)=3	72.98	24.327	5.018	3.16 5.09
				XX	
Interaction	(3-1)(4-1)=6	29.11	4.852	1.000	2.66 4.01
				NS	
Error (b)	n-18	87.27	4.848		
Total	35				

Conclusion

From the calculation above at the df = 2, 2, 3 and 6 there is no difference between replication, main treatment and interaction but there is highly significant difference between sub-treatment.

4.0 CONCLUSION

As seen from this unit, each design has its own uniqueness as to how it is being designed and calculated. Attention should be given to the way each design is being partitioned so as to arrive at a meaningful result.

5.0 SUMMARY

Summary: this unit, you have learnt how to calculate ANOVA for various designs in animal science, such designs as CRD, RCBD, Latin square nested design. You also learnt that when the calculated value at 5%, the effect of a particular treatment is said to be significant normally indicated by asterisk.

When it is greater at 1%, it is said to be highly significant and is indicated by two asterisks on the ANOVA table. On the other hand if the F-tabulated is greater than the F-calculated, the treatment effect is said to be insignificant and is indicated by N.S on the F-calculated on the ANOVA table.

6.0 TUTOR MARKED ASSIGNMENT

1. Observe the table below and answer the proceeding questions.

Trt1	Trt2	Trt3	Trt4
39	40	44	55
44	52	40	35
45	37	64	50
44	38	43	50

- (a) Suggest the Design for the table above,
- (b) Run an ANOVA for the design.

2.

	III	I	V	II	IV
5	C11	D7	D9	E10	B7
2	E12	C8	D9	B9	A8
4	B7	A8	C6	D3	E9
1	B12	A5	D8	A5	C5
3	E2	B10	A5	C4	D12

- a. What design is this?
- b. Write out the model for the design and run the analysis

3. Name the design and state suitable model for it

	I	II	III
Z _i y ₁	4	3	6
y ₂	4	5	8
y ₃	6	8	7
y ₄	5	6	2
Z ₂ y ₁	4	3	5
y ₂	4	7	6
y ₃	6	8	9
y ₄	9	2	3
Z ₃ y ₁	2	3	4

y ₂	6	5	2
y ₃	2	3	9
y ₄	5	5	5

7.0 REFERENCES/FURTHER READING

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MODULE 2

Unit 1	Introduction to Statistics
Unit 2	Descriptive Statistics
Unit 3	Probability
Unit 4	Measures of Relationships
Unit 5	Statistical Tests

UNIT 1 INTRODUCTION TO STATISTICS**CONTENTS**

1.0	Introduction
2.0	Objectives
3.0	Main Content
3.1	Scope of Statistics
3.1.1	Nature of Statistics
3.1.2	Descriptive Statistics
3.1.3	Inferential Statistics
3.2	Data Collection and Presentation
3.2.1	Data Collection
3.2.2	Why do we Collect Data?
3.2.3	How to Collect Data/Sources of Data
3.3	Basic Statistical Terms
3.4	Data Summarisation and Presentation
3.4.1	Tabular Presentation of Data
3.4.2	Guideline for Construction of Tables
3.4.3	Graphical Presentation of Data
4.0	Conclusion
5.0	Summary
6.0	Tutor-Marked Assignment
7.0	References/Further Reading

1.0 INTRODUCTION

Statistics deals with methods of collection, organisation, interpretation and presentation of information which are set in numerical form. Research or Experiment in animal science is aimed at careful collection of data that are subjected to statistical analysis in order to prove or refute the predetermined point (Hypothesis). In this unit, you will be introduced to the nature of statistics, collection and summarisation of statistical data as well as the basic concept/terms in statistics.

2.0 OBJECTIVES

At the end of this unit, students should be able to

- define statistics
- describe the two natures of statistics
- define data collection and why we collect data
- list and present data in a possible summarised format.

3.0 MAIN CONTENT

3.1 Scope of Statistics

Counting to numbers began right from the beginning of the world. Advancement in the way this counting (statistics) are now at the level of solving a lot of practical problems in animal experiments. With statistics, data on many animal experiments e.g Milk yield, weight gain of an animal and many other results are summarised into a meaningful and more readable state. The use of appropriate statistical techniques and sound statistical judgments to interpret data and results go as to reach reasonable decisions are now possible.

3.1.1 Nature of Statistics

3.1.2 Descriptive Statistics

This type of statistics summarises data so we can easily obtain an overview. It helps us to describe large number of data collected from our experiments to bring out information which is obscure.

3.2.3 Inferential Statistics

This nature of statistics involves the treatment of data leading to inference and predictions of what the population is likely to be. It allows us make claims or conclusions about a population based on a sample of data obtained from the population.

3.3 Data Collection and Presentation

3.3.1 Data Collection

Data collection is the method of gathering information on variables of interest in a systematic way that will enable the researcher/experimenter to answer his research questions (hypothesis) and to analyse outcome. It can also be said to be assembling of relevant information from sources in order to have a qualitative statistical analysis. Because of the importance data play in making decision in statistics, attention need to be given on how one collect and summarises his data. Data collected serve as a tool for rational evaluation and inferences.

Classifications of Data

- 1. Qualitative Data:** They are data collected on qualitative variable that cannot be quantified. Finding averages for such kinds of data does not really make sense. Example color of animals, Body conditions of animals and number of ticks on animals.
- 2. Quantitative Data:** These are data collected on quantitative variables. Such data can only be measured but not counted. Normal distribution curve are used to represent data. Examples are weight, length and heights.

3.3.2 Why do we Collect Data?

1. To confirm existing hypotheses
2. To refute the establish hypothesis with a backed up reasons for the observable differences.
3. To develop a new hypothesis. Such hypotheses are the null and alternate hypothesis. The null hypothesis is determined even before the data are collected while the alternate hypotheses are worked out tests statistics that are compared with the table value.

3.3.3 How to Collect Data/Sources of Data

1. **Primary Sources:** These are data gotten from a direct source or observation by the researcher.
2. **Secondary Sources:** Data collected from these sources are derived from already documented data. The researcher collects those data from organisations, Government establishment or farms without his/her taking part in the documentation.

3.4 Basic Statistical Terms

1. Variable: Is a value assigned to specific observation or a measurement? Examples are age, height, and length.

Variables can be divided into

- (a.) Discrete Variable: Is a variable that takes only integers as values, e.g. number of animals in a herd
 - (b.) Continuous Variable: Is a variable that takes any kind of real numbers and it is measured not counted, e.g. weight.
2. Observation: Is a value of a variable for a number of populations or a recording of information. It could be numerical or categorical.
 3. Sample: Is a subset of a population is that part of a population that are observed for the purpose making inference to the population. It could be random or purposive.
 4. Parameters: It is the characteristics of a population which helps to summarise information about the population with respect to the variable under study.
 5. Estimation: Is the process of summarising the information continued in a sample to make inference about the population.
 6. Sampling Size: This is the number of sampling unit taken from the population.
 7. Statistics: Any numerical value describing characteristics of a sample.

3.4 Data Summarisation and Presentation

Data are summarised and presented through tables and graphs.

3.4.1 Tabular Presentation of Data

Tabulation of a data reduces very big data into meaningful and readable form.

Data in tabulation are coded condensation of big information into a small range of meaningful categories to suit the requirement of the researcher. Examples of tabulation are:

1. Frequency distribution table
 2. Cross Tabulation
1. Frequency Distribution: Is a tabular arrangement of data by class that has its corresponding frequencies.

Example: Consider the weight of 100 goats in a herd

Weight	1-10,	11-20,	21-30,	31-40,	41-50	Total
Frequency	18	5	27	42	8	100

- Frequency is the number of appearance of a particular number of group or class of numbers that occur in a data set
- Cumulative Frequency: This is obtained by adding the frequencies of the succeeding class to that of the proceeding class. If for the example of goats above, we have:

Weight	1-10,	11-20,	21-30,	31-40,	and 41-50
Frequency	18	5	27	42	8
Cumulative Frequency	18	23	50	92	100

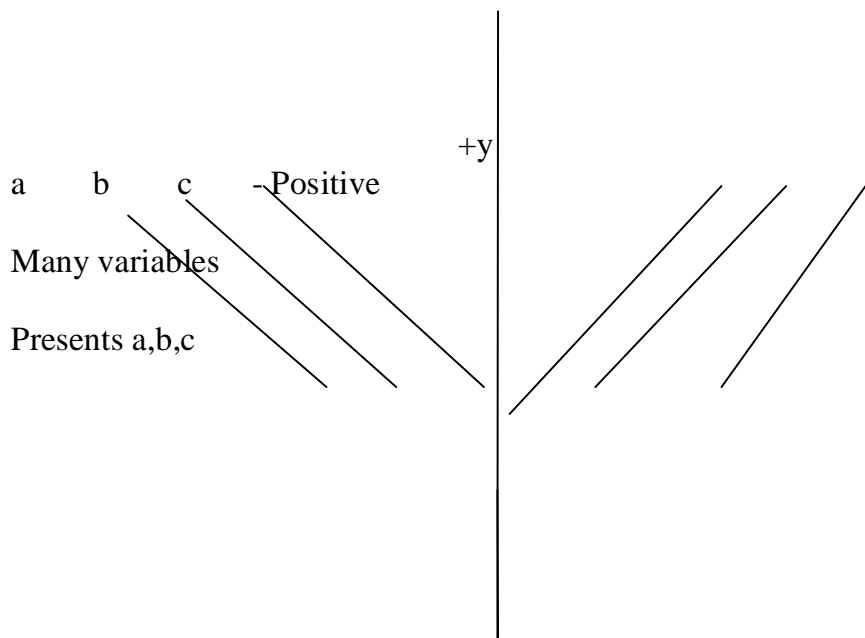
3.4.2 Guideline for Construction of Tables

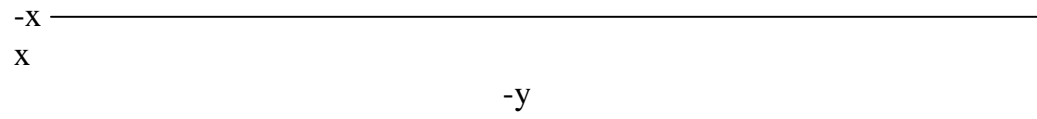
1. Title of the table should be self-explanatory and reflect the content
2. Figures to be compared should be adjacent of rows or columns
3. Figures should be from the same unit
4. Divide long rows and columns into sub heading
5. Use singular form for heading
6. Mention the unit of measurement
7. put sub-total where applicable
8. Put appropriate approximation for your data

3.4.3 Graphical Presentation of Data

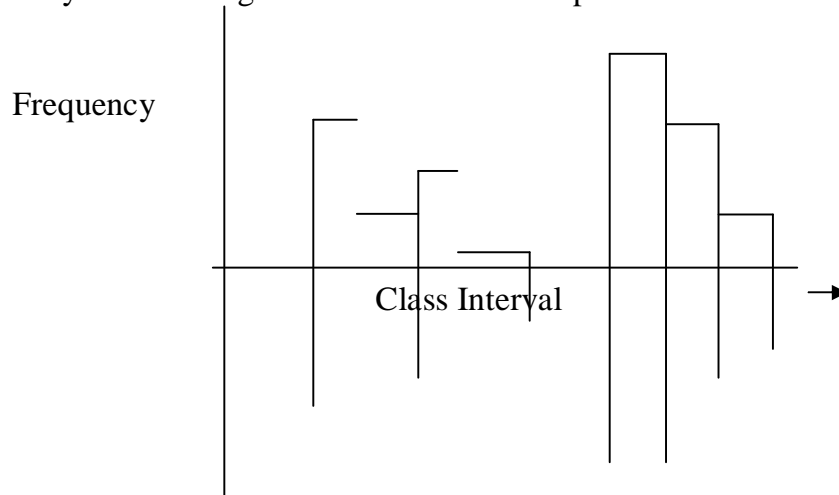
Presentation of data on graphs and diagrams makes it easier to understand and grasp.

Graphs are lines that relate dependent and independent variables: Graph can take a positive or negative format. Example

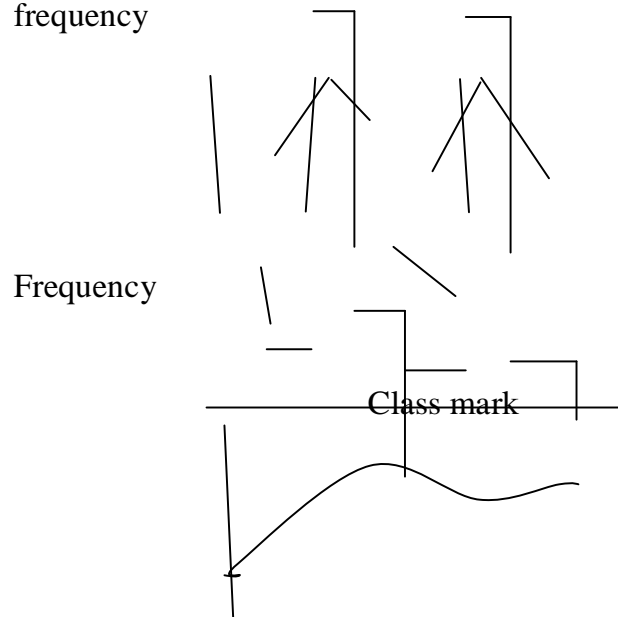




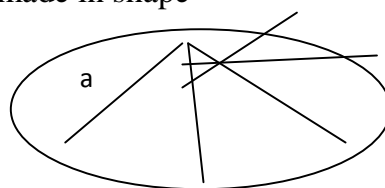
Histogram: It is pictorial representation of variables frequency which is done by constructing vertical bars that are separated from each other.



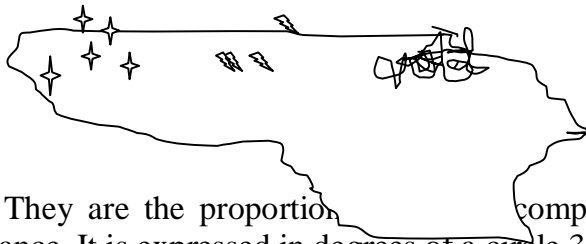
Frequency Polygon: It is gotten by joining the classmate against the frequency



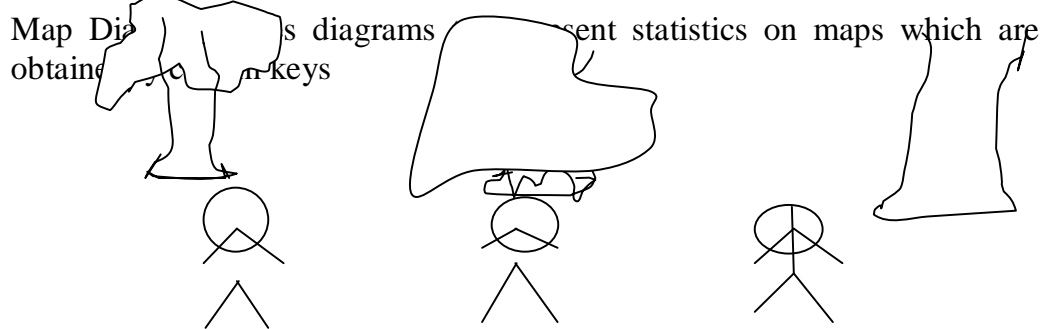
Ogive: It is obtained by drawing the curve of class interval against the frequency. It is made in shape



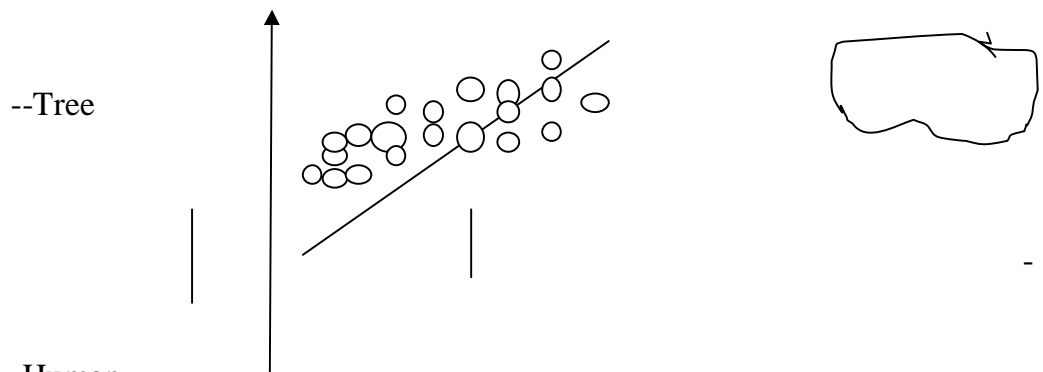
Frequency



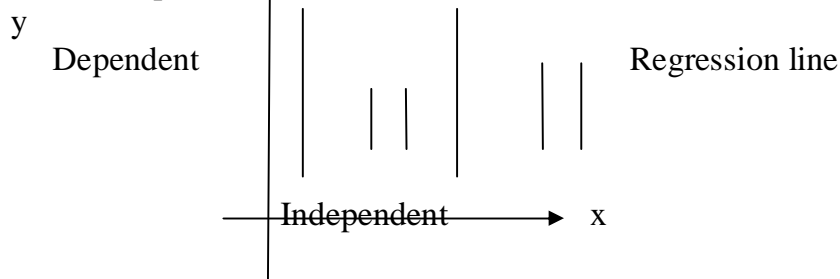
Pie Chart: They are the proportion components. They can be seen at a glance. It is expressed in degrees of a circle 360^0 .



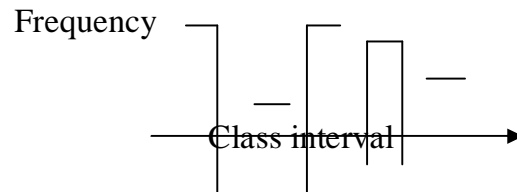
Pictograms: Uses sketches or shapes data representation



-Human
Scattered Diagrams: It is used for comparison of two variables it measures relationships between two variables



Bar Chart: They are rectangular bars plotted with equal gaps



SELF-ASSESSMENT EXERCISE

- (i) Think and mention the steps you took when collecting your data.
- (ii) Think and mention how many forms of graphs you have used before.

4.0 CONCLUSION

Statistics does not only involve the counting of numbers but collection of data and presenting it in the form pleasing and precise for the user.

5.0 SUMMARY

In this unit you have learnt the nature of statistics. You also learnt how to collect data and the source of data as well as the why we collect data. You have learnt how to summarise your data into pleasing formats.

6.0 TUTOR -MARKED ASSIGNMENT

1. List and explain the description and inferential statistics
2. Explain what data collection means
3. List and explain six basic statistical terms
4. Write on any four graphical presentations of data

7.0 REFERENCES/FURTHER READING

Miroslav. K. & William, R. L. (2004). *Biostatistics for Animal Science*. USA: CAB, Publishing.

