PRACTICAL MANUAL

PRINCIPLES OF ANIMAL NUTRITION AND FEED TECHNOLOGY

ANN (Unit – I)

Name of student …….

Roll No. …….

Batch No. ……..

DEPARTMENT OF ANIMAL NUTRITION

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PRACTICAL MANUAL

ANIMAL NUTRITION

CERTIFICATE

This is to certify the manual contains bonafide practical work of Mr./Ms. ……. bearing roll no.....student of the Mahatma Jyotiba Fule College of Veterinary & Animal Sciences Chomu, Jaipur, studying in the second year B.V.Sc. and A.H. degree course no. ANN (Unit – I) during the academic session 2025-2026.

Date: Signature of course teacher

(Assistant Professor)

Signature of Head of Department

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1. GENARAL PRECAUTIONS WHILE WORKING IN NUTRITION RESEARCH LABORATORY

General Instructions

Some general instructions for working in the laboratory are as follows.

1. Apparatus used should be kept neat and clean.
2. While pipetting rubber bulb should be used.
3. Small amount of liquid remaining in the pipette should not be blown out. In standardizing the pipettes, allowance is always made for this. However, a frosted ring near the top of latest designed pipettes indicates that the last drop is to be blown out.
4. Most measuring devices are made of glass which has a small temperature coefficient,

   Example: A soft glass vessel will lead to a change in volume by about 0.003 per cent per degree (°C) change in temperature; with heat resistant glass the change is about one-third of this. As a general rule, heating of calibrated equipment should be avoided. Rapid cooling can change the glass structure and causes change in volume.

Laboratory Safety

1. Laboratory should be well ventilated and fitted with exhaust fans for effective removal of fumes.
2. Use apron and other devices like gloves, goggles etc. depending upon the material to be handled.
3. Do not add water to acids. Keep acids off skin and protect eyes from spattering. If acids are spilled on the skin, wash off immediately with tap water. Gaseous nitrogen oxides fromHNO_3 can cause severe lung damage. Copious flow of fumes occurs when both concentrated HNO3and HC1 are mixed together.
4. If acid falls on clothes, neutralize the same with few drops of dilute ammonia solution or some other weak alkali solution.
5. If acid spills on the floor or the table, neutralize it with some weak alkali and wipe off with duster.

6.If you happen to suck acid into your mouth during pipetting wash your mouth quickly with water and then rinse with a weak solution of washing soda.

7. Use fume hood to protect against any type of fumes.

8. Avoid use of equipment for purposes other than intended.

Cleaning Laboratory Ware

The glass and porcelain ware should be thoroughly washed with some detergent and should be extensively rinsed with tap water followed by further rinsing with distilled water.

If a grease film remains after cleaning with detergent, a cleaning solution consisting of sodium or potassium dichromate in concentrated sulphuric acid may be used. After this, rinsing necessary in order to remove the last traces of dichromate ions which adhere strongly to glass or porcelain surface.

Preparation of Cleaning Solution

Weigh 60 g of potassium dichromate and transfer to a 1000 ml Pyrex beaker, add 300 ml tap water, and mix thoroughly with glass rod. Heat the solution to boil till potassium dichromate dissolves. Allow the solution to cool and then add slowly 640 m1 of commercial grade sulphuric acid while mixing thoroughly with glass rod. Transfer the contents to 1 litre capacity cylinder for cleaning glassware.