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UNIT 2 MEASUREMENT OF CENTRAL TENDENCY AND DISPERSIONS

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Measures of Central Tendencies
 - 3.1.1 The Mean
 - 3.1.2 Properties of Arithmetic Means
 - 3.1.3 The Median
 - 3.1.4 The Mode
 - 3.2 Measures of Variability/Dispersion
 - 3.2.1 The Range
 - 3.2.2 Mean Deviation/Mean Absolute Deviation
 - 3.2.3 Variance
 - 3.3 Standard Deviation
 - 3.3.1 Coefficient of Variation
 - 3.3.2 Standard Error
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

Measurement of central tendencies is values that points to the centrality within a given set of data. They form the backbone of descriptive statistics. The measures of variability measures how individual values are spread around the mean. In this unit therefore you will learn how to use the various measures of central tendencies as well as their advantages. You will also learn how data does spread around these central points.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- mention the various measurements of central tendencies and their applications in analysis of data
- measure the spread of variable around their mean.

3.0 MAIN CONTENT

3.1 Measures of Central Tendencies

They are statistical parameters that measure the central point of a data. The measures of central tendency are mean, median and mode. Their characteristics of good central tendency are;

1. it must be rigidly defined
2. it should be based on all figures
3. it should be easily computed
4. it should be least affected by sampling
5. it should be treated mathematically.

3.1.1 The Mean

The arithmetic mean is the most commonly used measure of central tendency. It is calculated by dividing the total number of observation by the number of observations. It is also referred to as index that describes the centrality of the set of observation irrespective of their magnitude. Outliers if not taken care of, can affect the validity of the arithmetic mean.

Arithmetic mean is denoted by the formula

$$\bar{X} = \frac{\sum Xi}{n}$$

For a given set of numbers of values x_1, x_2, \dots, x_n with frequencies f_1, f_2, \dots, f_n . The mean is computed as

$$\bar{X} = \sum \frac{xifi}{\varepsilon fi} = \frac{f_1x_1 + f_2x_2 + \dots + f_nx_n}{f_1 + f_2 + \dots + f_n}$$

Example: Find the arithmetic mean of lactation yield of 10 cows given their daily milk yield of 200kg, 250kg, 200kg, 150kg, 450kg, 300kg, 350kg, 100kg, 240kg and 420kg.

$$\bar{X} = \frac{\sum Xi}{n} = \frac{200+250+\dots+420\text{kg}}{10} = \frac{2660}{10} = 266\text{kg}$$

Example 2: Assuming the lactation yield above has frequencies as 3, 2, 5, 4, 7, 8, 1, 3, 4, respectively the mean is

$$\bar{X} = \sum \frac{fixi}{\varepsilon fi} = \frac{200(3)+250(2)+\dots+420(4)}{3+2+5+\dots+4}$$

Advantages of Arithmetic Mean

1. It is a single figure representing the data set. Therefore it is easy to understand.
2. It is easy to calculate.
3. It is least affected by sampling.
4. It is based on all values.

Disadvantages of the Mean

1. It is affected by the extreme figures.
2. It gives non existing figures at times.

3.1.2 Properties of Arithmetic Means

1. When all the derivations of measurement in a given set from mean is equal to zero
2. The mean affects values in the set under consideration
3. The sum of squares of derivation of the measurement from mean is always a minimum compared to the sum of square of the derivations
4. If x_1, x_2, \dots, x_n are the mean of observation and quantity $y = kx$ the mean of the new variable $y = y - \bar{k}x$
5. Adding or subtracting a constant a , from values x_1, x_2, \dots, x_n will affect the mean in the same direction.

3.1.3 The Median

It is a value that divides the whole series into equal parts when arranged in order.

For an odd number, the median = $\frac{1}{2}$ th term

For even the median = $\frac{1}{2}$ (item in the $\frac{n}{2}$)th position plus item in the $(\frac{n+2}{2})$ th position. Though median is easy to find it is non sensitive to others.

Example: The median of the data 4, 6, 8, 9, 12, 14 and 20 is 9.

For an even numbers of observation,. Example the median of 9, 15, 20, 25, 35, 40 is

$$\frac{20+25}{2} = \frac{45}{2} = 22.2$$

Median for Classified Group Data

It is a little bit difficult because one have to employ the use of class limit and frequencies

$$\text{Median} = L + \left(\frac{\frac{T_n}{2} - f_1}{f_2 - f_1} \right) i$$

Where; L= Lower limit of class in which the median is located when class are arranged in ascending order

T_n = Total Frequency

f_1 = Cumulative frequency before the median class

f_2 = Frequency of the median class

I = Class interval i.e. the difference between the lower and the lower boundaries of the median class.

Example; Find the median of the following data

Class	1-5	6-10	11-15	16-20	21-35
Frequency	4	2	9	5	6
Cumulative Frequency	4	6	15	22	26

Using the formula $L + \left(\frac{\frac{T}{2} - f_1}{f_2 - f_1}\right) i$ where, L= 11 the median class is 11-15
 $i= 5, f_1 = 6, f_2= 15, T =18$

$$11 + \left(\frac{\frac{18}{2} - 6}{15 - 6}\right) 5 = 11 + (5) = 16$$

Advantages of Median

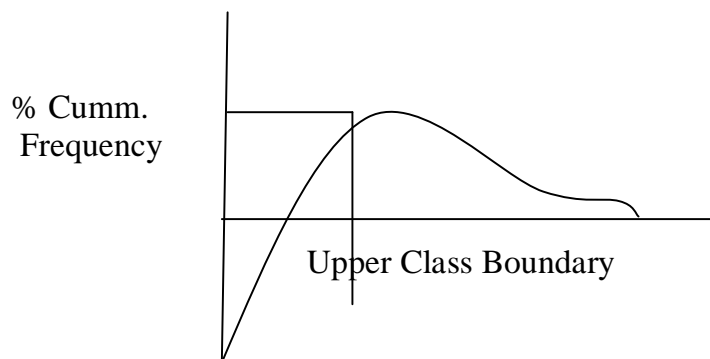
1. It is well defined
2. It is easy to calculate
3. It is not affected by outliers
4. It can be calculated even if the extreme values are not known but their numbers are known

Disadvantages of Median

1. It is affected by sampling
2. It cannot be treated mathematically
3. It gives equal weight age to all members
4. It lies between two values

Graphical Estimation of Median

A median value can be gotten from a graph when a cumulative frequency curve (Ogive) is drawn using the upper class boundary against the percent cumulative frequency. The value that corresponds to the 50th part is read down from the curve on the y-axis. The resulting value on the x-axis is the median.



3.1.4 The Mode

This is a measure of central tendency that shows the occurrence of a data. It is the number that has the highest frequency in a given series of data.

For an ungrouped data mode is simple to find. It is done by counting observing the number that has the highest appearance.

Example: Given the weight of animals

6, 4, 4, 7, 8, 9, 7, 8, 6, 7, 4, 5, 9, 10, 4, 5, 7, 5, 6, 7

The mode is 7 because 7 appeared 6 times.

If the set of observation has two modes, it is called a bimodal mode

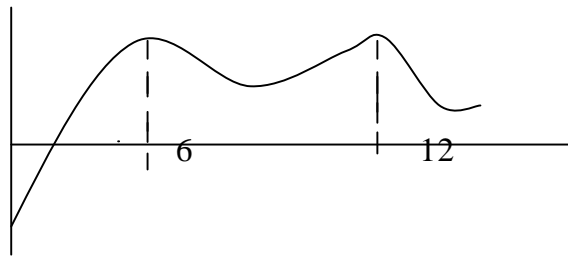
Bimodal data: to calculate a bimodal data, the first observed mode is called the primary mode. To calculate a bimodal data, add the two values of the mode then divide the result by two.

Example: Given the following data;

4, 5, 6, 6, 6, 6, 7, 7, 8, 8, 9, 9, 9, 9, 10, 11, 12, 12, 12

6 and 9 have the same number of recurrence;

$$\text{Mode} = \frac{6+9}{2} \left(\frac{\text{mode1}+\text{mode2}}{2} \right) = \frac{15}{2} = 7.5$$



For a group of classified data, the mode is given by the formula

$$\text{Mode} = L + \left(\frac{d_1}{d_1+d_2} \right) i \quad \text{or} \quad L + \left(\frac{f_0+f_1}{2f_0-(f_1+f_2)} \right) i$$

Where L= lower class boundary or the modal class

d₁= difference between the frequency or the modal class and the class before it (F₀ – F₁)

d₂= difference between the frequency or the modal class and the class after it

e= class interval

Example:

Class	0-10	10-20	20-30	30-40	40-50
Frequency	1	2	6	3	4
Cumulative Frequency	1	3	9	12	18

The modal class = 20-30; L=20, d₁=6-2, d₂=6-3, i=10

$$\text{Mode} = L + \left(\frac{d_1}{d_1+d_2} \right) i, = 20 + \left(\frac{4}{4+3} \right) 10 = 20 + \left(\frac{4}{7} \right) 10 = 20 + 5.7 = 25.7$$

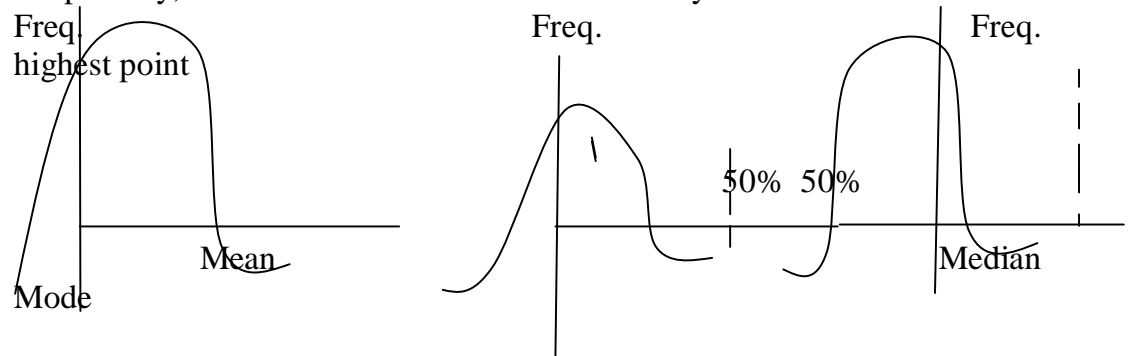
Advantages of Mode

1. It is not affected by others
2. A sample mode is a good representation of the pop
3. It is affected by skewedness of data

Disadvantage

1. It is a very poor measure of central tendency.

Graphically, the three measures of central tendency are shown below



3.2 Measures of Variability/Dispersion

To see how values are spread around the mean, we use measures of dispersion. The most used dispersions are range, mean deviation, standard deviation, variable, coefficient of variable, standard variable and standard error.

Properties of Dispersion

1. It gives an insight of true representative a sample is
2. It is used to compare two or more series
3. It is a good measure used for policy control

3.2.1 The Range

This is the simplest measure of dispersion. It is the difference between the largest value and the smallest value in a given series of observation.

Mathematically $R = L - S$ Where $L =$ Largest Value and $S =$ Smallest Value

Given the weight of sold goats; 2,5,6,7,2,8,10,15,11,13,14

The largest value = 15

The smallest value = 2

$\therefore R = 15 - 2 = 13$

Advantage

It is the most simplest to calculate to understand measures of dispersion

Disadvantages

1. It is affected by others
2. It does not give an insight of the mid values
3. It is not a presentation of all data.

3.2.2 Mean Deviation/Mean Absolute Deviation

This is the average of all the deviations of values from the mean.

Mathematically: $MD = \frac{\sum(x-x)}{n}$

Where $(x) = xi$ when $x \geq 0$

For a grouped data when the $MAD = \frac{\sum_{i=1} f_i(x_i - \bar{x})}{\sum f_i}$

MAD = 0 in a normal distributed data, therefore we use mean absolute deviation which ignores the algebraic signs.

Example: consider the following data

X_1 4, 5, 7, 11, 14, 22, 25 $\sum x = 88$; $n = 7$, $\bar{x} = 12.6$
 $x - \bar{x} = -8.6 -7.6 -5.6 -1.6 1.4 9.4 12.4$ $\sum(x - \bar{x}) = 0$ but $MAD = \frac{\sum(x_i - \bar{x})}{n} = \frac{46.6}{7} = 6.7$

Advantages

Calculation is done based on all values

Disadvantages

It is affected by sampling

3.2.3 Variance

It is the most widely used measure of dispersion. It is the average of sum of squared deviations from the mean. For population variance, that is a large number of data size.

Variance is denoted by

$\sigma^2 = \frac{\sum(\bar{x}_i - \bar{x})^2}{n}$

For sample variance with a small number of data size n, the variance is denoted by

$S^2 = \frac{\sum(\bar{x}_i - \bar{x})^2}{n-1}$

From the above data, our variance will be

$\frac{\sum(\bar{x} - x)^2}{n-1} = \frac{(4-12.6)^2 + (5-12.6)^2 + (7-12.6)^2 + \dots + (25-12.6)^2}{6} = \frac{409.7}{6}$

n-1 = 7-1 = 6

Variance = $S^2 = \frac{\sum_{i=1}^n f_i(x_i - \bar{x})^2}{\sum_{i=1}^n f_i} = \frac{409.7}{6} = 68.3$ For a grouped frequency:

$$S^2 = \frac{\sum_{i=1}^n f_i(x_i - \bar{x})^2}{\sum_{i=1}^n f_i}$$

Given the data below with the following frequencies

Class	Frequencies	Mid point (x)	Fx	d (x - \bar{x})	d ² (x - \bar{x}) ²	fd ² f(x - \bar{x}) ²
0-10	3	5	15	-19.1	364.81	1,094.43
10-20	4	15	60	-9.1	82.81	331.24
20-30	6	25	150	0.9	0.81	5.4
30-40	2	35	70	10.9	118.81	237.62
40-50	1	45	90	20.9	438.81	438.81
	$\sum f = 16$		$\sum fx = 385$			$\sum (x - \bar{x})^2 = 2107.47$

$$\bar{x} = \frac{\sum fx}{\sum f} = \frac{385}{16} = 24.1$$

$$\text{Variance} = \frac{\sum fd^2}{\sum f}$$

$$S^2 = \frac{2107.47}{16} = 131.72$$

3.3 Standard Deviation

This is the most widely and commonly used measure of dispersion, just like the mean in central tendency. It is defined as the average spread from the arithmetic mean. A small value gotten from the calculation of S.D indicates that the values of a given series are closely spaced.

S.D is denoted by S, which is the square root of variance. For a sample or small size, we divide



$$S = \frac{\sum_{i=1}^n (x - \bar{x})^2}{n-1} \quad \text{degree of freedom for large size,} \quad S = \frac{\sum_{i=1}^n (x - \bar{x})^2}{n}$$

ide the mean, spread deviation by

If it occurs with frequency, it is expressed as Type equation here.

$$S = \sqrt{\frac{\sum_{i=1}^n f(\bar{x} - x)^2}{N}}$$

From our example in variance

$$\text{S.D for unclassified data} = \sqrt{68.3} = 8.3$$

$$\text{The S.D for classified data in the table of frequency above} = S = \sqrt{131.72} = 11.48$$

Advantages of S.D.

1. It is well defined
2. It maintains the unit of an observation
3. It can be treated mathematically
4. It is not affected by sampling

Disadvantages of S.D

1. Difficult to calculate
2. It is affected by outliers

3.3.1 Coefficient of Variation

It is a relative measure of variability expressed as percentage. It is the ratio of standard deviation and the mean expressed as percentage.

$$CV = S/\bar{x} \times 100$$

From our example of unclassified data below

$$S = 8.3$$

$$\bar{x} = 12.6 \quad \text{Therefore} \quad CV = \frac{8.3}{12.6} \times 100 = 65.9\%$$

This is used for comparison of two or more series of data in a given set of series.

3.3.2 Standard Error

It refers to the ratio of standard deviation to the square root of the total number of observation. It is sometimes referred to as the error of the mean.

$$S.E = \frac{s}{\sqrt{n}} \quad \text{From our example above,}$$

$$S.E = \frac{8.3}{\sqrt{7}} = 3.1$$

Standard error of mean is usually presented with the mean of the data, example;

$$12.6 \pm 3.1$$

4.0 CONCLUSION

We have seen that the measures of central tendency and dispersion are two major mathematical treatment of data gotten from experiments that help us show how central our values are and how each value is spread around the mean.

5.0 SUMMARY

Measures of central tendency are mean, mode and median and measures of dispersion that are commonly used are the range, variance, mean deviation, standard deviation, coefficient of variation and the standard error. This helps us to present our data in a mathematical forms to locate how central and how each value are spread around a given mean of a given series.

6.0 TUTOR -MARKED ASSIGNMENT

1. Define the term measure of central tendency
2. List the three measures of central tendency
3. Calculate the mean the mean, mode and median of the following data;
1, 3, 3, 4, 4, 6, 6, 6, 6, 7, 8, 8, 9, 12
5. Define the term measures of dispersions and list the common measures of dispersion you know:
6. Given this set of data

Class	0-10	10-20	20-30	30-40	40-50
Frequency	7	2	8	6	5

Calculate the variance, standard deviation, coefficient of variation and standard error.

7.0 REFERENCES/FURTHER READING

Miroslav, K. & William, R. L. (2004). *Biostatistics for Animal Science*. USA: CAB, Publishing.

Singha P. (1992). *An Introductory Text on Biostatistics*. Zaria: Ahmadu Bello University Press Ltd. Nigeria.

UNIT 3 MEASURES OF RELATIONSHIP

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Correlation
 - 3.1.1** Scatter Graph
 - 3.1.2 Correlation Coefficient
 - 3.1.3 The Spearman's Rank Correlation Coefficient
 - 3.2 Regression Analysis
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

In the previous unit, you have learnt how values are spread around the mean. Those measures of dispersions only tell, you how the values in a series of data are spread. The measures of relationship we will discuss in this unit will open your mind to understand how two variables are related and the degree or magnitude of their relationship. Some of these relationships can easily be measured using a scattered diagram but beyond that, one needs a strong index to determine the strength of the relationship between two radiates.

In this unit, we will investigate, the relationship or association between two variables and the tool that is used to determine how one variable say y, depends on another variable say x, (i.e regression and correlation) because they describe the describe the association and the strength of such association between two variables.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain the concepts, correlation and regression
- draw and interpret a scalar diagram
- compute Pearson product moment correlation coefficient
- differentiate between positive and negative correlation using a well-defined diagram
- compute simple regression analysis problems.

3.0 MAIN CONTENT

3.1 Correlation

When two variables are at our disposal, as researchers the first thought that will come to us, is whether these variables are associated or not, if they are, how strong are their associations. The quantity that describes this is the correlation coefficient. In other words correlation techniques are used to evaluate relationships between bivariate variables. These bivariate variables are normally distributed.

In correlation analysis, the sign that is attached to correlation coefficient determines the direction of the relationships between the variables. Some variables even though they are associated, some of them will be negatively correlated meaning that an increase in one unit of one variable tends to result in a unit reduction of the other variable, while some bivariate variables tend to increase or decrease in the same direction. Some relationships that exist in animal production experiments are; body weight and egg production, milk yield and %fat, body weight and egg weight, body weight and height at wither sperm concentration and fertility and finally many other related associations in animal experiments. To determine such relationships the use of correlation coefficient is employed, which is a technique used for describing the strength of a linear relationship.

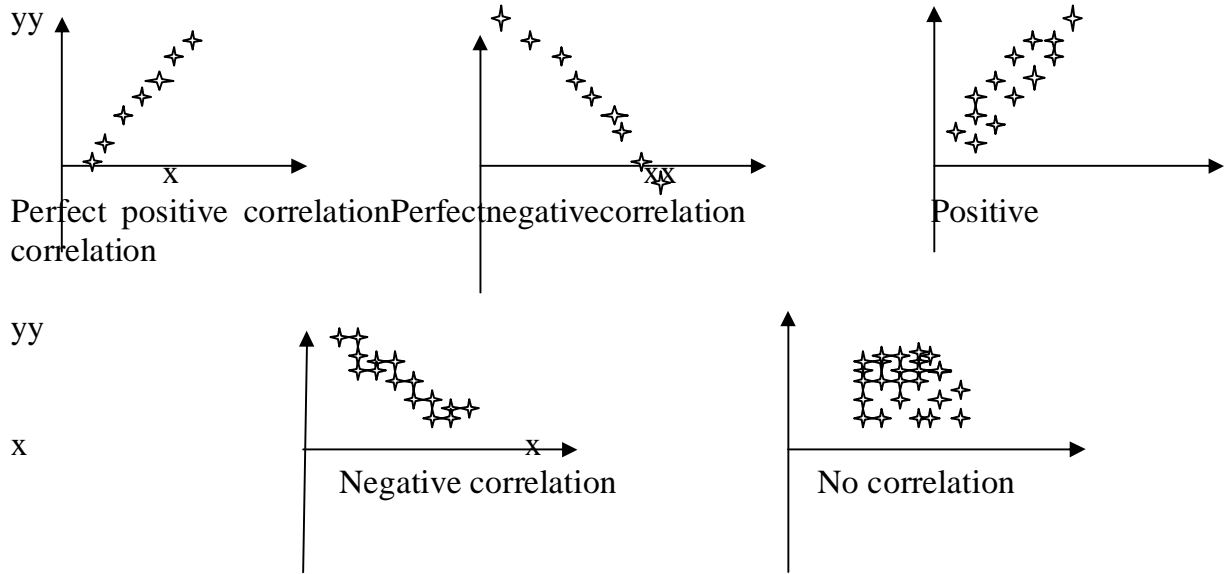
The easiest way to determine the extent of relationship between variables is by plotting the dependent variable (y) against the independent variable (x). The scatter plot helps us also to know the direction of the relationship that exists between the variables as mentioned earlier.

3.1.1 Scatter Graph

Here, data of the two variables are grouped and plotted using a single dotted line that shows individuals on the either dimension. For construction

of scatter diagram, the dependent variable are placed or arranged along the y-axis while the independent variable on the x-axis.

The figures below demonstrate the forms off relationships between the variable x and y.



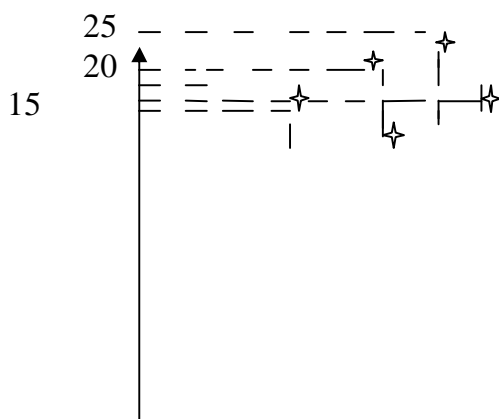
Example, use the following data obtained from height at withers and body weight of a red Sokoto goat taken on ten goats.

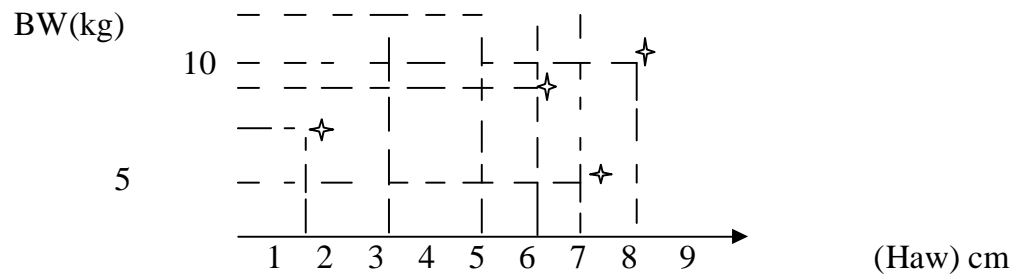
Height at wither (cm) (Xcm)	7	6	8	4	6	9	7	2	8
Body weight (kg) (Y)	21	19	17	15	13	11	9	7	5

Solution

1. First of all, determine your dependent and independent variables. The dependent variable on y-axis and the independent variables on x-axis.
2. Find the range of your variables so as to know the appropriate scales to use.
3. Plot the relationship using dotted lines.

For example above,





From the scatter plot above, it is obvious that it is difficult to determine the trend of relationship between height at wither and body weight. Therefore we can conclude that there is no relationship between them.

3.1.2 Correlation Coefficient

This is denoted by (r) and measures the strength and direction of association between two bivariate variables. Scatter plot only gives you an idea of how the relationship looks like but correlation coefficient gives you more precise association strength. Correlation strength is defined as an extent to which data points closely fit to each other on the margining line or scatter plot.

Correlation coefficient is always between -1 and 1 ($-1 \leq r \leq +1$)

Correlation coefficient value close to +1 indicates a perfect positive correlation or close to -1 indicates perfect negative correlation and r close to 0 indicates weak relationship and when $r = 0$. The variables are not correlated.

The correlation coefficient of a set of observations x_1, x_2, \dots, x_n are calculated

$$r = \frac{(\sum xy - (\sum x \cdot \sum y) / n)}{\sqrt{\sum x^2 - (\sum x)^2 / n} \sqrt{\sum y^2 - (\sum y)^2 / n}}$$

Example: Compute and give interpretation of the relationship between coefficient of correlation of the following.

Milk Yield (kg)	5	6	7	3	9
Fat Percentage (%)	8	8	7	7	10

Solution

X	Y	Xy	X ²	Y ²
5	8	40	25	64
6	8	48	36	64
7	7	49	49	49
3	7	21	9	49
9	10	90	81	100
$\sum x = 30$	$\sum y = 40$	$\sum xy = 248$	$\sum x^2 = 200$	$\sum y^2 = 326$

Using the coefficient of correlation, $r = \frac{(\sum xy - (\sum x \cdot \sum y) / n)}{\sqrt{\sum x^2 - (\sum x)^2 / n} \sqrt{\sum y^2 - (\sum y)^2 / n}}$

$$= \frac{248 - (30)(40)/5}{\sqrt{200 - \left(\frac{(30)^2}{5}\right)} \sqrt{326 - \left(\frac{(40)^2}{5}\right)}}$$

$$= \frac{248 - (1200)/5}{\sqrt{200 - \left(\frac{900}{5}\right)} \sqrt{326 - \left(\frac{1600}{5}\right)}}$$

$$= \frac{248 - 240}{\sqrt{(200 - 180)(326 - 320)}}$$

$$= \frac{8}{\sqrt{(20)(6)}} = \frac{8}{\sqrt{(120)}} = \frac{8}{10.95} = 0.73$$

3.1.3 The Spearman’s Rank Correlation Coefficient

The spearman’s rank correlation measures relationships between none normally distributed variable. We have that Pearson product moment measures relationships between a normally distributed variable, but in Spearman’s rank correlation, the variables are rank ordered. After ranking the values of individual observations, the mean of their ranks are obtained for each of the observation.

The spearman’s rank coefficient is given by the formula;

$$\sigma_s = \frac{6\sum di^2}{n(n^2 - 1)}$$

Just as in the product moment coefficient the spearman’s rank correlation can either be positive or negative. (1 ≤ rs ≤ +1)

3.2 Regression Analysis

Regression is a technique for quantifying the relationship among variable. The variables are divided into dependent and independent variable. Regression is an important tool for making predictions and inferences. In animal science researches, regression is used in various fields. For instance, in the field of breeding when a body weight is regressed on time, in the

field of physiology when a season is regressed on milk composition or the nutritionist may decide to regress feed conversion ratio to rate of grain. All these use regression to determine the extent of their relationships.

There are many types of regression, which include linear regression, multiple regression, polynomial and non-linear regression. But for purpose of this study, we will concentrate on linear regression;

Simple Linear Regression

The simplest linear regression involves only two variables;

- The dependent variable
- The independent variable

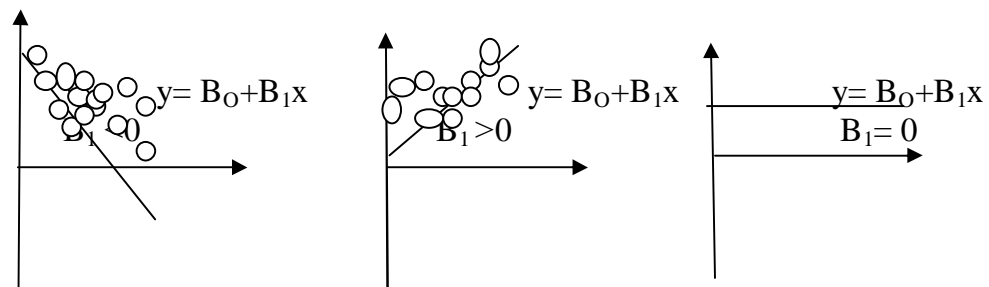
In this form of regression one of the independent variable is manipulated to obtain the value of dependent variable. Just like in correlation, Scatter plot can be used to serve as pretest in determining the direction of the regression line. The model used for linear equation is given below;

$$y = b_0 + b_1x_1 + e_i$$

Where b_0 = Intercept z which is the predicted value of y when $x = 0$ and this is gotten by finding the point at which the regression line intersects the y axis.

b_1 = The slope of the model can either be positive or negative. A positive slope indicates that when the independent variable increases, the corresponding dependent variable also increases. But when the slope is negative, it indicates that when the independent variable increases, it brings about a reduction in the value of dependent variables (opposite direction). When B_1 is close to either close to either -1 or +1, it is an indication of strong relationship but if it is equal to 0, just as we have seen in correlation it means that dependent and independent variables are not related.

Scatter Plot: The regression model can best be demonstrated by using a scatter plot to determine the direction of relationships that must be between the two variables.



Negative regression

Positive regression

No relationship

Regression coefficient b is denoted by the formula

$$b_1 = \frac{(\sum xy - (\sum x \cdot \sum y) / n) / \sum x^2}{\sum x^2 - (\sum x)^2 / n} \quad \text{and} \quad b_0 = \bar{y} - b_1 \bar{x}$$

SELF-ASSESSMENT EXERCISE

Give example of variables that their relationship can be computed using correlation and regression analysis.

4.0 CONCLUSION

Correlation and regression are the measures or relationship commonly used in agricultural experiments. They are important in estimating the direction of the relationships that exists between two variables, as well as the degree of the extent of the relations help that exist between the dependent and independent variables.

5.0 SUMMARY

In this unit, you have been taught the concept of correlation and regression where we said that correlation refers to the degree at which variables are related. We have also seen how to easily determine the direction of both correlation and regression using a fast method called scatter plot. It is easy because by just observing the spread of marked line you will know whether it is positive, negative or there is a relationship between the variables at all. With this in mind, we looked at how to calculate the correlation and regression coefficient using the most commonly used methods which is the Pearson product method for correlation and simple linear regression methods.

6.0 TUTOR -MARKED ASSIGNMENT

1. Define the concept correlation and regression
2. Give two methods of estimating correlation and regression
3. Using the Pearson product correlation coefficient. Calculate the relationship between the following data

X	20	34	42	39	25	40	52	38
Y	30	41	54	43	47	50	42	47

7.0 REFERENCES/FURTHER READING

Miroslav, K. & William, R. L. (2004). *Biostatistics for Animal Science*. USA:CABI Publishing

Singha, P. (1992). *Introductory Text on Biostatistics*. Zaria: Ahmadu Bello University Press.

UNIT 4 PROBABILITY THEORY

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Probability
 - 3.1.1 Basic Probability Terms
 - 3.2 Types of Probability
 - 3.2.1 Classical Probability
 - 3.2.2 Relative Frequency Approach (Empirical) Probability
 - 3.2.3 Subjective Probability
 - 3.3 Basic Probability Laws
 - 3.3.1 Additive Law
 - 3.3.2 Multiplicative Rule of Probability
 - 3.3.3 Compound Events
 - 3.3.4 Conditional Probability
 - 3.3.5 Bayes' Theorem
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

The concept of probability suggests the likelihood of an event to occur. It is obvious that in life we make predictions based on chances of it becoming true. Sometimes our predictions become true and sometimes they don't turn out the way we hope they would. We sometimes win and sometimes lose. Anytime we win we score ourselves one (1) and anytime we lose we score ourselves zero (0). In all experiments, we employ the use of probability to predict the occurrence of certain events.

For example we can predict that a dam will kid a male kid or a female kid. If the dam eventually kids a male kid we will score ourselves one (1) but if eventually she kid a female kid we score ourselves zero (0).

In this unit therefore, you will learn the basic probability types and laws as well as its applicability in research.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- define probability and the various probability types
- explain and compute the various probability laws
- distinguish between empirical, classical and subjective probabilities
- state and apply the basic probability laws to the different aspects of their experiments.

3.0 MAIN CONTENT

3.1 Probability

Probability is the measure of possibility that an event will occur. If it is certain that an event will occur, we say that its probability is 1.0, meaning that the probability of an event occurring is not certain. In most cases of experiments we assume that certain things will happen. If for instance we predicted that treatment A of a particular experiment will give a better performance than treatment B when subjected to the same chance, that can be expressed as the probability that A will be better than B, or that probability that B would be better than A. With this we can say that the probability that A will be better than B = 0.5 and the probability that B will be better than A = 0.5. Therefore probability of an outcome is the ratio of times the event will occur to the total number of all the observation.

$$P(\text{probability of outcome}) = \frac{m}{n}$$

Where m = number of times that an event will occur
n = total number of occurrence.

3.1.1 Basic Probability Terms

- 1) Experiment: Activity that produce an event. Or a process by which a measurement or observation can be obtained.
- 2) Outcomes: Is the resulting observation or measurement when an experiment has taken place. Simply put, is the result of an experiment.
- 3) Events: Is one or more of the possibilities outcome of an experiment.
- 4) Random Experiment: Is an operation that has an outcome that depends solely on chance.

- 5) Independent Event: Is the occurrence of an event in which the outcome of any one of the events does not affect the outcome of the other.
- 6) Mutually Exclusive Events: One or more events are said to be exclusive (or disjoint) when they have nothing in common.

3.2 Types of Probability

There are basically three types of probability;

1. Classical or Theoretical Probability
2. Relative frequency approach Probability
3. Subjective Probability

3.2.1 Classical Probability

This refers to situation when the possible outcome of an event is known and the probability of the event can be calculated without performing any experiment. This type of probability uses information that is not about physical situation.

Suppose an event (E) can happen in 10 possible ways out of 10 possibly equally likely ways, then the probability of the event occurring is denoted by.

$$P_E = \frac{\text{Number of possible outcomes in which event E occurs}}{\text{Total number of possible outcomes in the sample}}$$

$$Pr[E] = \frac{h}{n}$$

The probability of no occurrence of the event (failure) is denoted by

$$q = Pr(\text{not E})$$

$q = 1 - Pr(E)$ thus

$$p + q = 1$$

3.2.2 Relative Frequency Approach (Empirical) Probability

The empirical probability as the name suggests is based on observation. The empirical probability of an event is the relative frequency distribution based upon observation. In this type of probability, we don't have enough information about the ferial outcome of the event but we rather depend on experiments to determine it.

Here, the estimated probability of an event is taken as a relative frequency of occurrence of events when the number of observations is large. In an empirical approach of estimation, experiments are performed severally to obtain a result that can serve as a guide to what will happen with the occurrence.

Empirical Probability

$$= \frac{\text{Number of times event occurs}}{\text{Total number of times experiment is performed}}$$

For example, if 1000 tosses of a coin result to 600 heads, the relative frequency of the head is

$$\frac{600}{1000} = 0.6$$

If another 1000 tosses will give 300 heads the relative frequency will be

$$\frac{600+300}{2000} = \frac{900}{2000} = 0.45$$

3.2.3 Subjective Probability

This occurs in cases where the possible outcome is not known and it is important to experiment. In such cases the probability will be based on subject assessment or experience.

3.3 Basic Probability Laws

3.3.1 Additive Law

It is also known as “either” “or” rule. The probability that either A or B will occur mutually is the sum of their independent probabilities.

For example, two coins are tossed. The probability of either two heads or tails will occur is

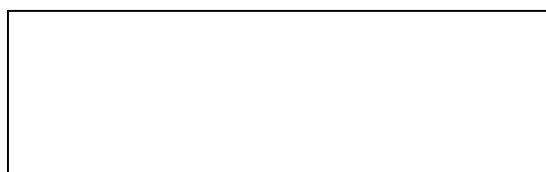
$$P(TT + HH)$$

Therefore, $P(A + B) = P(A) + P(B) - P(AB)$ for joint occurrence of events A and B at the same time

For mutually exclusive events, A and B;

$$P(A \cup B) = P(A) + P(B) - P(A \cap B)$$

This can further be explained by use of venn diagram



Example

What is the probability that in a mendelian cross between goats that are heterozygote for color Bb will produce offspring having heterozygote?

Solution

There are two ways in which a zygote may be produced

1. The dominant zygote allele (B) may be in the egg and the recessive (b) in the sperm or
2. The dominant allele may be in the sperm and the recessive in the egg.

Therefore the probability that the offspring will be heterozygous is the sum of the probabilities of those possible ways.

- a. The probability that the dominant allele will be in the egg with recessive in the sperm is;

$$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$$

- b. The probability that the dominant allele will be in the sperm and the recessive in the egg is

$$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$$

Therefore the probability that heterozygous will be produced is

$$\frac{1}{4} \times \frac{1}{4} = \frac{2}{4} = \frac{1}{2}$$

3.3.2 Multiplicative Rule of Probability

The multiplicative rule suggests that for event A or B that are independent. Their probability that they will both occur is equal to the product of their individual probabilities.

$$P(A \text{ and } B) = P(A \cap B) = P(A) \times P(B)$$

This is also referred to as the “Both” “And” rule.

Permutation

It is the number of ways the individual elements of interest are arranged in order. Those elements appear once. $n!$ Denotes the product of all number of elements ranging from 1⁻ⁿ

$$P_n = n! = (1, 2, 3 \dots n)$$

Example; $7! = 7 \times 6 \times 5 \times 4 \times 3 \times 2 \times 1 = 5040$

If we want to select only few objects in n group then permutation will be the best for it.

$${}^nPr = \frac{n!}{(n-r)!}$$

Example; if we have 10 cows on our farm, how many different ways can 5 cows be selected from the farm.