Note

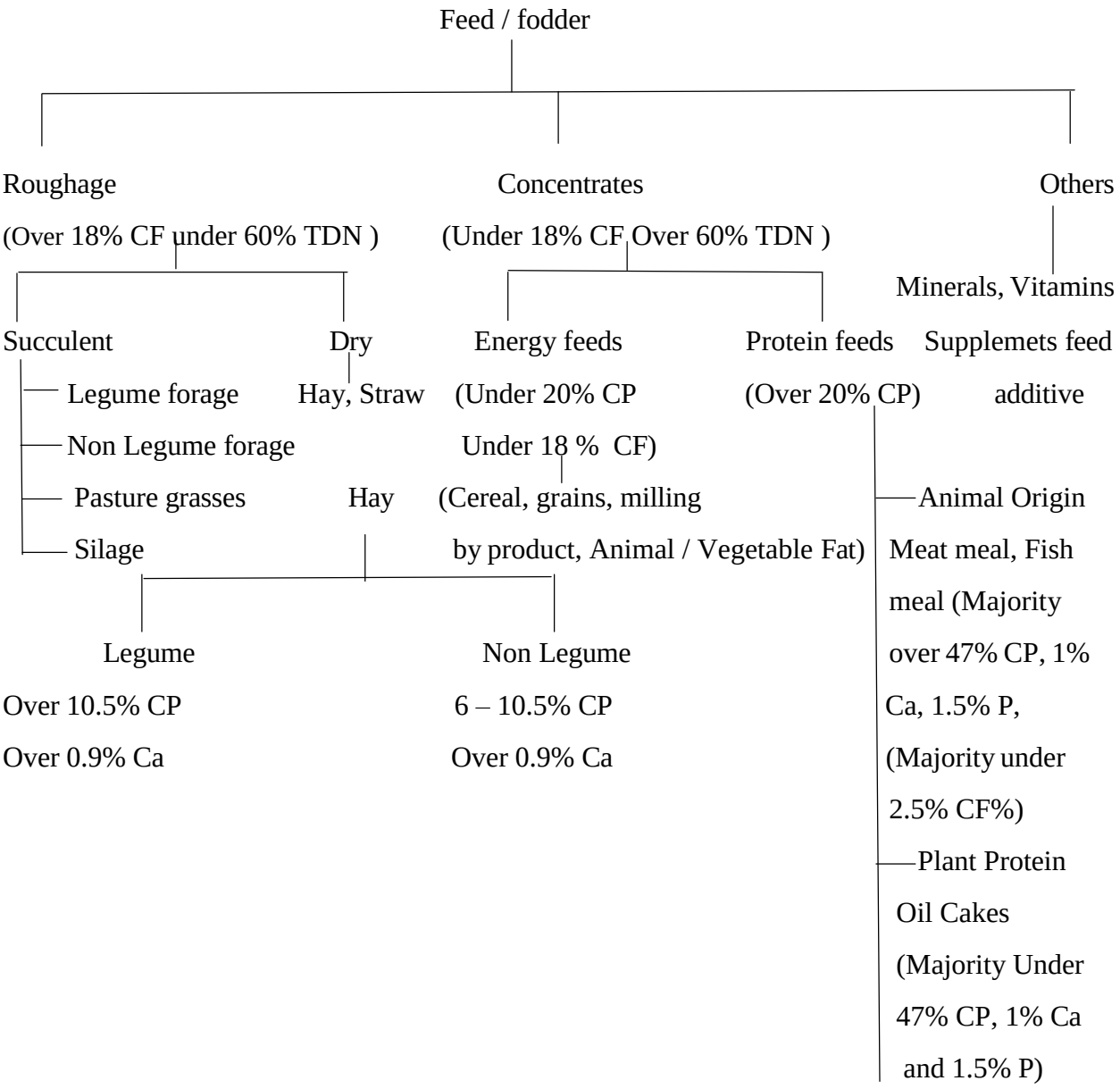
1. Cleaning solution should be discarded when it acquires green colour of chromium ion.

2. Cleaning solution is most effective when warmed up to about 70°C.At this temperature it rapidly attacks plant and animal matter and thus it is potentially dangerous preparation.

3. Spillage, if occurs, should be cleaned with water. and clean. Working place table should also be cleaned daily.

2. FAMILIARIZATION OF VARIOUS FEED STUFFS, FODDER AND THEIR SELECTION

Classification of feed/fodder



High Moisture Feed

Molasses (CF = 0%, CP = Under70%)	60%
Silage	45 – 60%
Haylage	25 – 45%
Green fodder	15 – 30%
Fresh whole skimmed milk	9 – 13%

The following green fodders are commonly used for feeding of livestock

Non-Legume fodder crops:

1. Jonna or Jowar (*Sorghum bicolor*):

- It is a fast growing, high yielding, palatable fodder crop.
- Most popular fodder for cattle and buffaloes.
- Ideal crop for silage making.
- Economical and a rain fed crop.
- If growth is stunted as seen under drought conditions, the plant may contain HCN.
- **Seed rate:** Line sowing 15-20 kg/ha, Broad casting 40-50 kg/ha.
- **Yield** about 15,000 to 20,000 kg / acre.

2. Bajra (*Pennisetum americanum*):

- Millet crop and can grow even on very poor soil.
- Several cuttings can be taken during the year.
- **Seed rate:** 10-15 kg/ha.
- **Yield** about 10,000 kg / acre.

3. Maize (*Zea mays*):

- Requires good soil and much irrigation.
- It is not as hardy as jowar.
- It is palatable and nutritious.
- Ideal crop for silage making.
- Stems are softer than jowar stalks.
- **Seed rate:** Line sowing 50-60 kg/ha, broad casting 100 kg/ha.
- **Yield** about 15,000 to 20,000 kg / acre.