Solution: Using

$${}^nPr = \frac{n!}{(n-r)!}$$

Our n = 10 cows

$$\begin{aligned}
 {}^{10}P_5 &= \frac{10!}{(10-5)!} = \frac{10 \times 9 \times 8 \times 7 \times 6 \times 5 \times 4 \times 3 \times 2 \times 1}{5 \times 4 \times 3 \times 2 \times 1} \\
 &= \frac{3,628,800}{120} = 30,240
 \end{aligned}$$

r = 5 possible ways hence

Combination

Just like the permutation, combination involves the number of ways individual members of the n group are arranged but in combination. The order by which they are arranged is not important/ considered. ${}^nC_r = \frac{n!}{(n-r)!r!}$

That is the combination of n taken at a time

Example; If 7 cows are taken from a herd of 20, how many cows combination exists.

$${}^{20}C_7 = \frac{20!}{(20-7)!7!} = \frac{20 \times 19 \times 18 \dots 1}{13 \times 7 \times 6 \times 5 \dots 1}$$

3.3.3 Compound Events

These are events that have more than one event. Assuming A and B occur at the same time. A and B could be said to have an intersection of the event(A ∩ B)

Just like the addition rule, an instance where “either” A “or” B occur is called union of events (A ∪ B)

The probability of A ∩ B = P(A ∩ B)

The probability of A ∪ B = P(A ∪ B)

The probability of an event that A does not occur (A complement) denoted by A^c is

$$P(A^c) = 1 - P(A)$$

Example

Let the event B be such that the result of a throw of die is an even number, let event A be such that the number is less than 4

$$B = [2, 4, 6] \text{ find } B \cap A$$

$$A = [1, 2, 3] \text{ find } P(A \cap B)$$

Solution

$$B \cap A = 2$$

$$P(A \cap B) = P(A) + P(B) = 1/6$$

3.3.4 Conditional Probability

Is the probability of an event B happening when one have a prior knowledge of the probability that some other event A has occurred conditional or statistical dependence exist when the probability of an event is dependent upon the occurrence of some other event.

$$P\left(\frac{B}{A}\right) = \frac{P(A \cap B)}{P(A)}$$

For independent probability, the occurrence of event A is not affected by the occurrence of B. Therefore the probability that both occur is the product of their individual probability

$$P(A \cap B) = P(A) P(B)$$

But for dependent event, the probability of B occurring is affected by the occurrence of A

$$P\left(\frac{B}{A}\right) = \frac{P(A \cap B)}{P(A)}$$

Example of independent and dependent probability

1. If we throw a coin and A and B are the possible occurrences, then

$$P(A \cap B) = P(A)P(B) = 1/2 \times 1/2 = 1/4$$

2. For dependent probability: if in a pen of 40 rabbits, divided into 4 colors (black 10, grey 10, white 10, and brown 10) if the first draw is black and the second is black again, then $P(A \cap B)$.

$$P(A \cap B) = P(A)P\left(\frac{B}{A}\right)$$

= If black is drawn and the second draw is black.

$$= \left(\frac{10}{40}\right)\left(\frac{9}{39}\right) = \frac{90}{1560}$$

The probability of drawing two black rabbits = $\frac{90}{1560}$

3.3.5 Bayes' Theorem

The theorem is useful in evaluating the probability of an event A with provided information on the probability of another event B that had occurred after the occurrence of A.

$$P\left(\frac{B}{A}\right) = \frac{P(B) P(A/B)}{(P(B)P\left(\frac{A}{B}\right) + (P(B)P(A/B))}$$

P(B) = probability of event B compliment

$$P\left(\frac{A}{B}\right)$$

= probability of event A having known that the complement B has occurred

Example

If two strains of cock A and B are used in a flock of chickens, if cock A is used, 80% of the hens and cock B, 20% of the hens. We assume that recorded fertility of eggs fertilized by cock A and B are 45% and 95% respectively. For a certain egg, the information about the cock is missing, what is the probability that the cock has fertilized the egg is cock B.

$P(A_1) = 0.8$ is the probability of using cock A

$P(B_1) = 0.2$ is the probability of using cock B

E = The event that an egg was layed from a cock

$$P\left(\frac{E}{A_1}\right)$$

= 0.45 The probability of successful making or fertility of cock A

$$P\left(\frac{E}{B}\right)$$

= 0.95 The probability of a successful making or fertility of cock B

Therefore the probability that the egg is fertilized by the cock is

B

$$\begin{aligned}
 P\left(B_2/E\right) &= \frac{P(B \cap E)}{P(E)} = \frac{P(B) P(E/B)}{P(A)P(E/A_1) + P(B) P(E/B)} \\
 &= \frac{(0.2)(0.95)}{(0.8)(0.45) + (0.2)(0.45)} = \frac{0.19}{1.25 + 0.09} = 0.142
 \end{aligned}$$

The probability that the cock that fertilize the egg is cock B = 0.142

SELF-ASSESSMENT EXERCISE

Name the types of probability.

4.0 CONCLUSION

Looking at how probabilities help us in predicting the outcome of some events even before they happen. You must have seen how we can apply probability to most of our day to day lives and our experiment to help us predict the outcome of some events.

5.0 SUMMARY

In this unit, you learnt the meaning of probability, the basic terms used in probability, the various types of probability as well as various probability rules. These are tools we use for predicting the outcome of an occurrence so that we will prepare for them.

6.0 TUTOR- MARKED ASSIGNMENT

1. Define the term probability
2. Discuss the types of probability
3. Differentiate between dependent and independent probability
4. State the Baye's theorem and its application in animal production
5. Differentiate between permutation and combination
6. If in a herd of 400 cows, 50 females were drawn. What is the probability that the cow is lactating. Assuming we have 75 lactating cows in the herd?

7.0 REFERENCES /FURTHER READING

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UNIT 5 STATISTICAL TEST OF HYPOTHESIS

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Hypothesis Test of a Population Mean
 - 3.1.1 Hypothesis
 - 3.1.2 Error in Hypothesis Testing
 - 3.2 Z-Test of Significance
 - 3.3 Hypothesis Testing for Sample (T-Test)
 - 3.4 One Sided and Two Sided Test
 - 3.5 F-Test
 - 3.6 Chi-Square Distribution (X^2)
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

In the previous unit we learnt the various probability laws and how it can be applied to animal production researches. In this unit, you will learn the various tests of hypothesis that experiments are subjected to, to determine whether to accept or reject the null hypothesis. You will be introduced to different tests including the

1. Z and t tests
2. χ^2 Distribution test

2.0 OBJECTIVES

At the end of this unit, students should be able to

- define hypothesis and the types of hypothesis used in animal researches
- name the various tests of hypothesis and explain how each one works
- practically solve some problems in researches using the various test.

3.0 MAIN CONTENT

3.1 Hypothesis Test of a Population Mean

From unit one, we defined sample as a subset of a population. The main reason we test for hypothesis is to enable us judge whether our sample mean is different from the population mean. Remember that population consists of all researchable items the researcher wants to study. Therefore we can have the population of cattle in Nigeria or the population of sheep, goat, poultry, and so on and so forth. This could be countable figure or uncountable. Be reminded that sample is the proportion of the population that truly represents the population and can be used to make inference about the population. Since we say population can be uncountable or too large to measure, they are determined by parameters which are estimated from a sample.

Attention should therefore be given to how sample are selected so that it does not affect inference on the population. What inferential statistics does in general is to estimate population parameters when the size is too large to be measured. A rule that describes how to calculate a simple estimate using observations from a sample is called a rule point estimator. For a normally distributed population the sample mean is approximately normal with a mean μ and standard deviation from which the estimate is made.

3.1.1 Hypothesis

Hypotheses are postulated assumptions on the nature of population. They are just statements that could be true or not about the population. For instance we can postulate that the population of sheep in Nigeria is 30,000. This could just be an assumption or a record that is obtained from previous or present reports or journals.

Two types of hypothesis are

1. Research Hypothesis
2. Statistical Hypothesis

The former is the hypothesis postulated the researchers before starting the research. These are postulated on the bases of previous studies, investigation and sometimes experience. While the latter follows the researcher's hypothesis that proves or disproves the hypothesis.

The statistical hypothesis comprises of the null hypothesis and the alternating hypothesis.

The null hypothesis suggests no difference between some observations and is denoted by H_0 .

The alternative hypothesis suggests the opposite that suggest a difference between observations denoted by H_a or H_1 . In all researches or experiments, the statistical alternative (H_1) is the expected outcome that the researcher hopes to observe at the end of the experiment.

In testing statistical hypothesis, a level of confidence or significance is needed to enable the researcher make a conclusion on whether to accept or reject the null hypothesis, then we can conclude that the alternative hypothesis is true; otherwise the null hypothesis becomes true.

A researcher can choose to test the effect of temperature on the fertility of say rabbit. The null hypothesis will suggests that temperature has no effect on the fertility of rabbit, while the alternative hypothesis H_a will contradict it by suggesting that temperature affects the fertility in rabbit. The null and alternative hypothesis is always opposite.

3.1.2 Error in Hypothesis Testing

There are two types of error that a researcher may likely make in the course of testing the hypothesis.

I. Type One Error

Type one error is committed when a null hypothesis is rejected when it is true or is supposed to be accepted.

II. Type Two Error

Type two errors are committed when the null hypothesis is accepted, when it is supposed to be rejected.

To eliminate type one error,

- a. Use a high level of significance (at 5% or 1 %.)
- b. Choose a stronger test.

To eliminate type two error

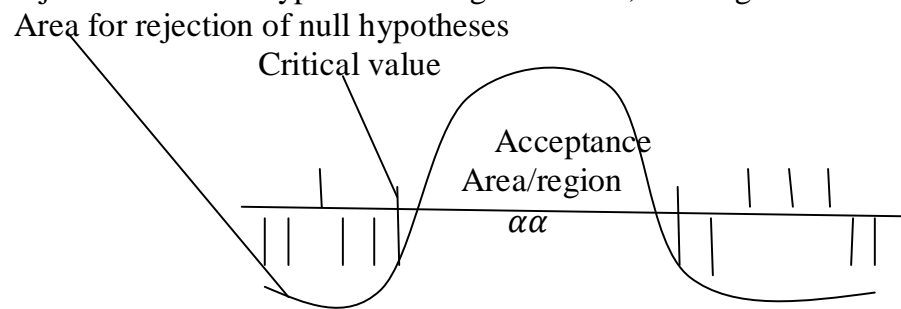
- a. Chose a stronger test.
- b. Use more efficient design of an experiment.

Level of Significance

It is the probability with which we are willing to risk a type one error. It is denoted by α and it is specified before the samples are even drawn.

When a test is chosen, if the calculated value gotten is higher than the value of F read on the table, the null hypothesis is rejected, if the opposite null is accepted. 1% level of level of significance means that we are 99% confident the hypothesis is true and 1% confident the hypothesis is false, at 5% level of significance. We are 95% confident that the hypothesis is true and 5% that it is false.

In most animal science design/researches, the 5% or 1% level of significance is adopted. To illustrate the area that permits the acceptance of rejection of a null hypothesis at a given value, the diagram is drawn below.



Procedures for the testing of Hypothesis

To test the hypothesis, the following procedures have to be followed.

1. Set up hypothesis (H_0 and H_a)
2. Determine the level of significance at which to test the hypothesis.
3. Test the hypothesis
4. Draw a conclusion

3.2 Z-Test of Significance

Z-test is denoted by the formula

$$Z = \frac{y - \mu}{\sigma / \sqrt{n}}$$

Where y = sample mean, μ = Population mean, σ / \sqrt{n} = Standard error

Z. Test can either be one tailed or two tailed. One tailed test, the α valued is not divided by 2 because it is not normally distributed and is denoted by

$$H_0: \mu \leq \mu_0$$

$$H_A: \mu > \mu_0$$

Two tailed is a normally distributed population. Therefore α is divided by two ($Z\alpha/2$) to get the critical value.

$$H_0: \mu = \mu_0$$

$$H_A \neq \mu_0$$

Supposing an n number of samples with mean \bar{Y} . The population of animals with μ with means μ (which is assumed to be known and standard deviation is known.) The question will be whether the sample mean \bar{Y} is necessarily different from the population mean μ , at α level of significance.
 Example

A random sample of 64 goats is taken from a population of goats in Zaria and their average weight is 14.0kg. Assuming the population standard deviation is 2.5 kg, does the hypothesis belong to the population of goat in Zaria with the mean 16kg?

Solution

Step 1: Set up the hypothesis

$$H_0 : \mu = 16\text{kg Or } 14\text{kg} = 16\text{kg}$$

$$H_1 : \mu \neq 16\text{kg } 14\text{kg} \neq 16\text{kg}$$

Step 2: The level of significance to use will be $\alpha = 0.05$ (95%)

Step 3: Calculate/Test the hypothesis

Our known values are $n = 64$ $n = \text{Sample Size}$

$\bar{Y} = 14\text{kg}$ $\bar{Y} = \text{Sample Mean}$

$\mu_0 = 16\text{kg}$ $\mu_0 = \text{Population Mean}$

$\alpha = 0.05$ $\alpha = \text{Level of Significance}$

$\sigma = 2.5\sigma = \text{standard deviation}$

$$Z = \frac{y - \mu}{\sigma / \sqrt{n}}$$

Therefore:

$$Z = \frac{14 - 16}{2.5 / \sqrt{64}} = \frac{-2}{0.198} = -10.1$$

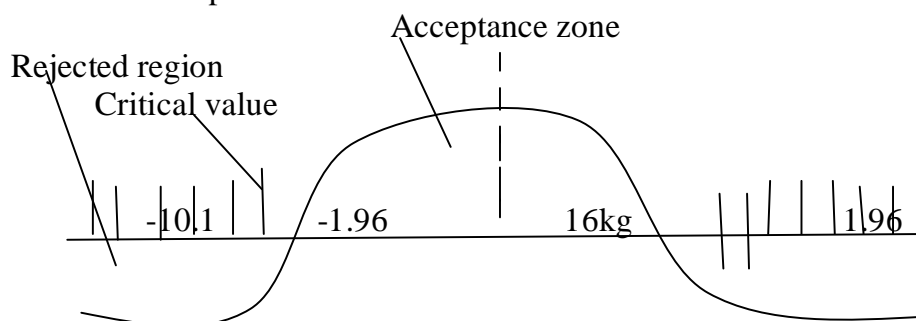
Our $|Z| = -10.1$

Since it is a tailed test, we will divide the α by Z such that

$$Z_{\alpha/Z} = Z_{0.05/2} = Z_{0.025}$$

Which corresponds to 1.96 on Z table $|Z| = -10.1$, Z critical = ± 1.96

Check the critical zone using the normal distribution table to check if 10.1 fall in the acceptable area of H_0 .



The table value at 5% level of significance = 1.96

Decision: As the value $|Z|$ is 10.1, falls within the rejection zone, (which is more than Z critical) 1.96 we will reject H_0 and conclude that the probability is less than 0.05 that the sample belong to the population mean of 16kg with a standard deviation of 2.5kg.

3.3 Hypothesis Testing for Sample (T-Test)

For t-test, the only difference with z-test is that z-test requires a large number of samples ($n > 30$). For sample size ($n \leq 30$), drawn from a normal population, the test to use is t-test. For t-distribution, the shape of the distribution depends on the degree of freedom which $n-1$ where $n =$ sample size.

Example1. A random sample of 24 broiler chickens of 4 weeks were with the average weight of 2.6kg is chosen. Does this belong to the population of broilers with the mean 1.8kg and standard deviation of 0.9kg?

Solution

$$\bar{Y} = 2.6\text{kg}, \mu = 1.8\text{kg}, n = 24, S = 0.9\text{kg}$$

Step 1

Set up the hypothesis

$$H_0 : U = 1.8\text{kg}$$

$$H : U = 1.8\text{k}$$

Step 2

Calculate the t-statistics

$$t = \frac{\bar{Y} - \mu_0}{\frac{s}{\sqrt{n}}} = \frac{2.6 - 1.8}{\frac{0.9}{\sqrt{24}}} = \frac{0.8}{0.18} = 4.44$$

Since it is a two tailed test, we will divide the $\alpha/2$ our $\alpha = 0.05$, $t_{\alpha/2} = 0.025$

Our degree of freedom $df = n-1$, $n=24$

Therefore $df = 24 - 1 = 23$.

We will check the table at $\alpha = 0.025$ under the degree of freedom 23

Since our (t) (4.44) is greater than the t-critical 2.069, we will reject the null hypothesis and accept the alternative hypothesis.

3.4 One Sided and Two Sided Test

One sided or one tailed test or hypothesis has only one critical value at which hypothesis is to be tested. It is either right or the left and it uses terms like less than or greater than.

$$H_0 : U \leq U_0$$

$$H_i : U > U_0$$

H_a : U is rejected when $|t|$ or $|z| > t_{\infty}$ depending on whether it is skewed to the right or left.

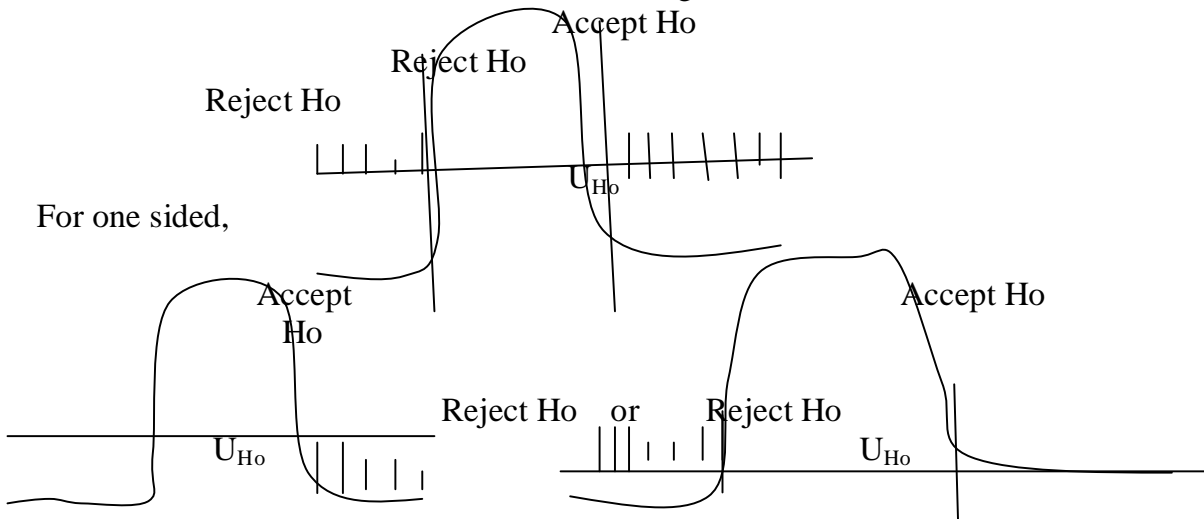
For two tests as seen above, the point is that whether the sample mean \bar{Y} is different from some value μ_0 .

$$H_0 : U = U_0$$

$$H_a : U \neq U_0$$

Such that $H_0 : U = U_0$ is rejected when $|z|$ or $|t| > Z_{\infty/2}$ or $t_{\infty/z}$

One side test that can be illustrated with the diagram



For one tailed t and z, the same calculation is applied except x is not divided by 2.