4. Guinea grass (*Panicum maximum*):

- It is an exotic, perennial fodder grass.
- Is one of the famous tropical grasses well adapted to our country.
- Leaves are finer and more nutritious than Napier grass.
- Can be grown under varied soil and climatic conditions.
- First cutting is obtained in $2^1/_2$ months and later on every 4 weeks.
- Fields should be replanted after at least 6 years.
- 8 to 10 cuttings can be taken in a year.
- **Seed rate:** 10-12 kg/ha in case of direct sowing, for transplanting 2-3 kg/h, rooted slips 25,000-30,000 / ha.
- **Yield** about 30,000 kg / acre.

5. Napier grass or Elephant grass (*Pennisetum purpureum*):

- It is an exotic, perennial fodder grass.
- Native of Africa.
- Gives very high yield but is not as palatable as other fodder grasses.
- Can be cut for every 40 or 45 days after first cutting, first cutting will be obtained in 75 days.
- Makes good silage.
- Stem cuttings (slips) - 30,000-37,500/ha
- 7 to 8 cuttings can be taken in a year.
- **Yield** about 180-220 tonnes/ha.

6. Hybrid Napier:

- Commonly known as Pusa giant napier.
- It is a cross between bajra and indigenous napier grass.
- Under irrigated conditions the yield is about 20 to 25 tons.
- Stem cuttings – 30,000-37,500/ha.
- The oxalate content of the crop is rather high.
- Varieties – CO_1, CO_2, CO_3, CO_4 NB-21, APBN-1 and IGFRI – 3,6,10.
- **Yield** about 200-250 tonnes/ha/year.

7. NB-21:

- It is also a cross between bajra and napier grass.

- It is specially bred to reduce the oxalate content of the crop.
- Stem cuttings (slips) – 30,000-37,500/ha.

8. Rhodes grass (*Chloris gayana*):

- It is a perennial grass.
- It has fine soft leaves and relished by cattle and horses because of its high palatability.
- First cutting can be obtained 2 months after planting.
- Can be used as pasture and can also be made into hay.
- 5 to 7 cuttings may be taken in a year.
- **Yield** about 25,000 kg / acre.

9. Para grass or Buffalo grass or Water grass (*Brachiara mutica*):

- Propagation is through slips.
- Growth is quick and yield is high compared to other grasses.
- Grows well in water logged areas.
- First cutting is obtained in 3 months.
- 6 to 8 cuttings can be taken in a year.
- Stem cuttings 62,500-75,000/ha.
- **Yield** about 35,000 kg / acre.

Legume fodder crops:

1. Berseem (*Trifolium alexandrium*):

- Also referred to as Egyptian clover.
- It is an annual shrub and grows to a height of 60 to 90 cm.
- Can be grown on all soils except on sandy soils.
- Cannot withstand drought.
- Ideal time of sowing is from last week of September to 1st week of October.
- **Seed rate:** 25-30 kg/ha.
- First cutting can be obtained in 50 to 60 days when the plants are about 20 to 30 cm in height.
- Next cutting can be obtained at 35 to 40 day intervals.
- **Yield** about 700 to 800 quintals / hectare.

2. Lucerne (*Medicago sativa*):

- Commonly known as alfalfa in USA.
- It is a perennial herb characterized by deep roots and grows to a height of 0.6 to 1.6 m.
- Can be grown under diverse climatic conditions but cannot thrive in alkaline soils.
- September to November months are ideal for sowing.
- **Seed rate:** 10 kg/ha.
- First cutting is obtained within 90 days and subsequent cuts after 30 to 40 day intervals.
- 8 to 10 cuttings can be obtained in a year.
- **Yield** about 335 to 450 quintals / hectare but with liberal manuring 800 quintals / hectare can be obtained.

3. Cowpea (*Vigna anguculata*):

- It can be used as a fodder crop as well as a green manure crop.
- It is not a season bound crop and can be grown in any season.
- Can be grown both on sandy and heavy soils.
- If the fodder is to be used as green manure crop, the first cut can be taken for feeding animals and the remaining stubble is ploughed to enrich the soil.
- Ideal as intercrop in young orchards.
- **Yield** about 90 to 120 quintals / hectare.

4. Pillipesara (*Phaseolus trilobus*):

- One of the popular legume fodder crops grown in coastal A.P.
- Can be used both as green fodder and as a manure crop.
- Can be cultivated in all soils from sandy loams to heavy black soils.
- It is a crop of 4 to 5 months duration.
- Best crop grown in rice fallows.
- Initial 2 cuttings will yield about 150 quintals green fodder / hectare after which the crop can be ploughed to be used as green manure.
- Excellent quality hay (CP 20 %) can be prepared from this fodder.

5. Sun hemp (*Crotalaria juncea*):

- It is an annual fodder.
- It is indigenous to India and most widely grown fibre crop next to jute.
- It can be grown as a green manure crop and forage crop.
- Grown as a Kharif and rabi crop.
- Commonly grown in rice fallows as 2nd crop after harvesting paddy crop.
- Can also be grown as inter crop in orchards.
- **Seed rate:** 28 kg/ha and the seed is broad casted.
- **Yield** about 20-35 tonnes / ha.
- Good quality hay can be prepared with 11.5% CP.

6. Horse gram (*Marcotyloma uniflorum*):

- One of the most widely grown legumes in India.
- Can be grown on any type of soil except alkaline.
- Can be grown as Kharif as well as rabi crop.
- **Seed rate:** 28-34 kg/ha.
- **Yield** about 9 tonnes/ha.
- Good quality hay with 10 – 15% CP can be prepared.
- Best for feeding of small ruminants.

Roughages

A. Crop residues

1.	Wheat straw	(*Triticum vulgare*)
2.	Paddy straw / Rice straw	(*Oyza sativa)*
3.	Maize straw	(*Zea mays*)
4.	Bajra straw / Kadbi	(*Pennisetum typhiodeum*)
5.	Jowar	(*Sorghum vulgare*)
6.	Ragi straw	(*Eleusine coracona*)
7.	Gram / Chick	(*Cicer arienum*)
8.	Groundnut haulms	(*Archis hypogaea*)
9.	Guar / Phalgati	(*Cyamopsis tetragonoloba*)

10. Moth / Dew bean (*Phaseolus aconitifolius*)
11. Black gram / Urad (*Vigna munga / Phaseolus radiates*)
12. Green gram / Mung (*Phaseolus munga)*
13. Soybean (*Glycine max)*
14. Mustard/ Rapeseed (*Brassica campestris*)
15. Arhar / Tur / Peageon (*Cajanus cajan / indicus*)
16. Horse grain / Kulthee (*Dolihos billows*)
17. Lentil / Masoor (*Lense ulinaris*)

B. Cultivated Fodder

1. Jowar (*Sorghum vulgare*)
2. Maize (*Zea mays*)
3. Bajra (*Pennisetum typhoideum*)
4. Barley (*Hordium vulgare*)
5. Oat (*Avena sinensis)*
6. Lucerne / Alfalfa (fodder queen) (*Medicago sativa*)
7. Bersocm- (King of fodder) (*Trifolium alexndrinum*)
8. Cow pea / Lobia (*Vigna sinesis*)