T- Test of Significance of Two Means

It is used to compare two treatments to see if they are significantly alike or not. There are two types

1. Paired T-test
2. Independent T-test

1. Paired T-test: It is calculated where one object is subjected to two treatments. It test for significance of paired observations occurring to a common linking factors that are treated alike.
2. Independent T-test: It is calculated in a situation where object is subjected to one treatment.

$$T = \frac{\text{Difference between means}}{\text{Standard Error}}$$

For paired T-test $\bar{X}_1 - \bar{X}_2$

SE

For a given sets of data, calculate \bar{X}_1 and \bar{X}_2 ; Find $S_1 = S_2$ and $X_1^2 = (S_1 - S_2)^2$;

$$SE = \sqrt{\frac{Ex_1^2 - (Ex_1)^2/n}{n(n-1)}}$$

$$t = \frac{x_1 - x_2}{\sqrt{\frac{Ex_1 - (Ex_1)^2/n}{n(n-1)}}}$$

Example: Two types of feed A and B were given to beef cows and the following results were computed

Feed A	Feed B
n = 6	n = 6
x = 8	X = 7
X ₁ = 6	X ₁ = 6
X ₂ = 10	X ₂ = 10

Test whether there is any difference on daily weight gain of cows fed these types of feed A and B

$$t = \frac{8 - 7}{\sqrt{\frac{10 - 6^2/6}{6(6-1)}}} = \frac{1}{\sqrt{\frac{4}{30}}} = \frac{1}{0.365} = 2.74$$

Degree of freedom since they have same sample size;

$$df = k - 1 = 6 - 1 = 5 = t_{\infty}/2 = t_{0.025}$$

Check the degree t 0.05 under the degree of freedom 5

t_{0.025}
df

5	2.571
---	-------

The calculated value /t/ is 2.74 is greater than /t critical/ = 2.571 and difference is 5. Therefore feed A is significantly different than (higher) than feed B. therefore feed A resulted to higher body weight gain than feed B.

2. Independence Test

$$t = \frac{\text{differences between mean}}{\text{SE of difference between mean}}$$

SE of difference between two means

$$\sqrt{SE_1^2 + SE_2^2}$$

$$SE_1^2 = \frac{Ex_1^2 - (Exi)^2/n}{n(n-1)}$$

$$SE_2^2 = Ex^2 - (Exi)^2/n$$

Using our data above

$$SE_1^2 = \frac{1018 - 1014}{30} = \frac{4}{30}; SE_2^2 = \frac{868 - 864}{30} = \frac{4}{30}$$

$$SE \text{ of difference between means} = \sqrt{\frac{4}{30} + \frac{4}{30}} = 0.516$$

$$T \text{ independence} = \frac{8-7}{0.516} = \frac{1}{0.516} = 1.936$$

$$\text{Degree of freedom} = (m_1 - 1) + (m_2 - 1) = (6 - 1) + (6 - 1) = 10$$

$$t_{\alpha} = t_{0.05} = 2.228$$

t is less than t_{α} ($0.516 < 2.228$) at $\alpha = 0.05$

Under t-paired test the effect between feed A and B was significantly different but in t independence test. The effect is not significantly different. This shows that a proper choice of test to use in a specific condition is important to avoid wrong conclusion.

Example

Same animals different feed = Paired t-test

Different animals, different feed = Independent –t test

3.5 F-Test

The F-distribution/test gives the ratio of the mean of treatment over the mean square of error

$$F = \frac{Ms \text{ treatment}}{Ms \text{ error}} = \frac{\text{Means of square treatment}}{\text{Mean of square error}}$$

The F-test is used to test the effects of treatments. It tests the effect of groups of mean where randomly selected from the same population or whether treatments which have affected each group separately have resulted in shifting the mean sufficiently to the point where they are considered to come from different population. The use of analysis of variance in calculating total variance helps in easing F-test calculation.

The analysis of variance is divided into four columns;

1. Source of variation 2. Degree of freedom, 3. Sum of squares, 4. Mean squares

The different steps used in calculating the F-test are as follows;

Step 1

Compute the correction factor (CF) which is the summation of all the individual values under observation divide by the number of observation;

$$CF = \frac{(\sum xi)^2}{N}$$

Step 2

Compute the crude sum of squares; square individual value under observation then minus the C.F;

$$CSS = \sum x^2 - CF$$

Step 3

Compute the treatment sum of square = sum of squared treatment divided by number of replications per treatment minus the correction factor;

$$TSS = \frac{\sum T^2}{r} - CF$$

Step 4

Compute the replication sum of squares; sum of squared replication divided by the number of treatment – CF

Step 5

Sum of square of error = total sum of squares – Treatment sum of squares – Replication sum of squares

$$SSE = CSS - TSS - RSS$$

Step 6

Compute the mean squares for each of the source of variation as follows

$$\begin{aligned} \text{Rep Ms} &= \frac{\text{Rep SS}}{r - 1} \\ \text{Trt Ms} &= \frac{\text{Trt SS}}{t - 1} \\ \text{Ems} &= \frac{\text{error SS}}{(t - 1)(r - 1)} \end{aligned}$$

Step 7

Compute f-test for testing treatment value as

$$F = \frac{\text{Trt Ms}}{\text{Error Ms}}$$

Step 8

Compare the computed value in the tabulated value

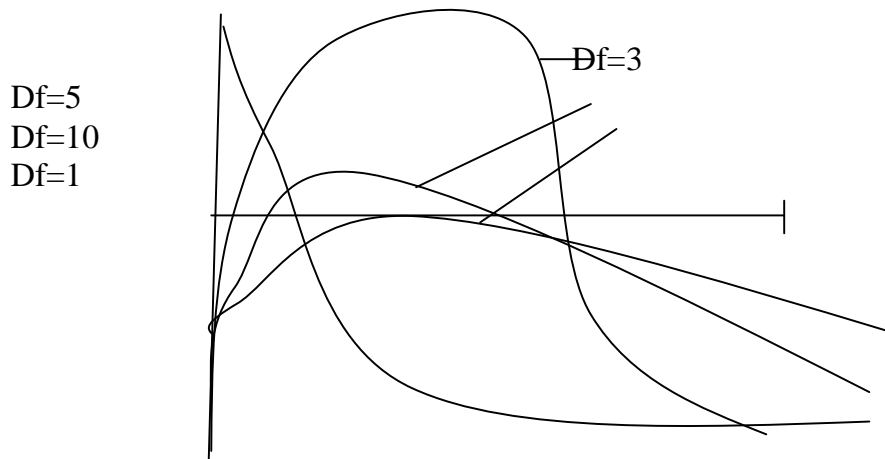
f1 = Treatment difference

f2 = error df

Example of an F- test is seen in unit 4 of module 1.

3.6 Chi-Square Distribution (X^2)

Chi-square distribution is used to perform hypothesis testing on qualitative data (nominal or ordinal). Chi-square test compares the statistical difference between the observed (O) and the expected (E) frequencies. The null hypothesis in chi-square suggests that the observed and expected frequencies are the same. While the alternative hypothesis with state the opposite. It assumes that samples are drawn randomly and each drawn sample is independent on each other. Chi-square is always skewed to the right and it depends on degree of freedom for its operation. The higher the degree of freedom the normally distributed the chi-square is



Characteristics of Chi-Square

1. It is always skewed to the right (has no negative value)
2. It is non-symmetrical
3. Its parameter depends on degree of freedom (n-1)

Uses of Chi-Square

1. Goodness of fit list
2. Test of independence using frequency table
3. Display of contingency

Example

Supposing you cross a pure breeding black pig with a pure white pig and all the progeny were black. Then you cross the offspring of the first mating and knowing Mendelian ratios, then you predicted 3:1 ratio of black to white will occur. But after the cross was born, you find 156 piglets in all with 100 black and 56 white.

You will expect 117 black and 39 white based on 3:1 Mendelian ratio. That is;

$$3/4 \times 156 = 117$$

$$1/4 \times 156 = 39$$

From this cross, what you observed is different from what is expected but are these differences statistically different? The statistical test to use is chi-square;

$$\begin{aligned} \chi^2 &= \sum \frac{(\text{observed} - \text{expected})^2}{\text{Expected}} \\ \chi^2 &= \sum \frac{(101 - 117)^2}{117} + \frac{(36 - 39)^2}{39} \\ &= \frac{(-17)^2}{117} + \left(\frac{17}{39}\right)^2 = \frac{289}{117} + \frac{289}{39} \\ &= 2.47 + 7.4 \\ \chi^2 &= 9.87 \end{aligned}$$

To determine whether to accept or reject the hypothesis, we compute the degree of freedom $V=K-1$. For example, $n=2$, $DF=2-1=1$, our $\alpha = 0.05$. Check $\chi^2_{.050}$ at 1 df

		$\chi^2_{.050}$	
df	1	3.841	

Since $\chi^2 (9.87)$ is greater than $\chi^2_{cm} (3.841)$ we conclude that the difference in the observed and the expected is significantly different.

SELF-ASSESSMENT EXERCISE

Name three statistical tools you know.

4.0 CONCLUSION

Looking at how hypothesis testing is paramount in making a valid conclusion on whether to accept or reject a hypothesis. Attention need to be given to the choice of test statistics to use so as to avoid making a wrong conclusion.

5.0 SUMMARY

In this unit, you learnt what hypothesis testing is, the type of error in statistics and how to avoid them and the tests of hypothesis using different statistical tests tools like Z and t-test, F distribution test and chi-square distribution test.

6.0 TUTOR- MARKED ASSIGNMENT

1. Differentiate between research hypothesis and statistical hypothesis
2. Name and state the two types of error in hypothesis testing
3. State the condition for using either t-test or z-test
4. Given a sample of 50 rabbits with the average weight of 10kg. Does this belong to the population with the population mean of 8kg and statistical deviation 1.5kg?
 - a. Name the test statistics to use
 - b. Test for significance
5. In a population of 400 cows, 70 are polled white horned. Assuming 295 were observed to be polled and the rest horned. Compute X^2 for this to check for significance.

7.0 REFERENCES / FURTHER READING

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MODULE 3

Unit 1	Principle of Volumetric Data Analysis
Unit 2	Gravimetric Data Analysis
Unit 3	Thermometric Data Analysis
Unit 4	Electrochemical Data Analysis
Unit 5	Optical Method of Chemical Analysis

UNIT 1 PRINCIPLE OF VOLUMETRIC ANALYSIS

CONTENTS

1.0	Introduction
2.0	Objectives
3.0	Main Content
3.1	Basic Concept of Volumetric Analysis
3.1.1	Basic Terms in Volumetric Analysis
3.1.2	Methods of Determining End Point in Titration
3.2	Requirements for Volumetric Treatment of Sample
3.3	Forms of Volumetric/Titrimetric Analysis
4.0	Conclusion
5.0	Summary
6.0	Tutor-Marked Assignment
7.0	References/Further Reading

1.0 INTRODUCTION

Volumetric analysis is one of the core chemical methods of analysis employed in animal production. It is defined as the quantitative analysis of an unknown chemical solution by determining the quantity of reagent of unknown volume of a solution. This involves reactions between two substances in a given solution (titrate and the analyte). Therefore, in this unit, we will discuss the general principle of volumetric analysis and various types of volumetric filtration and their uses in animal production.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain the concept of volumetric analysis
- list the requirement for volumetric treatment of sample
- list and explain the forms of volumetric analysis.

3.0 MAIN CONTENT

3.1 Basic Concept of Volumetric Analysis

Volumetric analysis, also known as titrimetry reaction deals with reaction between the volume of a reagent and that of the analyte (sample). It measures the volume of a reagent reacting stoichiometrically with the analyte of which an indicator is used to determine the end product or actual volume of the analyte. This is done by a continuous addition of the titrant of a known concentration to analyse up to the level of change in colour that is observed as an indicator of the completion of the reaction.

3.1.1 Basic Terms in Volumetric Analysis

1. Titration: The process of determining the volume of a reaction that as a reagent is added to a solution to give an expected outcome.
2. Standard Solution: A reagent of known concentration
3. Indicator: A substance that change colour at the end of the consumption of analyte near the equivalence point of known concentration
4. Equivalent Point: Is the point at which a stoichiometric titrant is added with the analyte or the titrant.
5. End Point: The point at which the reaction is observed to be completed.
6. Titration Error: The difference between the equivalent point and the end point of titration.
7. Concentration: is a determined quantity of a substance in a defined volume of solution.

3.1.2 Methods of Determining End Point in Titration

1. The use of PH indicator which change in colour as a result of change chemical property of an analyte during an acid-base reaction.
2. Colour change which occur in some reactions without necessarily adding any indicator. This is common in redox reaction.
3. Precipitation: This happens at the end of certain reaction when an ion is precipitated as an indication of a completion of the reaction.
4. Potentiometer: This involves the test for a change in the working electrode at the end of the reaction.

3.2 Requirements for Volumetric Treatment of Sample

The basic conditions or requirement for an efficient titration are:

1. The reaction should relatively be fast
2. At the end of the reaction, there should be a clear change on the physical or chemical features of the solution.
3. The reaction should be conducted in an controlled environment to prevent unnecessary interference.
4. The indicator to be used must be carefully chosen and must be available at the point of analysis.
5. The end point should be reproducible and have a large equilibrium constant at the end of the reaction.

3.3 Forms of Volumetric/Titrimetric Analysis

There are basically three types of volumetric analysis or method. Each method is based on the type of reaction included. These are:

1. **Acid Base Titration**
This type of titration involves the use of acid or a base to determine the concentration of the reaction by neutralisation. This is carried out by acid or base of a known concentration. In most laboratory analysis, acid and base titration is used to determine the acidity or alkalinity of solutions using a particular indicator. Such indicator could be a litmus paper which serves as visual indication that shows red colour in an acidic medium and blue colour in a basic medium. Acid base titration is applicable in samples that have acid or basic contents and analysis that involve the use of kjeldahl digestion or analysis like the determination of nitrogen in milk.
2. **Precipitation Titration**
This kind of titration is based on reactions that yield ionic compounds in form of insoluble salt at the end of the reaction. The production ion makes it a little hard to determine the end point with high level of precision. Precipitation titration is commonly used in the determination of chlorine bromide and cyanate in a sample. In most cases, the reagent used is silver nitrate or sometimes potassium chromate.
3. **Redox Titration**
This is a titration method based on oxidation and reduction between the filtrant and analyte. The principle of redox reduction is based on

reduction or oxidation of electron from substance to another which is determined by calorimetric or potentiometric end point. Redox reaction employs the use of oxidising or reducing agent. The former can be a specie that removes electron from another specie or a chemical that transfer electronegative atom (oxygen) while the later act as an electron donor by losing some electrons. Example of the reaction is the reaction of glucose with oxygen in an aerobic condition and the reduction of iodine to iodide.

4.0 CONCLUSION

Volumetric analysis often referred as titration is the method of chemical analysis in which substance amount is determined by analysing the volume that the substance occupies by employed the different specific known titration like acid base, redox and precipitation titrated

5.0 SUMMARY

Volumetric analysis deals with reaction between the volume of reagent and that of the analyte and that the most common types of volumetric analysis are acid-base titration, precipitation titration, and redox titration. We also learnt the various methods of determining end point of volumetric analysis and some conditions of volumetric analysis.

6.0 TUTOR- MARKED ASSIGNMENT

1. Define volumetric analysis
2. List and explain the various forms of volumetric analysis
3. List the condition necessary for titration
4. List the method of determining end point in volumetric analysis
5. List five basic terms in volumetric analysis

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UNIT 2 GRAVIMETRIC DATA ANALYSIS

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Basic Concept of Volumetric Analysis
 - 3.1.1 Basic Terms in Volumetric Analysis
 - 3.1.2 Methods of Determining End Point in Titration
 - 3.2 Requirements for Volumetric Treatment of Sample
 - 3.3 Forms of Volumetric/Titrimetric Analysis
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

Another method of chemical analysis is gravimetric analysis which describes a set of methods that make use of quantitative procedures to measure the weight of an unknown quantity of an analyte in a mixture. This takes into consideration many treatments that employ processes like heating and drying that will help in evaluating a particular substance of interest. Gravimetric analysis is one of the chemical methods that guarantee precision and accuracy of measurement even though they are manually determined and laborious.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- define gravimetric analysis
- list out the types of gravimetric analysis
- give the advantages and disadvantages of gravimetric analysis
- explain the general procedure for gravimetric analysis.

3.0 MAIN CONTENT

3.1 The Concept of Gravimetric Analysis

Gravimetric analysis is defined as one of the methods of analytical chemistry that measures the mass of a solid in a mixture by calculating the differences in weight. If for instance, one wants to know the weight of a solid that are in suspension in a liquid medium, it is easy to do that by simply filtering the solid suspension with a known weight of a filter paper and dry them together to remove the moisture that states that still stick to the solids and filter paper. Calculating the difference between the final weight of the sample and the weight of the filter gives you the actual weight of the sample (solid). In the case of aqueous ions, the analyte needs to be converted into a solid form before proceeding with the analysis; this could be done by applying enough positive potential between the pair of electrodes in the sample.

An example of application of gravimetric analysis in animal production is in determination of total solids in a milk sample. The method is done by directly heating the sample and taking the difference between their weight before and after heating. Whatever result is gotten, indicates the total solid of the composition.

3.2 Types of Gravimetric Methods

There are four types of gravimetric analysis even though two are the most commonly used in animal production.

1. Precipitation gravimetric analysis
2. Electrogravimetric analysis
3. Volatilisation gravimetric analysis
4. Particulate gravimetric analysis

For the purpose of this course, we will concentrate on the two most commonly used in animal production: Precipitation gravimetric analysis and Volatilisation gravimetric analysis

3.2.1 Precipitation Gravimetric Method

In precipitation gravimetric, appropriate reagent is used to obtain an insoluble compound in a mixture. Insoluble compound collected is filtered and further treated to a point where there is observation of a constant

weight over time. The use of precipitating reagent to the solution contain say ion of interest leads to several steps before the final larger filtered crystals are obtained. These stages involve the induction, nucleation and digestion. Digestion is commonly used in animal production for heating the social and mother liquor for some period of time.

Steps in Precipitation Gravimetric Analysis

1. **Sample Preparation:** Sample collected for precipitation analysis are first of all pre-treated to fasten the rate of precipitation. For example samples with high or low solubility are pre-treated with reagents to make it into solution that favours precipitation. The P^H, volume, temperature and the concentration of the solution should be in the state that favours precipitation of the solution.

2. **Precipitation:** In precipitation, smaller particles are difficult to filter but it is the opposite with the larger particles. Therefore, the addition of precipitating agent to react with the analyte helps increase the size of the precipitate which settles fastly and readily filtered. The larger particles obtained are core prone to re-adsorption and they can be easily be washed to remove impurities. We use relative super saturation RSS to understand the condition favouring particle growth

$$RSS = \frac{Q-S}{S} \quad \text{Where} \quad \begin{array}{l} Q = \text{Concentration} \\ S = \text{solubility} \end{array}$$

Solutions with high super saturation yield smaller particles with high surface area, while low super saturation yields larger particles with low solubility.

For a successful precipitation, Q must always be kept low while S be kept high. This is achieved by production of precipitate from dilute solution and storing slowly while adding the dilute precipitating reagents. The precipitate should be obtained from a unit solution and ensure the process of precipitation is done at low P^H to discourage solubility of the precipitate.

3. **Washing of Impurities:** The next step after filtration is washing of the precipitate. The precipitate is washed to remove the co precipitate (impurities). This is done by electrolyte that is added o the washing liquid. Avoiding the use of water for washing reduces the formation of colloids (peptisation).