9. Cluster bean / guar (*Cyamopsis psoraloides/Tera gonalobus*)

10. M. P. Chari (*Sorghum biocolor*)

11. Senji / Indian clover (*Mellilotus parviflora*)

12. Methi (*Trigonella Foceum*)

13. Metha (*Trigonella polysoxta*)

C. Pasture grasses

1. Anjan / African fox tail (*Cenchrus ciliaris*)

2. Dhaman / Kala dhaman (*Cenchrus setigerus*)

3. Bhurut (*Cenchrus catharticus)*

4. Sewen (*Lasiurus sindicus*)

5. Murut (*Panicum tugidum*)

6. Guinea (*Panicum maximum*)

7. Bur (*Andropogan laniger*)

8. Lamp (*Aristida depressa*)

9. Karda / jerga (*Diacanthium annulatum*)

10. Doob / Bermuda (*Cynodon dactyla*)

11. Jharan (*Eleusine verticellata*)

12. Gantil (*Eleusine flegillifera*)

13. Makara (*Eleusine aegyptica*)

14. Bhoobra (*Eleusine sindica*)

15. Bandra (*Seteria gluca*)

16. Napier / Elephanta (*Pennisetum purpureum*)

17. Thin napier (*Pennisetum polystachyon*)

18. Dinanath (*Pennisetum pedicellatum*)

19. Kikuya (*Pennisetum clandestnum*)

20. Sudan (Sorghum sudanse)

21. Johnson / Baru (*Sorghum halepnese*)

22. Cocks foot (*Dactylus glomurata*)

23. Para (*Brachiaria mutica*)

24. Signal (*Brachiaria decumbens*)

25. Chotijergi (*Bothriochloa pertusa)*

26. Spear / Sarval / Sarvan (*Heteropogan contortus*)

27. Rhodes (*Chloris gayana*)

28. Pulongi (*Sporabolus orientalis / indicus*)

29. Charikachawablav (*Sporobulus pallidilus*)

30. Stylo (*Stylosanthes guianesis*)

31. Cham (*Chocharan antichocharan*)

32. Rye (*Lolium perenne*)

33. Tall fescue (*Festuca arundinacia*)

34. Jaragua (*Hyparrhenia rufa*)

35. Dallis (*Paspalum dilatatum*)

36. Reel canary (*Phalaris arundinacia*)

37. Teosinte / Mak chari (*Euchlanea mexicana*)

38. Purple moor (*Molinea caerulea*)

39. Bent (*Agrostis spp*.)

D. Shrubs, Herbs and Bushes

1. Ker (*Capris aphylla*)
2. Sinia (*Cotularia buyhia*)
3. Bui (*Aerua tomentosa*)
4. Khemp (*Leptidenia spartium*)
5. Phog (*Calligonum polyginoides)*
6. Aak (*Calatropis percera / zigentica*)

E. Other vegetation

1. Motha (*Ciprus ritandus*)
2. Bekeria (*Indigofara cordifolis*)
3. Kanti (*Tribulus tarristoris*)
4. Ghokru (*Tribulus allantus*)
5. Ghantia (*Tribulus)*
6. Matria / Water melon (*Citrullus valgaries*)
7. Kachar (*Citrullus lanatus)*
8. Dachab (*Cyperus ratendus*)

F. Top feed / Tree leaves

1. Khejri (*Prosopis cinelaria*)
2. Pardesi kheri / mesquite (*Prosopis juliflora*)
3. Babool (*Acacia arabica*)
4. Lzryl Babool (*Acascia tortalis*)
5. Kikar / Khair (*Acacia catechu*)
6. Jal (*Salvidora aeoides*)

7. Pelu (*Salvidora persica*)

8. Sirus (*Albizzia lebbeck*)

9. Sisum (*Albizza sisu*)

10. Neem (*Azardirachta indica*)

11. Burgad / Banyan (*Ficus bangalensis*)

12. Ardu (*Ailanthus ecelsa*)

13. Ber (*Zizyphus zujuba*)

14. Bari (*Zizyphus rotundifolia*)

15. Jhar beri / Pala (*Zizyphus numularia*)

16. Subabul / Ipill (*Leucena leucocephala*)

17. Bamboo (*Dendrocalamins strictus*)

18. Peepal (*Ficus religiosa*)

19. Mopen (*Chlorostyle mopen*)

20. Nutan (*Dicrostyle neutan*)

21. Teak (*Tictona grindis*)

22. Mango (*Mangifera indica*)

23. Pakar (*Ficus infectoria*)

Concentrate

A. Cereal grains

1. Maize (*Zea mayes*)
2. Barely (*Hordium vulgare*)
3. Oat (*Avena sativa*)
4. Bajra- Pearl millet (*Pennisetum typhoideum*)
5. Jowar- Great millet (*Sorghum vulgare*)

6. Ragi- Finger millet (*Eleusine coracana*)

B. **Mill by products**

Cereal by products

1. Wheat bran (*Triticum Vulgare*)
2. Rice bran (*Oryza sativa*)
3. Corn (*Zea mays*)
4. Sorghum gluten (*Sorghum vulgare*)

3.PREPARATION AND PROCESSING OF SAMPLES FOR CHEMICAL ANALYSIS - HERBAGE, FAECES, URINE AND SILAGES

Objective:

Objective of sample to be draw small amount of a representative material from bulk quantity.

Preparation and processing of samples for chemical analysis herbage, faeces, urine and silages

Biological material is not homogenous in nature. Therefore, to obtain representative sample for analysis, systematic procedures have to be followed.

Sampling of green fodders

Green fodders are collected from field or form bulk supply at various places randomly. Remove the contamination like soil and other material by gentle brushing. Never wash the plant material with water. Cut the sample immediately in small pieces and make thoroughly and sampled.

Sampling of silage

Collect the sample from different part of silo pit cut into pieces of 3 to 5 cm length with help of a hand chopper on clean surface. Then representative sample for analysis of nitrogen, pH, volatile acids, ammonia nitrogen and dry matter.

Sampling of concentrates

Concentrates from bags shall be collected at the time of loading and unloading of the cart or from godown. The following rule is to be applied for collecting samples:

No. Of bags in lot	**Minimum number of bags to sampled**
Upto 30 bags	All bags
31 to 100	30 bags
301 to 500 bags	30 bags + 10 % of excess

A metallic tubular slotted probe is used for collecting of samples from bags. The bag from which samples to be taken are identified at random on the basis of a simple N/n formula, where n is the number of total bags and n is the number of bags to be sampled.

Concentrates from bulk

Sample should be selected from various depths in case of bulk feeds. Preferable sampling can be done the time of when feed is moved out or in.

Quantity of consignment	**No. of spot from where sample to be drawn**
Upto 300 tons	30
301 to 1000 tons	50
1001 tons and above	100

Sampling of bulk feed ingredient is done either with a thermo sampler or a deep bin probe. Mix the sample thoroughly and determine its dry matter content. Grind the sample and preserve for further analysis.

The following preparations should be observed while drawing, preparing, storing and handling of samples.

- Sample should not be taken in a placed exposed to dampness, dust, breeze etc.
- The sampling probe should be clean and dry.
- The sampling material, sampling probe, sample container and sample should be free from contamination.
- The sample container should be sealed air tight and labeled accordingly.
- Sampling of ingredients should be placed in two sealed packets. Out of two sealed packets of sample one is sent for analysis and the other is retained for further reference.

Sampling of faeces

large animal

Mix daily collection on a thoroughly clean surface by hand. Take $1/100^{th}$ part of dm estimation and $1/500^{th}$ part of dm and $1/50^{th}$ part for nitrogen estimation. For nitrogen estimation

Preserve the sample in bottle with 40% w/v H_2SO_4 acid solution after through mixing. Stored the dried and ground faeces in bottles for further analysis.

Small animals

Mix the faeces pellets thoroughly and sub sample of 5% and 1% for dry matter & nitrogen analysis representative as above.

Poultry

Collect dropping on polythene sheets. Separate contamination like feathers and feet and mix thoroughly and take sub sample 5% and 1% for DM and nitrogen estimations respectively. For nitrogen determination samples should be preserved with 5% w/v H_2SO_4. Preserved dried & ground samples for further analysis.

Sampling of urine

Sampling of urine (5% to 20% depending on the size of the animal) are collected daily in bottles and evaporated of ammonia is prevented by adding about 5 ml of toluene or 25 ml of dilute sulphuric acid. The urine collected is mixed and take 200 ml sub sample for further analysis.

Milk sampling

Uniform and thoroughly mixes milk samples are taken in bottle and preserve by adding potassium dichromate or mercury chloride. The samples are stored in refrigerator with analyzed.

Processing of sample

The sample received in the laboratory is the first to be labelled.

Each packet of sample should contain the following information

- Name of sample
- Code number of sample
- Date of procurement
- Date of sampling
- Batch number in case of processed feeds
- Signature with date

Processing

A portion of the samples received in the laboratory may be subjected for moisture estimation. Take the rest of sample is dried in a hot air oven at 60°C, ground in mill to about 1mm particle size, mixed thoroughly and transferred into a clean dry air tight container for further analysis. In case of silages immediately after collection of sample, it is taken in a polythene bag.

Exercise question

- Why should the processed sample to be stored in an air tight container?

4. PREPARATION OF STANDARD AND PERCENTAGE S OLUTIONS AND REAGENTS USED IN NUTRITION LABORATORY

Standard solution: Solutions of accurately known strength are called standard solutions. A standard solution contains a known weight of reagent in a definite volume of solution.

Normal solution: Normal solution is one which contains one gram-equaivalent weight of the solute per litre of the solution. Normality of a solution is usually designated as **N**.

Determination of gram equivalent weight of an acid and an alkali:

Equivalent weight (EW) of a substance is its weight equivalent in reacting power to an atom of hydrogen. It is calculated as follows:

$$\text{EW of an acid} = \frac{\text{Molecular weight of an acid}}{\text{Basicity}}$$

Where, basicity of an acid is equivalent to the number of replaceable hydrogen atoms present in one molecule of the acid. Thus, EW of H_2SO_4 is 98/2=49 and of HCl is 36.46/1=36.46.

$$\text{EW of an alkali} = \frac{\text{Molecular weight of an alkali}}{\text{Acidity}}$$

Where, acidity of an alkali is equal to the number of replaceable hydroxyl groups present in one molecule of alkali. Thus, EW of NaOH is 40/1=40.

EW expressed in gram is called gram-equivalent weight.

Determination of gram equivalent weight of oxidizing agents:

Gram-Equivalent weight of an oxidizing agent is the weight of the substance which is equivalent to 8 g of available oxygen.