4. **Drying:** The precipitates gotten and washed are dried to a constant weight by heating at 110 – 120 °c for one to two hours. The time taken on heating depends on the stability of the weight of the precipitate.
5. **Weighing:** The dried sample will then be placed in a desiccator in most cases and weighed to ascertain the actual weight of the sample in question.
6. **Calculation:** This is done on a weight bases and expressed in percentage. For example, if P is our analyte of interest then

$$\% P = \frac{\text{weight of P X 100}}{\text{sample weight}}$$

3.2.1 Volatilisation Gravimetric Analysis

This type of gravimetric involves the separation of the substance of interest in a gas form using thermal or chemical decomposing agent. It can also be trapped and weighed. This is done by weighing the volatile part and then comparing with the non volatile part. With technological advancement, instrument used in thermal conductivity, infrared absorption or coulometry are used to measure the product of volatilisation. The most important thing in volatilisation gravimetric irrespective of method or instrument employed, the product of the decomposing reaction should be known. The practical application of the volatilisation gravimetric depending on the method of analysis is the determination of ash in a sample of feed or milk, where the sample is placed in a crucible and be allowed to decompose at fixed temperature in an oven or furnace. The difference between the sample mass and that of the residue is measured on a balance to the quantity or ash by trapping and weighing the volative product heated in a closed container as the gas is trapped in selective absorbent traps.

3.3 Advantages of Gravimetric Analysis

1. It is one of the most accurate and precise methods
2. It readily gives room for experimental error
3. It is a direct method that requires less standardization
4. Sample equipments are used for the method

3.4 Disadvantages of Gravimetric Analysis

1. It only analyse one or few elements at a time
2. Colloid can basically be formed if mistakes are made during the analysis
3. Labour intensive and time consuming

3.5 General Gravimetric Analysis Procedure in Animal Research

The practical procedure is seen in fibre (crude fibre) determination of a feed sample. The procedure involves:

1. Fresh samples should be subjected to addition of petroleum ether three times and store gently to extract fat in the sample. This done by string gently, allowing to settle and decent each time. The fat free material should be transferred in a beaker and 200ml of pre-heated 1.25% H₂SO₄ should be added and the material be boiled for exactly 30 minutes. Constant volume should be maintained by adding water.
2. While the heating process is going, a filter paper fitted to funnel should be pre-heated by pouring hot water into the funnel.
3. The boiled acid mixture should be filtered hot under sufficient suction so as to make it possible to filter 200ml of the mixture within 10 minutes.
4. The filtered residue should be washed several times with boiling water until it becomes neutral to litmus paper.
5. The residue should be transferred back to a beaker and 200ml of pre-heated sodium hydroxide be added and boiled for another 30 minutes and the content volume be maintained by constantly adding water.
6. After boiling, the mixture should be filtered hot through a filter crucible this time around, under gently suction.
7. It should be washed thoroughly with hot water and twice with alcohol.
8. The crucible and its content should be dried to 100% cooled and weighed.
9. Place the crucible in a muffle furnace for about 3 hours at a temperature of 500-600°C then cool in desiccators and weigh.

$$\% \text{ fiber} = \frac{\text{dry wt of residue before ashing} - \text{wt of residue after ashing}}{\text{original sample weight}}$$

SELF-ASSESSMENT EXERCISE

Give one example of the application of gravimetric analysis in animal production

4.0 CONCLUSION

Gravimetric analysis is one of the most reliable and precise method of analysis even though it is laborious and time consuming. The method can be used for analysis of both organic and inorganic substance with assurance of good precision.

5.0 SUMMARY

In this unit, you have learnt:

1. Definition of gravimetric analysis
2. Application of gravimetric analysis to animal production
3. The types of gravimetric analysis
4. The advantages and disadvantages of gravimetric analysis.
- 5.

6.0 TUTOR-MARKED ASSIGNMENT

1. Define and give one practical application of gravimetric analysis in the animal production lab.
2. List and describe the various steps in precipitation gravimetric.
3. Distinguish between the precipitation and volatilisation gravimetric analysis.
4. List three advantages and disadvantages of gravimetric method of analysis

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UNIT 3 THE CONCEPT OF THERMOMETRIC DATA ANALYSIS

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Concept of Thermometric Analysis
 - 3.1.1 Different Types of Thermometric Analysis
 - 3.2 Thermometric Titration
 - 3.3 The Principles of Thermometry
 - 3.4 The Advantages and Disadvantages of Thermometric Analysis
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

Thermometric technique is one of the old techniques used in chemical analysis, even though improvement over years has brought a lot of change in the instruments employed and the speed with which it operates.

Direct-reading thermometric analysis is one of the recent techniques which utilize the use of heat of reaction i.e. the amount of heat consumed or emitted during a chemical reaction. Some other methods include the titration aspect of thermometric which uses a change in temperature to determine the end point of the reaction.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- define the term thermometric analysis
- list different types of thermometric
- itemise the principles of thermometric.
- list the advantages and disadvantages of thermometric method.

3.0 MAIN CONTENT

3.1 Concept of Thermometric Analysis

Thermometric analysis is a form of thermal analysis in which a temperature change is used to cause reaction. It is a method used for determination of the transformation a substance undergo while being heated or cooled at an essentially constant rate. In real sense, thermometry involves the practice of temperature measurements, this is how to say, materials and processes are studied under conditions of changing in their temperatures.

3.1.1 Different Types of Thermometric Analysis

Thermo analytical methods or analysis can be grouped into various classes depending on what it measures as well as the kind of instrument it uses. Some of the classifications are:

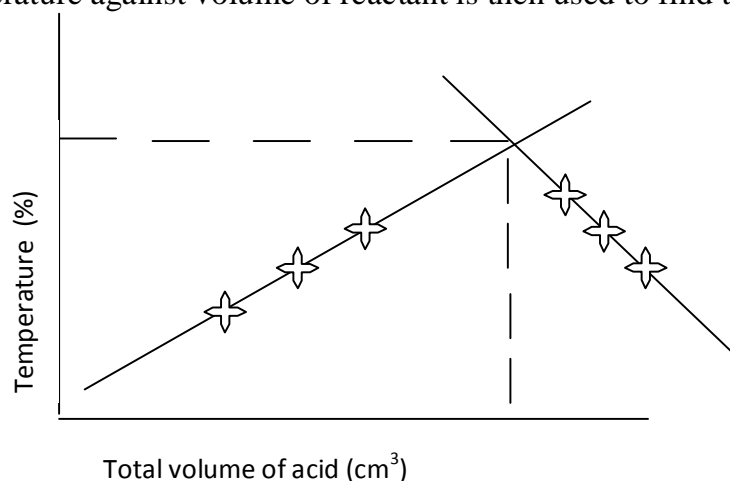
Thermo Gravimetric Analysis: This is an analysis that measures change in weight of a substance with respect to change in temperature. The sample of interest is subjected to continue increase in temperature on a linear scale of time (thermodynamics) while a condition where the sample is placed on a constant temperature for given period of time to observe change in weight is called static thermal thermo-gravimetry. The basic instruments employed for this analysis are the precision balance furnace (that have a linear rise temperature) and recorder. The graph of change in weight is plotted against change in temperature which is sometimes affected by heating rate and temperature of the samples environment. This method of analysis is mostly suitable for determining the range of temperature of drying and in so many analytical analyses like the evaluation of kinetic measures of weight changes in a chemical reaction.

Differential Thermal Analysis (DTA): In this method, the changes in temperature of the analyte sample and that of a reference material is observed and recorded over a period of time with respect to the furnace temperature. In this technique, the apparatus used include sample holder, furnace, flow control system, preamplifiers and recorder, furnace power. The basic methodology behind this method is as follows:

1. A thin thermo couple should be inserted into a disposable sample tube which can hold 0.1 – 10mg of sample.
2. Place an identical tube containing a reference material
3. Put the two tubes in a sample block. Side by side
4. Heat or cool at a uniform rate
5. Then measure the difference between the sample and the reference material

3.2 Thermometric Titration

This method of thermal analysis which is also called enthalpimetric titration. This method of titration we measure temperature against volume of a titrant. The change in temperature is normally used to indicate the end point of the titration. This is done by recording a temperature change each time a reactant (titrant) is added to another reactant. The graph of temperature against volume of reactant is then used to find the end point.



The simplest method of thermometric titration is the acid base thermometric titration. The equipment required for such test are:

1. Thermometer (0-100^oc)
2. Two polystyrene cups
3. Beaker (250 cm³)
4. 50cm³ Burette
5. A burette stand
6. A change and its stand
7. A cork that will fit the thermometer
8. Pipette
9. Pipette safety filter

The procedure for Thermometric analysis depends on the sample and analyte of interest.

3.3 The Principles of Thermometry

Any property of a material which changes with temperature can be used to indicate or measure temperature. For example, the expansion of solids, liquids and gases are all used to make thermometers. Other examples are: an electrical resistance thermometer, in which the resistance of a length of

wire changes with temperature; a thermocouple thermometer, in which junctions between two wires of different metals generate a voltage when the junctions are at a different temperature; a pyrometer in which high temperatures are judged by comparing the colour of a hot object with a reference colour scale.

The type of thermometer which is chosen for a particular application will be decided by some of the following points:

- Does the thermometer work over the range of temperatures required?
- Is the thermometer sensitive enough (Can it detect small enough changes of temperature)
- How quickly does the thermometer respond?
- How small, portable and convenient is the thermometer?
- Can the thermometer give continuous reading and be connected to an electrically operated chart-recorder or warning device
- How expensive is it?

3.4 The Advantages and Disadvantages of Thermometric Analysis

Some of the advantages of thermometric titration are that it is rapid, simple, and precise and can be used for various analyses in biochemical studies (enzyme catalysed reactions, proteins and cupids) and in titration of metals such as calcium (Ca^{2+}) and Fe^{3+} . The use of temperature verses concentration instead of logarithms makes it possible to separate reactions that are run simultaneously as long as their temperature of reaction differs.

However, the major disadvantages is that the inability of the method to obtain multiple endpoint for multi-component mixture. Lack of selectivity associated with mixture. The method requires a sensitive instrument for measuring the system.

SELF- ASSESSMENT EXERCISE

- (i) Name four basic instruments for Thermometric analysis.
- (ii) List out the disadvantages of thermometric analysis

4.0 CONCLUSION

Thermometric techniques are therefore important instrumental techniques that are used in many fields of studies including physics, biological, biochemical and many other fields of sciences. It is fact, accurate and precise.

5.0 SUMMARY

In the end of this unit, you have learnt about:

1. The definition of thermometric techniques
2. Classifications of thermal methods and their operations
3. The differences between each methods
4. The basic instruments of each methods

6.0 TUTOR-METHOD ASSIGNMENT

1. Define thermometric analysis
2. List the basic instrument for each of the following method
 1. Thermo gravimetric
 2. Differential Thermal Analysis
 3. Thermometric titration
3. List out the advantages of thermometric analysis

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Christian, G.D. (1980). *Analytical Chemistry*. (3rd ed.). New York: John Wiley and Sons.

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UNIT 4 ELECTROCHEMICAL ANALYSIS

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Concept of Thermometric Analysis
 - 3.1.1 Different Types of Thermometric Analysis
 - 3.2 Thermometric Titration
 - 3.3 The Principles of Thermometry
 - 3.4 The Advantages and Disadvantages of Thermometric Analysis
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

Electrochemistry is a general term that describes the various techniques of determining an analyte concentration by measuring potential charge or current or reaction. It involves the migration of electron in a redox reaction. Electrochemical methods comprise of different categories which is determined by the cell that is acting as a reference and the other different electrochemical methods and how they are applied in animal production researches.

2.0 OBJECTIVES

At the end of this unit, you should be able to;

- illustrate the principle of electrochemical cells
- list and differentiate the various categories of electrochemical methods
- list the advantages of electrochemical methods
- apply the principles of electrochemical methods to animal researches.

3.0 MAIN CONTENT

3.1 Concept of Electrochemical Analysis

It is a method of quantitative analysis that is based on electrical properties of an anlyte solution by using an electrochemical cell. In electrochemical methods, the electrochemical properties of solution depend on the nature of the components of the solution such as the concentration.

Basic Terms in Electrochemical Analysis

1. **Electrochemical Cell:** A device that produces an electric current from energy released by a spontaneous redox-reaction
2. **Redox Reaction:** Is the reaction that involves the release of uptake of electrons(s).
3. **Redox Potential:** Electrical potential that exist in a redox reaction.
4. **Electrode:** Combination of metals immersed in a salt solution (it could be salt, acid or base).
5. **Reference Electrode:** Electrode with a constant electrochemical potential that is used as a reference point when measuring the voltage between the reference and sensing electrode.
6. **Solutions:** Homogeneous mixture of two substances.
7. **Potential:** Electrical charge distribution established between two media of different compositions.
8. **Electrical Conductivity:** Substance with mobile charge carrier (electrons ions) at which is acting as electrical conductors when a current is applied.
9. **Concentration:** Amount of dissolved substance based on solvent or the entire solution.
10. **Oxidation:** The lost of electron from an atom or molecule.
11. **Reduction:** The gain of electron from an atom or molecule.

3.2 Methods of Electrochemical Analysis

There are basically four methods of electrochemical analysis. The choice of these methods depends on the structure and chemical concentration of material matrix that is to be analysed, they are:

1. Potentiometric method
2. Voltametry method
3. Coulometry method
4. Conductometric method

3.2.1 Potentiometric Method

This method allows the potential of electrochemical cells to be measured under zero current. This is done by determining the difference between the potentials of two electrodes (The working or indicator electrode and the reference electrode). The former responds to analyte actively while the latter has a fixed potential. The most common potentiometric electrode is the one used in the P^H meter (glass membrane electrode). The electrodes are identical by convention; the electrode on the left is referred to as anode while that on the right is the cathode. When conducting Oxidation-Reduction reaction, oxidation occurs at the anode while reduction occurs in the cathode. In potentiometric analysis, the reference electrode is the anode cell while the indicator electrode is the cathode. While the reference electrode provides a stable known potential, the indicator electrode measures any change in the reaction that is attributable to analytes effect. This method is applicable in many redox titration, ionmetry and potentiometric titration in the field of sciences.

3.2.2 Voltametric Method

This method uses current to measure potential of the electrochemical cells. It measures the current flowing through an electrode clipped in a solution which contain electroactive compound when the potential is measured. The method is used for analysing any chemical specie that is electroactive by causing a reduction or oxidation of the solution. The current flowing in this method is due to electron transfer that happens during a reduction oxidation on the electrode surface. This method is principally based on the study of the dependence of polarization current applied to electrons when their potentials differ significantly.

3.2.3 Conductometric Method

This method measures the conductivity of the electrolyte which is used to measure the progress of a reaction in titration experiments, the electrolytes conductivity of the mixture is monitored as the reactant is been added. A sudden observable change in the conductivity indicates the equivalent point and sometimes, electrical conductance measurements can also be used to determine the end point of the reaction.

3.2.4 Culometric Method

The basis of coulometry is Faraday's law of electrolysis. Coulometry as one of electrochemical method it measures the amount/quantity of materials deposited on an electrode during a reaction. Also recall that Faraday's law states that the weight of substance liberated during electrolysis is directly proportional to the quantity of electricity passed through it. In coulometry, the electrodes used have potentials which is different from the equilibrium value which measures that amount of matter transformed during electrolysis reaction by determining the quantity of electricity in coulombs lost or gained (complete oxidation or reduction). The two forms of coulometry are the controlled potential coulometry in which a constant potential to the electrochemical cell is applied and the controlled current coulometry in a constant current is passed through the electrochemical cells. This method solely depends on current.

3.3 Basic Principle of Electrochemical Methods

One basic principle of electrochemical methods is the transfer of electron from one atom or molecule to another during a redox reaction which is brought about changing the oxidation state of both the atom that is going out electron (donor) and the one receiving (recipient).

Any property of a material which changes with temperature can be used to indicate or measure temperature. For example, the expansion of solids, liquids and gases are all used to make thermometers. Other examples are: an electrical resistance thermometer, in which the resistance of a length of wire changes with temperature; a thermocouple thermometer, in which junctions between two wires of different metals generate a voltage when the junctions are at a different temperature; a pyrometer in which high temperatures are judged by comparing the colour of a hot object with a reference colour scale.

The type of thermometer which is chosen for a particular application will be decided by some of the following points:

- Is the thermometer working within the range of temperatures required?
- Is the thermometer sensitive enough (Can it detect changes of temperature)
- How speedily does the thermometer respond?
- How little, handy and convenient is the thermometer?
- Can the thermometer give continuous reading and be connected to an electrically operated chart-recorder or warning device

- How costly is it?

Electrochemical Cells

An electrochemical cell is a tool that is able of either generating electrical energy from chemical reactions or facilitating chemical reactions through the introduction of electrical energy. A common example of an electrochemical cell is a standard 1.5-volt battery meant for consumer use.

An electrochemical cell consists of two half-cells an each of these cells consists of electrode and an electrolyte. The two cells may use the same electrolyte, or they may use different electrolytes. The chemical reactions in the cell may involve the electrolyte, the electrodes, or an external substance (as in fuel cells that may use hydrogen gas as a reactant). In a full electrochemical cell, species from one half-cell lose electrons (oxidation) to their electrode while species from the other half-cell gain electrons (reduction) from their electrode.

Selected Principles: Electrochemical Cells

An electrochemical cell changes chemical energy to electrical energy when it is part of a complete electrical circuit.

For instance, an exothermic reaction occurs when a piece of zinc metal is placed in aqueous copper (II) sulfate. In the reaction, electrons are transferred from zinc atoms to copper (II) ions because zinc is a better reducing agent (i.e., it is more easily oxidized);

Advantages and Disadvantages of Electrochemical Methods

Advantages

The tools used for the reaction can be devised from a cheap electrical conductive substance.

Disadvantages

1. It require constant electric current
2. It is liable to corrosion
3. Only electric conductive material can be used.

4.0 CONCLUSION

Electrochemical methods are techniques used in determining an analyte concentration by measuring the potential charge of the reaction.

5.0 SUMMARY

In this unit, you have learnt that:

1. Electrochemical analysis is a quantitative analysis that is based on electrical properties or analyte solution by using electrical cells.
2. Methods of electrochemical analysis are:
Potentiometric, Voltametric, Coulometric and Conductometric
3. The basic principles of electrochemical analysis are based on oxidation and reduction reactions.

6.0 TUTOR- MARKED ASSIGNMENT

1. Define the term electrochemistry
2. Distinguish between the different method of electrochemical methods
3. State the basic concept of electrochemical analysis

7.0 REFERENCES / FURTHER READING

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UNIT 5 OPTICAL METHOD OF ANALYSIS

CONTENTS

- 1.0 Introduction
- 2.0 Objectives
- 3.0 Main Content
 - 3.1 Definition of Optical Methods
 - 3.2 Principle and Instrumentation of Coulometry
 - 3.3 Principles and Instrumentation of Spectrophotometer
 - 3.4 Principles and Instrumentation of Atomic Absorption
 - 3.5 Principle of Fluorescent
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

Some analyses are determined by the amount of radiation they either absorb or display. Generally, the optical methods of analysis make use of light intensity to measure the concentration of analyte. Certain substances in a sample have the ability to absorb or emit light radiation under certain situations. Thus the absorption or emission of the various degree of light radiation is achieved by the use of different instruments depending on the analyte of interest.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- define optical method of analysis
- explain the different methods of optical analysis
- state the laws of light absorption.