EW of $KMnO_4$ in acidic medium is determined as follows:

$2\ KMnO_4 + 3\ H_2SO_4 = K_2SO_4 + 2\ MnSO_4 + 3\ H_2O + 5\ (O)$

$2\ (39 + 55 + 64) = (5 \times 16)$

or

316 g = 80 g

Hence, 316 g of $KMnO_4$ will yield 80g of oxygen for oxidation. Therefore, 8 g of oxygen will be displaced by 31.6 g of $KMnO_4$.

Equivalent weight of $KMnO_4$ in alkaline medium is determined as follows:

$2\ KMnO_4 + H_2 = 2MnO_2 + 2\ KOH + 3\ (O)$

In this case, 316 g of $KMnO_4$ will yield 48 g of oxygen for oxidation.

$2\ KMnO_4 = 3(O) = 6H$

So, Equivalent weight of $KMnO_4$ in alkaline medium is:

316/48 x 8= 52.67

Molar solution:

Molar solution is one that contains one mole or one molecular weight in grams of a substance in each litre of the solution, whether the substance is in the form of molecules, ions or any other species. It is designated as **M.**

Molality:

Molality of a solution is the number of moles of solute per 1000g of solvent. It is designated as **m.**

Titration:

A form of analysis by means of comparison with solutions of standard strength.

Acid base Indicators:

Acid base indicators are those substances whose color in a solution is dependent on the pH of the medium. They are generally complex organic compounds of fairly high molecular weight. In water or other solvents they behave as weak acids or bases and thus participate in equilibrium reactions involving hydrogen ions. The change of color is due to internal structural re-arrangement which is responsible for indicator property.

$Indicator^- H$ ---------- H^+ + $Indicator^-$
(Acid)
$Indicator^- OH$ ---------- OH^+ + $Indicator^+$
(Base)

Some of the important titration and choice of indicators are given below.

Reactions of Indicators

pH range	***Indicator***	***Acid color***	***Base color***
3.1 to 4.4	Methyl orange	Pink	Yellow
4.2 to 6.2	Methy1 red	Red	Yellow
6.0 to 7.6	Bromothymol blue	Yellow	Blue
8.2 to 10.0	Phenolphthalein	Colorless	Pink
10.0 to 12.0	Alizarine yellow	Yellow	Violet

Use of indicators

Acid	***Alkali***	***Indicator to be used***
Strong	Strong	Any indicator can be used
Strong	Weak	Methyl orange (or) methyl red
Weak	Strong	Phenolphthalein

Standard solutions

Few standard solutions which are used in proximate analysis of feedstuffs are

1. N/10 H_2SO_4
2. N/10 NaOH
3. N/10 $KMnO_4$
4. 1.25% H_2SO_4 (w/v) or 0.256 N H_2SO_4
5. 1.25% NaOH (w/v) or 0.313 N NaOH

Other solutions used commonly in nutritional analysis of feed stuffs are

1. 40% NaOH (w/v)
2. 3% KNO_3 (w/v)
3. 20% Ammonium molybdate (w/v)
4. 50% HCl (w/v)

Certain primary standard solutions are also required for standardization of above solutions. These are:

1. N/10 Na_2CO_3
2. N/10 oxalic acid [$(COOH)_2.2H_2O$]

Preparation of N/10 Na_2CO_3

Calculations

MW of Na_2CO_3 = 106 g

$Na_2CO_3 + 2\ HCl = 2\ NaCl + H_2O + CO_2$

So, acidity of $Na_2CO_3 = 2$

EW of $Na_2CO_3 = 106/2=53$ g.

Therefore, 5.3 g Na_2CO_3 is required for each litre of solution to make N/10 Na_2CO_3. Since it is hygroscopic, it must be made perfectly anhydrous before it is weighed.

Procedure:

Take 6.7g of Na_2CO_3 (AR) in crucible and heat it in a hot air oven at about 100^0C for few hours so as to drive out any moisture and to convert any preformed $NaHCO_3$ to Na_2CO_3. Cool in a dessicator and weigh exactly 5.3g dried salt and dissolve it in a little quantity of freshly boiled distilled water. Transfer it to one litre volumetric flask and make volume up to the mark. Shake well and label it as 0.1N Na_2CO_3 solution.