3.0 MAIN CONTENT

3.1 Definition of Optical Methods

They are spectroscopic techniques that use absorption or emission of electromagnetic the radiation (visible or infrared) within a spectrum to evaluate the energy changes within a matter.

Types of Optical Methods

1. Colorimetry
2. Spectrophotometry
3. Atomic absorption
4. Fluorimetry
5. Atomic emission analysis

1. The colorimetry involves the measurement of concentration of a compound in a solution by the dictate of the colour of light it absorbs.
2. Spectrophotometry measures the absorption of light by measuring the amount of light the sample absorbs in molecules as beam of light passes through a sample and the intensity of the light that reaches the detection.
3. Fluorimetry. This method measures a fluorescent of a given sample after excitation with a beam of light.
4. Atomic absorption: This method involves the emission of light in an electronic medium that is executed.

3.2 Principle and Instrumentation of Coulometry

The principle behind colorimetric analysis is the estimation of colour of a coloured substance or it should be capable of forming a coloured complex by adding of reagents. Once the light in relation to its colour intensity which is proportional to the concentration of the substance, this happens when a monochromatic light passes through them. The quantity of light absorbed or emitted is in agreement with the two laws of light absorption. Beers and Lambert laws, Beer's law states that the amount of light absorbed by a molecule in a solution is directly proportional to the concentration of substance in the solution. This is an experimental decrease in transmittance with an increase in concentration.

Mathematically, Beer's Law is given as

$$C \propto \log_{10} \frac{I_0}{I} \text{ or } \epsilon C = \log_{10} \frac{I_0}{I}$$

Where C = Concentration, ϵ = Constant, I_0 = Transmitted light and \log_{10} = Absorption

Lambert's law: states that there is an exponential decrease in transmittance of light with an increase in thickness of coloured solution as light passes through the sample.

Mathematically, Lambert law is given as

$$L \propto \log_{10} \frac{I_0}{I} \quad \text{Where } K = \text{Constant, } KL = \log_{10} \frac{I_0}{I}, I = \text{Path Length}$$

Colorimetric measurement is expressed mathematically by combining the two laws as shown below

$$ECl = \log \frac{I_0}{I}$$

Where I_0 = Intensity of incident light, I = Intensity of transmitted light, E = Absorption coefficient, C = Concentration, L = Depth of absorbing length

Instrumentation: The standard part of coulometer is composed of:

1. Light source (visible or ultraviolet)
A monochromator which is holding a prism that help disperse the light from the source and slit which is used for selection of desired wavelength.
2. A optical cuvelt for holding the sample
3. Filter (made of coloured glass)
4. Detector
5. Output

3.3 Principles and Instrumentation of Spectrophotometer

The basic principle of spectrophotometry is the fact that substance absorbs or transmits electromagnetic radiation energy as a function of a wavelength. That is to say each substance have specific wavelength it absorb or emit. For example, a substance can absorb a particular colour or light while transmit other coloured wavelengths. However, the amount of energy absorbed or transmitted must meet the required energy to cause a movement of electron from one level to another. The instrumentation spectrophotometry is smaller to that colorimetry except that the filter in coulometer is substituted by prism grating and that the detection of light which passes through the sample is detected photocells rather than photo multiples in coulometer.

3.4 Principles and Instrumentation of Atomic Absorption

The principle of atomic absorption is not too different from that of spectrophotometry because light in both forms is absorbed in atomic absorption light energy is absorbed by particles of analyte vapourised in a flame. The absorption follows Beer's Law in which the absorption of light and it quantitative correlation to concentration of the atomic vapour as well as the path length in the flame.

A fine sample solution is allowed to pass through a flame where it is vapourised and atomised into a gaseous solution with the analyte of interest. The radiation from the light source emits a wavelength that provide an energy level to the higher level so that some of the light may be absorbed by the vapourised atoms while other travel through monochrometer and detector.

The Atomic Absorption Consist of Three Steps

- | | |
|--------|--|
| Step 1 | The flaming of a sample into a vapour form |
| Step 2 | Irradiation of vapour (atomic) at a wavelength that corresponds to the analyte of interest. |
| Step 3 | Absorption of radiation by the flamed sample which is proportionate to the concentration of the analyte of interest. |

The Instrumentation Consists of:

1. Light source
2. Monochrometer
3. Sample container (flame)
4. Detector
5. Amplifier indicator

3.5 Principle of Fluorescent

The basic principle of fluorescent is the excitation of electron to a high energy level. As it reaches the highest elevation it returns to the normal state by emitting part of the energy it absorbed as a specific light wavelength. The intensity of radiation fluorescent (f) is in direct proportion to the radiant power of the absorbed exciting beam in the system.

$$F = K (I_0 - I)$$

Where I_0 = Incident beam, I = Transmitted radiant, K = Constant

Fluorescent radiation is in direct proportion with the concentration of fluorescent substance just as seen in Beer's Law.

Hence $F = KC$ where C = concentration

Fluorescent method can be applied in many organic and inorganic analyses. It is used in determining the level of amino acid, protein co-enzymes and many other analyses.

SELF- ASSESSMENT EXERCISE

1. State the Beer's and Lambert laws.
2. List the types of optical methods

4.0 CONCLUSION

From what we have learnt, optical methods are important methods used in determination of many analytes of interest using various methods which includes, colorimetry, spectrophotometry atomic absorption and fluorescent method

5.0 SUMMARY

In this unit, you have learnt that:

- Colorimetric method involves the measurement of concentration by colour detectors.
- The basic principle of spectrophotometry is the absorption of electromagnetic radiation as a function of a wavelength.
- The principles of atomic absorption involves the absorption of light by particles of a vapourised analyte

Atomic absorption has three steps:

1. The flaming step
2. Irradiation step
3. Absorption state.

6.0 TUTOR -MARKED ASSESSMENT

1. State Beer's and Lambert law
2. Explain the principle of colorimetry and its instrumentation
3. Differentiate between colorimeter and spectrophotometer.

7.0 REFERENCES / FURTHER READING

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MODULE 4

Unit 1	Sample Preparation for Chemical Analysis
Unit 2	Some Selected Analysis in Animal Science Researches
Unit 3	Concept of Separation Techniques

UNIT 1 SAMPLE PREPARATION FOR CHEMICAL ANALYSIS

CONTENTS

1.0	Introduction
2.0	Objective
3.0	Main Content
3.1	Basic Principles of Sampling
3.2	Methods of Sampling
3.3	Sample Collection
3.4	Types of Sample
3.5	Composite Sample
3.6	Sample Treatment and Size Reduction
4.0	Conclusion
5.0	Summary
6.0	Tutor-Marked Assignment
7.0	References/Further Reading

1.0 INTRODUCTION

Samples are representative of a given quantity that is routinely collected for analyses. Sampling is major aspect of a successful chemical analysis because the way sample is collected and handled affect to a great extent the result of the analysis. Wrongly collected and handled samples can affect the precision of the analysis even when the right method of analysis is used. Therefore the importance of correct sampling technique and documentation as well as treatment of various sampled materials cannot be over-emphasized. In this unit therefore, you learn the basic principles or sampling for laboratory analysis.

2.0 OBJECTIVES

At the end of this unit, you should be able to;

- explain the basic principle of sampling

- list and explain the various methods of sampling
- state the various treatments required for samples collected for chemical analysis.
- explain how to document samples made for analyses.

3.0 MAIN CONTENT

3.1 Basic Principles of Sampling

Sampling involves the activities employed in gathering acceptable laboratory size materials that can serve as a true representative of a population. Sample collection must be done in a manner that guarantees the maintenance of the physical and chemical nature of the sample well as prevents contamination of the product being sampled. In most analysis, the samples composition does not necessarily need to be identical to that of the substance being analysed provided the samples are large enough to be a true representative of the substance. Depending on source of the sample, different methods of sample collection are employed.

3.2 Methods of Sampling

The two most common sampling techniques are:

1. **Random Sampling:** In this method of sampling, each material of the population has equal opportunity of being collected. This can be achieved by dividing the target population into equal proportions so that each proportion can be collected randomly by the use of a random number table or any randomisation technique. This kind of sampling can be from a finite or infinite population.
2. **Stratified Sampling:** In this method of sampling, population is divided into various strata (homogeneous unit) and each sample is collected randomly for each stratum. This type of sampling is employed when the population is heterogeneous so that each sample collected from the stratum is more homogenous than that of the entire population.
3. A good sample irrespective of the method of collection should possess the following characteristics:
- 4 It must have the same characteristics with the original population from which it is taken from.
- 5 Sample nature must be maintained throughout the analysis period.
- 6 The number of sample collected should be large enough to reduce experimental error.

Sample handling implies how sample are managed or processed. After collection, samples collected should be properly managed so as to avoid compromise in the original state of the sample. Before looking at how to handle and document a sample, let us look at the criteria for accepting or rejecting a sample.

Criteria for Rejection of Analytical Sample

Reject any samples that are:

1. Not true representation of the population from where they are collected
2. Faulty label
3. Samples that are duplicate
4. Samples from faulty and leaky containers
5. Contaminated samples
6. Samples that their sources do not conform to the type of sample needed for the requested tests.
7. Samples that have overstayed the needed time for their analyses
8. Samples that have insufficient size number for the require

3.3 Sample Collection

Any sample collected for analyses must be well labeled or documented so that if any problem develops during the analyses, the source can easily be traced. Also, sample documentation helps when a researcher wants to make future use of the sample. Some information to be documented when collecting samples are as follows:

1. Source of the sample
2. Sample identification number
3. Time or season the sample was collected
4. The purpose of sample collection
5. Location of the sample collected
6. Method used to collect the sample
7. method of sample presentation

3.4 Types of Sample

Grap Sample: This is a type of analytical sample that is collected in a specific population over a given time interval at different locations. For a population with fairly the same homogeneous property, time and space, a single sample collected at random can fully serve as a true representative of the population. Entire properties grap sampling can also be viewed as a single sample or measurement taken at a specific time over a given period and space.

3.5 Composite Sample

This consists of collection of series of grab sample to form a single sample. When this is done, the average composition of a population is known over a period of time and space. The sample can be collected in systematic manner using continuous and constant instruments. Examples of such samples are analysis of feed ingredients in the lab that are collected and done over a period of time and intervals.

3.6 Sample Treatment and Size Reduction

Since all samples collected are directly subjected to chemical analysis, treatment with various methods are done to reduce the size into a homogeneous form and to ensure that the composition is not compromised, treatment helps in reducing all sources of contamination and to convert samples into forms that can be analysed. Some of the methods employed for sample treatment are;

1. **Mixing:** This is done to increase the homogeneity of sample materials and to have a random distribution of sample components,
2. **Oven Drying:** Some samples that have moisture content are subjected to various forms of drying to reduce the moisture content of such samples before analysing it.
3. **Sieving:** It involves the reduction of some particles size so as to remove unwanted large particles that can affect analysis. Sieving also help in reducing a bulky particles into smaller homogenous form that can still represent the total sample.
4. **Heating:** Some samples undergo heating so as to soften them for effective analysis, heating helps in breaking down of some tough and fibrous materials as well as to deal with some to fasten the analysis of a particular sample.

4.0 CONCLUSION

Sample preparation for effective chemical analysis cannot be over emphasized. This is because except one is able to secure the right sample and document it properly every other effort in chemical analysis will be meaningless. Therefore, sampling methods are to be chosen with carefulness and the choice of treatment must be carefully selected so as to achieve the desired result.

5.0 SUMMARY

In this unit, we have learnt about

- Sampling collection and handling
- Sampling techniques
- Sample documentation and
- Sample treatments

6.0 TUTOR- MARKED ASSIGNMENT

1. Justify the statement, sampling is the bedrock of successful analysis
2. List out the criteria for rejecting a sample
3. List three methods of treating a sample
4. List and explain the types of sample

7.0 REFERENCES/FURTHER READING

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UNIT 2 SOME SELECTED ANALYSIS IN ANIMAL SCIENCE RESEARCHES

CONTENTS

- 1.0 Introduction
- 2.0 Objective
- 3.0 Main Content
 - 3.1 Proximate Analysis
 - 3.1.1 Moisture Determination
 - 3.1.2 Ash (Mineral Content Determination)
 - 3.1.3 Kjeldahl Nitrogen Determination Method and Apparatus
 - 3.1.4 Crude Protein
 - 3.1.5 Crude Fibre
 - 3.1.6 Crude Fat (Ether Extract)
 - 3.2 Qualitative Analysis
 - 3.2.1 Calcium
 - 3.2.2 Phosphorus
 - 3.2.3 Blood Urea
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor-Marked Assignment
- 7.0 References/Further Reading

1.0 INTRODUCTION

In the last module, we have discussed the various chemical methods used in animal science analyses. This chapter is therefore aimed at discussing some analyses that are mostly employed in animal science researches. Such analysis as proximate composition analysis and some other biochemical analyses; for instance is useful in determining the quality and quantity of a given material using one or the combination of this quantitative and qualitative analysis.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- list the different classification of feed under proximate analysis
- describe the various techniques of feed analysis

- describe how to analysis of some qualitative techniques in animal researches.

3.0 MAIN CONTENT

3.1 Proximate Analysis

The proximate composition is obtained through proximate analysis. Proximate analysis divides the feeds into six (6) different fractions. These are:

1. Moisture,
2. Ash,
3. Crude protein,
4. Ether extract,
5. Crude fiber and
6. Nitrogen free extract.

3.1.2 Moisture Determination

Procedure

- Weigh 5g of the sample into a crucible
- Weigh and record the weight of crucible fresh sample
- Dry to constant weight at 100°C preferable in an oven fitted with controlled ventilation.
- Remove and weigh the crucible + dry sample

Calculation

Moisture content (%)

$$= \frac{\text{weight of crucible+fresh sample}-\text{weight of crucible+dry sample}}{\text{weight of fresh sample}} \times 100$$

3.1.2 Ash (Mineral Content Determination)

Procedure

- Weigh a dry small crucible and required as W_1
- Weigh a 2.0g sample into the dry crucible
- Then place in a muffle furnace at 600°C for 6 hours
- Cool in desiccators and weigh as W_2

Calculation

$$\text{Ash (\%)} = \frac{W_2 - W_1}{\text{weight of sample}} \times 100$$

3.1.3 Kjeldahl Nitrogen Determination Method and Apparatus

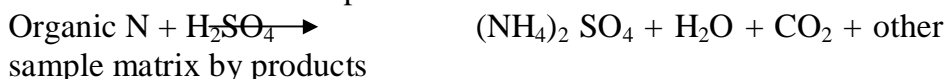
Kjeldahl Method

The kjeldahl method is used in determining the nitrogen content of organic and inorganic matter. Although the technique and apparatus have advanced considerably over the years, the basic principles introduced by Johan Kjeldahl still stand today.

The kjeldahl method is divided into three main steps;

1. **Digestion:** The decomposition of nitrogen in organic samples utilizing a concentrated acid solution and a digestion tablet, this is accomplished by boiling a homogeneous sample in concentrated sulfuric acid in which the end result the end result gives an ammonium solution.

A general equation for the digestion of an organic sample is shown below as one basic example:



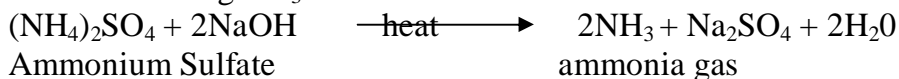
Acid Consideration: Sulphuric acid has been used for the digestion of organic samples. The amount of acid required is relative to sample size and amount of carbon and hydrogen in the sample, as well as amount of nitrogen.

Catalyst additions: Mercuric oxide has been widely used but because of environmental effects order the handling and disposal, other catalyst have gain more favour e.g. Titanium oxide and copper sulfate, 5mgSe and 50kg₂SO₄.

2. **The Distillation Process**

Adding excess base to the acid digestion mixture aid to convert NH₄ to NH₃, then followed by boiling and condensation of the NH₃ gas in receiving solution.

The acid digestion mixture is diluted is made strongly alkaline with NaOH liberating NH₃ as follows:



Digestion mixture dilution: The acid digestion mixture is usually cooled and diluted with ammonia-free water.

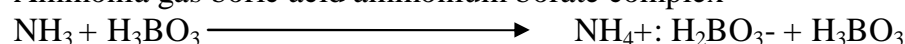
NaOH Addition: Concentrated NaOH is added slowly down the neck of the flask.

Distillation: In the majority of the NH_3 is distilled and trapped in the receiving acid solution within the first 5 or 10 minutes of boiling. But depending on the volume of the digestion mixture and the method being followed, 15 to 150ml of condensate should be collected in receiving flask to endure complete recovery of nitrogen.

Receiving solutions: When Boric acid is used, the exact concentration is not needed because the titration directly measures the amount of ammonia in the distillate by neutralizing the 1:1 complex formed by ammonia and boric acid.

The chemical reaction is;

Ammonia gas boric acid ammonium borate complex



Colour Change

The boric acid captures the ammonia gas, forming an ammonium borate complex. As ammonia is collected the colour of the receiving solution changes.

3. Titration

Ammonium borate complex, sulfuric acid, ammonium sulfate and boric acid



The addition of sulfuric acid exactly neutralized the ammonium borate complex, and a reverse colour change is produced.

The boric acid method has two advantages: only the one standard solution is necessary for the determining and the solution has a long shelf life.

3.1.4 Crude Protein

Reagents

1. Sulphuric acid (98%)
 2. 5mg se, 5g K_2SO_4 (catalyst)
 3. NaOH, 40% solution (10N solution)
 4. Boric acid solution
 5. 0.1N H_2SO_4
1. Digestion and distillation units

2. Digesting tubes (500ml capacity or larger)
3. Conical flasks, 250ml.

Method

Weigh accurately approximately 1.0g of sample into a digesting tube and add 1 tablet of catalyst, and 12.5ml sulphuric acid while in the fume cupboard. Load into the Digester, set the timer to 1 hour 15 minutes. At the end of this time, unload from the digester and allow to cool for about 30 minutes.

On cooling add distilled water, make then distilled collecting distilled ammonia in 25ml boric acid solution contained in a conical flask. On completion of distillation remove and titrate against standard acid solution.

Calculation:

$$\% \text{ protein} = \frac{14.01 \times \text{normality of acid} \times (\text{vol of acid} - 0.1) \times 6.25}{\text{weight of sample} \times 1000} \times 100\%$$

3.1.5 Crude Fibre

Crude fibre is determined as that fraction remaining after digestion with standard solutions of sulphuric acid and alkali (Potassium hydroxide) under carefully controlled conditions.

Reagent

1. Sulphuric acid solution (1.25%)
2. Potassium hydroxide solution (1.25%)
3. Petroleum ether, boiling range 40 to 60°C
4. Hot water

Instruments:

1. Hotplate
2. Beaker glass without spout
3. Fibre caps – capsule tray for 6 fibre caps + boiling stand & drying stand
4. Accessory: crucible for incineration
5. Drying chamber (oven) 105°C
6. Muffle Furnace, Temp, 600°C
7. Dessicator
8. Timer

Principle

When doing a crude fibre analysis the components neither soluble with sulphuric acid nor with potassium hydroxide such as cellulose,

hemicellulose and lignin are determined. The residual, undigested material is dried, weighed and then incinerated. The differences between the content and undigested part as the crude fibre is determined.

Procedure

Weigh 1.0g into the empty fibre caps which has been weighed and insert in the capsules tray. De-fating of the sample is especially important for samples with a high fat content. To de-fat, the capsules tray is immersed three times in a row into 100ml 40-60 petroleum ether. By turning it as well as moving it up and down with the help of the boiling stand, the sample is de-fatted, apply heat until sample is dry.

Pour sulphuric acid solution into the beaker, lower the capsules tray gently using the handle of the boiling stand. Mix it by stirring the boiling stand for about 1 minutes so that the sample is entirely soaked and make sure the fibre cap is filled with washing solution and place the beaker on the hotplate which has been pre-heated for about 5 minutes. Bring it to a boil by setting it full. During this boiling stage the sample should floating freely in the fibre-cap. This can be done by gently stirring the boiling stand with the handling tool or by softy swirling tool thus draining the acid from the fibre-caps. Discard the acids with the solubles in the beaker. Rinse the fibre-caps contained in the capsule tray several times with hot water.

Pour potassium hydroxide solution into the beaker, repeat the above procedure with (KOH).

The fibre-caps are taken and dried in the oven with the use of drying stand. After which each fibre-cap is put into a crucible, weighed and recorded. They are then placed in the muffle furnace at 600°C to ash about 4 hours. They are then brought out into the dessicator for another 30 minutes, and weighed.

Calculation

$$\% \text{ fibre} = \frac{W_3 - (0.9981 W_1) - (W_5 - W_4) - 0.002 \times 100}{W_2}$$

Where W_1 = weight of fibre caps

W_2 = weight of fibre caps with grinded sample

W_3 = dried sample + fibre cap

W_4 = weight of small crucible

W_5 = weight of crucible removed from the furnace

3.1.6 Crude Fat (Ether Extract)

Reagent and Equipment:

1. Petroleum ether (b.p 40 – 60°C)

2. Extraction thimbles
3. Soxhlet extraction apparatus
4. Fat cups
5. De-fatted cotton wool

Method:

Weigh into an extraction thimble 1.0g of the dried grinded sample cover with defatted cotton wool weigh the fat cup to be used. Insert the sample thimble in the extraction unit 50mls of the solvent (petroleum ether) is added into the fat cups. The cups are heated by the electrical heating plate after control unit has being programmed extraction. The 4-step extraction procedure consists of boiling, rising recovery and pre drying.

After completion (it takes 1hr 30mins), remove the fat cup and place in the oven for some minutes. Then put in the dessicator, after which you take the weight of the extracted fat + fat cups.

Calculation

$$\frac{\text{weight of fat + fat cup} - \text{weight of fat cup}}{\text{weight of sample}} \times 100$$

Free Fatty Acid

Acid value or FFA: The acid value of an oil or fat is defined as the number of mg of potassium hydroxide required to neutralize the free acid in 1g of the sample. The result is often expressed as the percentage of free acidity.

Reagents and Apparatus

1. Diethyl ether
2. Alcohol
3. Phenolphthalein solution (1%)
4. 0.1 ml sodium hydroxide

Method

Mix 25ml diethyl ether 25ml alcohol and 1ml phenolphthalein solution (1%) and carefully neutralized with 0.1 M sodium hydroxide. Dissolve 1.0g of the oil in the mixed neutral solvent and titrate with aqueous 0.1M hydroxide shaking constantly until a pink colour which persists for 15sec is obtained. The titration should preferably not exceed about 10ml or other two phases are liable to separate.

Calculation

$$\text{Acid value} = \frac{\text{titrate value (ml)} \times 5.61}{\text{Wt of sample used.}}$$

$$\text{FFA} = \frac{\text{acid value}}{2}$$

FFA = free fatty acids

Ph Measurement

PH meter was switched on and allowed to warm for 5 minutes. The PH was then first standardised with PH buffer solutions of PH4, PH7, PH9 to ensure the sensitivity and accuracy of the meter this was done by dipping the same electrode of the meter into the respective buffer solution with thoroughly rinsing after each dipping.

The pH of sample was taken by dipping the same electrode after through rinsing in distilled water into the sample solution and pH value consequently read out on the meter readout unit.

3.2 Qualitative Analysis

These are Classical qualitative inorganic analysis in which elemental composition of inorganic compounds is determined. The identification of the constituents, e.g. elements or functional groups, present in a substance is achieved through qualitative analysis. Some few examples of these analyses are.

3.2.1 Calcium

Reagent

1. Hydrochloric acid
2. Murexide indicator
3. 5N NaOH solution
4. EDTA solution

Apparatus

1. 100 ml volumetric flask
2. Beakers
3. Qualitative filter paper and & funnels
4. Burette
5. Pipette

Methods

Weigh 2.0g finely ground feed into 100ml volumetric flask; add 10ml of hydrochloric acid. Allow to stand for 20 minutes and shake.

Add distilled water and make it up to 100ml mark. Filter using the funnel and filter paper into a beaker. Take 10ml of the filtrate into another beaker. Add 4ml 5N NaOH and murexide indicator, dilute with distilled water and mix. It gives a pink colour. Titrate using EDTA.

Calculation

$$\text{Calcium (\%)} = \frac{\text{Vol of EDTA} \times 50}{\text{weight of sample} \times 25.5}$$

NB: Limestone sample does not require filtrations and 1.0g is taken for estimation of calcium.

3.2.2 Phosphorus

Reagents and Equipment

1. Molybdovanadate reagent
2. Phosphorous standards
3. Digesting tubes
4. Digestor
5. Catalyst
6. Volumetric flask (100ml)

Procedure

Digest 1.0g of sample as in protein. Pour the digested sample into 100ml volumetric flask, wash down with distilled water and make up to 100ml mark. Form that take 10mls and add 25mls molybdovanadate solution. Shake and leave for about 30 minutes. Then read the absorbance at wavelength 470mm.

Calculation:

Phosphorous (%) = Dilution factor (1000) x Slope x Absorbance

Preparation of Standard Graph

To a series for 100ml volumetric flasks add 0, 2, 5, 5, 10, 20, 30, 40 and 50ml of standard phosphate solution and dilute each with water. Repeat the above procedure for determination of phosphorus. Plot a standard curve. Calculate slope.

3.2.3 Blood Urea

Reagents

Urease solution

EDTA buffer

Phenol solution

NaOH

Apparatus

Colorimeter

Water bath at 37⁰C

Cuvettes with 1-1.6 cm light path

Test tubes

Micropipettes (0.2ml)

Pipettes

Timer

Procedure

Dilute the plasma 1:10 with ammonia free saline

Pipette 1 ml of the diluted urease into test tubes(3 test tubes)

Pipette in the 0.2ml ammonia free water as blank, diluted urea standard and diluted plasma and mix Cork each bottle and allow to stand for 15 minutes in the water bath at 37⁰c

Add into each of the 3 tubes 5ml dilute phenol solution and 10 ml of hydrochlorite solution and mix methodically

Allow the corked tubes rest in water bath for 30 minutes

Put the content into the cuvettes and read off optical density for each at 630nm

Calculation

$$\text{Mg urea/100 ml plasma} = \frac{\text{O.D plasma} \times 50}{\text{O.D standard}}$$

4.0 CONCLUSION

Quantitative and qualitative analysis are some of the major analyses employed in animal science experiments. Therefore, mastering of these techniques is important as most analysis that has to do with lab employ one or more of the methods.

5.0 SUMMARY

In this unit, you have learnt about proximate analysis which divides the feeds into six (6) different fractions. Namely:

1. Moisture
2. Ash
3. Crude protein
4. Ether extract
5. Crude fiber
6. Nitrogen free extract.

And how each of these are determined Also some few qualitative analysis like Ca, P and Urea determination.

6.0 TUTOR -MARKED ASSIGNMENT

1. Differentiate between qualitative and quantitative analyses

2. Enumerate the steps in Crude protein determination
3. List the reagents for Calcium determination.
4. State the procedures for Phosphorus determination.

7.0 REFERENCES /FURTHER READING

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UNIT 3 CONCEPT OF SEPARATION TECHNIQUES

CONTENTS

- 1.0 Introduction
- 2.0 Objective
- 3.0 Main Content
 - 3.1 Concept of Filtration
 - 3.1.1 Filtration Technique
 - 3.1.2 Application of Filtration
 - 3.2 Concept of Crystallisation
 - 3.2.1 Crystallisation Techniques
 - 3.2.2 Application of Crystallisation
 - 3.3 Concept of Distillation
 - 3.3.1 Distillation Technique
 - 3.3.2 Areas of Application of Distillation
 - 3.4 Concept of Extraction
 - 3.4.1 Extraction Technique
 - 3.4.2 Application of Extraction
 - 3.5 Concept of Chromatography
 - 3.5.1 Techniques involved in Chromatography
 - 3.5.2 Application of Chromatography
- 4.0 Conclusion
- 5.0 Summary
- 6.0 Tutor- Marked Assignments
- 7.0 References/Further Reading

1.0 INTRODUCTION

Separation techniques deals with the separation of mixtures to enhance purity of substances, is a process employed to achieve any mass_transfer phenomenon that converts a mixture of substances into two or more distinctive product mixtures, at least one of which is enriched in one or more of the mixture's constituents. In some cases, a separation may completely separate the mixture into its uncontaminated constituents. In this unit therefore, students will be exposed to different methods and techniques of separation components of mixtures, to appreciate the importance of such separation techniques, to apply different methods and techniques in separating components of mixtures.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- define the concept of separation techniques
- list and explain the separation techniques
- apply the relevance of separation techniques to their researches.

3.0 MAIN CONTENT

3.1 Concept of Filtration

Filtration is generally considered a physical operation which is used for the separation of solids from fluids (liquids or gases) by interposing a medium through which only the fluid can pass. The fluid that passes through is called the filtrate. Oversize solids in the fluid are collected, but the separation is not complete; solids will be contaminated with some fluid and filtrate will contain fine particles

3.1.1 Filtration Technique

There are several filtration techniques depending on the desired outcome namely, hot, cold and vacuum filtration. Some of the major purposes of getting a desired outcome are, for the removal of impurities from a mixture or, for the isolation of solids from a mixture.

- a. Hot Filtration Method: is mainly used to separate solids from a hot solution. This is done in order to prevent crystal formation in the filter funnel and other apparatuses that comes in contact with the solution.
- b. Cold Filtration Method: is the use of ice bath in order to rapidly cool down the solution to be crystallised rather than leaving it out to cool it down slowly in the room temperature. This technique results to the formation of very small crystals as opposed to getting large crystals by cooling the solution down at room temperature.
- c. Vacuum Filtration Technique: is most preferred for small batch of solution in order to quickly dry out small crystals. This method requires a Büchner funnel, filter paper of smaller diameter and rubber tubing to connect to vacuum source.

3.1.2 Application of Filtration

Filtration, as a physical operation is very important in agric science for the separation of materials of different chemical composition in other to purify compound. Filtration is also important in feed stream, as in the biofilter, which is a combined filter and biological digestion device.

3.2 Concept of Crystallisation

Crystallisation is the process of formation of solid crystals precipitating from a solution, melt or more rarely deposited directly from a gas. Crystallisation is also a chemical solid–liquid separation technique, in which mass transfer of a solute from the liquid solution to a pure solid crystalline phase occurs. In chemical engineering crystallisation occurs in a crystallizer.

3.2.1 Crystallisation Techniques

- A) Crystal Growing: The aim is to grow single crystals of suitable size in at least two of the three dimensions. The size of the crystals can be influenced by a number of factors, for example the solubility of the sample in the chosen solvent, the number of nucleation sites and time. If possible a solvent should be chosen in which the sample is moderately soluble.
- B) Solvent Evaporation: This is the simplest technique for air stable samples. A near saturated solution is prepared in a suitable solvent. The sample can then be left in a sample vial that has a perforated cap.
- C) Slow Cooling: This is done by allowing a saturated solution that is heated to cool for several days at a temperature below the boiling point.
- D) Solvent Diffusion: This method is applicable to samples that are air and/or solvent sensitive. A solution is placed in a sample tube then a second less dense solvent is carefully dripped down the side of the tube using either a pipette or a syringe to form a discreet layer.
- E) Vacuum Sublimation: A large number of compounds can be sublimed to form excellent crystals. There are numerous variations of this method using either static or dynamic vacuum.

3.2.2 Application of Crystallisation

Crystallisation is applied in many industrial manufacturing companies: table salt, sugar, sodium sulfate, urea, and many more, are produced by

crystallisation from solutions, for example, production of sucrose from sugar beet, where the sucrose is crystallised out from an aqueous solution.

3.3 Concept of Distillation

Distillation is a Process in which the components of a substance or liquid mixture are separated by heating it to a certain temperature and condensing the resulting vapors. Some substances (such as crude oil, oil and water mixture) have components that vaporize at different temperatures and thus can be separated by condensing their vapors in turn.

3.3.1 Distillation Technique

A) Fractional Distillations: Mixtures of liquids whose boiling points are similar (separated by less than 70°C) cannot be separated by a single simple distillation. In these situations, a fractional distillation is used.

B) Vacuum Distillations

Vacuum distillation is distillation at a reduced pressure. Since the boiling point of a compound is lower at a lower external pressure, the compound will not have to be heated to as high a temperature in order for it to boil. Vacuum distillation is used to distill compounds that have a high boiling point or any compound which might undergo decomposition on heating.

3.3.2 Areas of Application of Distillation

A) Purified Water and Ethanol: Distillation can be used to separate water from salt; obviously it can also be used to separate water from microbes and other impurities. Distilled water, Ethanol, the alcohol in alcoholic beverages, grapes for wine; barley and hops for beer; juniper berries for gin; potatoes for vodka, and so on.

B) Industrial Distillation: In contrast to ethanol, methanol or "wood alcohol" is a highly toxic substance whose ingestion can lead to blindness or death. It is widely used in industry for applications in adhesives, plastics, and other product.

3.4 Concept of Extraction

Transferring a solute from one solvent into another is called extraction. The solute is extracted from one solvent into the other because the solute is more soluble in the second solvent than in the first. The two solvents must

be immiscible (not mix freely), and they must form two separate phases or layers, in order for the extraction to work.

Extractions using a separatory funnel are essentially the only kind of extraction performed in the organic analysis.

3.4.1 Extraction Technique

- A) Soxhlet extraction: This method is adequate for both initial and bulk extraction. The product is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. The main advantage of Soxhlet extraction is that it is a continuous process.
- B) Pressurised solvent extraction: The material is loaded into an extraction cell, which is placed in an oven. The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurised at programmed levels for a set period of time. The cell is flushed with nitrogen gas, and the extract, which is automatically filtered, is collected in a flask. Offers a more economical and environment-friendly alternative to conventional approaches.

3.4.2 Application of Extraction

- DNA purification: The ability to purify DNA from a sample is important for many modern biotechnology processes.
- Food industry: The system can be used by the food industry to isolate or eliminate particular flavors.
- Analytical chemistry: Often there are chemical species present or necessary at one stage of sample processing that will interfere with the analysis.
- Drug industries and Perfumeries.

3.6 Concept of Chromatography

The term chromatography literally means color writing, and denotes a method by which the substance to be analysed is put into a vertical tube containing an adsorbent, the various parts of the substance moving through the adsorbent at varied rates of speed, according to their degree of attraction to it, produce bands of color at different levels of the adsorption column. This is done in two phases, the mobile phase which is referred to the fluid that carries the mixture of substances in the sample through the adsorptive material and the stationary or adsorbent phase which is a solid material that takes up the particles of the substance passing through it.

3.5.1 Techniques involved in Chromatography

The technique is a valuable tool for the research biochemist and is readily adaptable to investigations conducted in the clinical laboratory. For example, chromatography is used to detect and identify in body fluids, certain sugars and amino acids associated with inborn errors of metabolism.

1. Adsorption Chromatography: that in which the stationary phase is an adsorbent.
2. Affinity Chromatography: that based on a highly specific biologic interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand.
3. Column Chromatography: the technique in which the various solutes of a solution are allowed to travel down a column, the individual components being adsorbed by the stationary phase. The most strongly adsorbed component will remain near the top of the column; the other components will pass to positions farther and farther down the column according to their affinity for the adsorbent.
4. Exclusion Chromatography: that in which the stationary phase is a gel having a closely controlled pore size. Molecules are separated based on molecular size and shape, smaller molecules being temporarily retained in the pores.
5. Gas Chromatography: a type of automated chromatography in which the mobile phase is an inert gas. Volatile components of the sample are separated in the column and measured by a detector. The method has been applied in the clinical laboratory to separate and quantify steroids, barbiturates, and lipids.
6. Gas-Liquid chromatography: gas chromatography in which the substances to be separated are moved by an inert gas along a tube filled with a finely divided inert solid coated with a nonvolatile oil; each component migrates at a rate determined by its solubility in oil and its vapor pressure.
7. Ion Exchange chromatography: that utilizing ion_exchange_RESINS, to which are coupled either cations or anions that will exchange with other cations or anions in the material passed through their meshwork.
8. Paper Chromatography: a form of chromatography in which a sheet of blotting paper, usually filter paper, is substituted for the adsorption column. After separation of the components as a consequence of their differential migratory velocities, they are stained to make the chromatogram visible. In the clinical laboratory, paper chromatography is employed to detect and identify sugars and amino acids.

9. Partition Chromatography: a process of separation of solutes utilizing the partition of the solutes between two liquid phases, namely the original solvent and the film of solvent on the adsorption column.
10. Thin-Layer Chromatography: that in which the stationary phase is a thin layer of an adsorbent such as silica gel coated on a flat plate. It is otherwise similar to paper chromatography.

3.5.2 Application of Chromatography

This technique is widely applied in various fields, few of which is the manufacturing plant – to purify a chemical needed to make a product. Biotechnology industry: establishing the purity or concentration of compounds in biotechnological research.

Biological application – Chromatography has many applications in biology. It is used to separate and identify amino acids, carbohydrates, fatty acids, and other natural substances.

4.0 CONCLUSION

Separation techniques are an important part of chemistry. However, their importance is not just limited to chemistry; they are also used in our daily lives. Separation techniques are methods used to separate and or purify mixtures. There are many kinds of separation techniques that are used in our daily life; Crystallisation, Filtration, Distillation etc

Separation methods are processes that involve the separation of chemical mixtures that are crucially important in many areas of analyses. There are as many uses for separation as there are techniques to accomplish it, as well as their applications in various fields of research

5.0 SUMMARY

In this unit, you have learnt the various separation techniques, their principles as well as their applications. These techniques include:

1. Filtration
2. Extraction
3. Distillation
4. Crystallisation and
5. Chromatography.

6.0 TUTOR- MARKED ASSIGNMENT

1. describe the principle of filtration and give two areas where it can be applied
2. describe the techniques behind extraction
3. how is colour detected in chromatography
4. describe crystallisation technique

7.0 REFERENCES/FURTHER READING

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