Preparation of N/10 oxalic acid

Calculations

Oxalic acid [$(COOH)_2$. $2H_2O$] has molecular weight of 126 and basicity 2. So, EW of oxalic acid = 126/2=63g

Thus, weigh exactly 6.3g of $(COOH)_2$. $2H_2O$ and transfer it to a volumetric flask (1litre capacity) half filled with distilled water. Shake well and make the volume up to the mark. Label it as N/10 oxalic acid solution.

Note: If anhydrous $(COOH)_2$. $2H_2O$ is available then dissolve 4.5 g of the acid in one litre of distilled water to get 0.1N oxalic acid solution.

PREPARATION OF STANDARD SOLUTIONS

Procedures for the preparation of commonly used standard solutions in Animal Nutrition laboratory are furnished below.

1. Preparation of N/10 H_2SO_4 solution.

EW of H_2SO_4 = 49g

Specific gravity = 1.84 g/ml

So, volume of 49g H_2SO_4 = 26.6ml

Concentrated H_2SO_4 (Reagent grade) is about 97% pure.

Therefore, actual amount of concentrated H_2SO_4 required for one litre of 1N H_2SO_4 solution = 100/97 x 26.6 = 27.42 ml. Thus, for one litre of N/10 H_2SO_4 solution, 2.74 ml concentrated. H_2SO_4 is required.

Procedure

Take 2.74 ml of sulphuric acid in a beaker half-filled with distilled water. Transfer the contents and washings to a volumetric flask (1 litre) and make volume up to the mark. Shake well and titrate this solution with 10 ml of 0.1 N Na_2CO_3 using methyl orange as indicator. Repeat the titrations to get at least three concordant readings.

Standardization

Suppose 10 ml of 0.1 N Na_2CO_3 = 9.5 ml of N/10 H_2SO_4

$V_1N_1 = V_2N_2$ $N_1V_1 = N_2V_2$

or, 10 x 0.1 N = 9.5 x N_2 0.1 x 1000 = 0.10526 x V_2

or, N_2 = 0.10526

To prepare 1 litre N/10 H_2SO_4, the volume of 0.10526 N acid required is 100 x 0.1/0.10526 = 950 ml of 0.10526 N acid which should be diluted to one litre. Check it again with N/10 Na_2CO_3 for three times. It must neutralize equal volume of N/10 Na_2CO_3 solution. Label it as 0.1 N H_2SO_4 solution.

Precaution: Never add water to an acid.

2. Preparation of N/10 NaOH solution

Calculations:

Molecular weight of NaOH = 40g

Acidity (number of replaceable OH group) = 1

Equivalent weight of NaOH = 40g

Therefore, 4 g of NaOH dissolved in one litre solution will give N/10 NaOH solution.

Procedure:

Weigh quickly 4 g of NaOH in a beaker (as it is hygroscopic) and dissolve it in dissolve it in distilled water. Transfer the contents and the washings to a volumetric flask (1 litre). Cool and then make volume upto the mark. Shake well and standardize this solution against N/10 oxalic acid using phenolphthalein as an indicator. Label it as 0.1N NaOH solution.

3. Preparation of N/10 $KMnO_4$ solution:

Dissolve 3.2 g of $KMnO_4$ in one litre of distilled water. Boil it for 10-15 minutes and then allow standing for few days and then filtering it through glass wool.

Take 10 ml of N/10 oxalic acid in a beaker. Add 5 ml of sulphuric acid, warm it to 60-70°C and titrate against $KMnO_4$ from the burette till a light pinkish colour appears. Take three concordant readings.

Suppose, 10 ml 0.1 N oxalic acid = 9.75 ml of $KMnO_4$

$$10 \times 0.1\ N = 9.75 \times N_2$$

$$N_2 = \frac{10 \times 0.1\ N}{9.75}$$

$$= 0.10256$$

To prepare 1000 ml of 0.1 N $KMnO_4$ solution, the volume of $KMnO_4$ to be taken:

$$\frac{1000 \times 9.75 \times 0.1}{10 \times 0.1} = 975 \text{ ml}$$

Now take 975 ml of prepared $KMnO_4$ solution and make it to 1000 ml by adding distilled water.

Note

- Ordinary or even pure distilled water contains traces of organic matter which reduces the $KMnO_4$ solution. That is why the solution is boiled and kept for some time for standardization.

- In the absence of sufficient amount of dilute H_2SO_4 or due to the rapid addition of $KMnO_4$ in titration flask, brown turbidity (manganous oxide) may appear.

EXERCISE

1. Prepare 1 litre of N/10 sulphuric acid standard solution.
2. Prepare 1 litre of N/10 of sodium hydroxide standard solution.

PREPARATION OF COMMON REAGENTS AND INDICATORS

The following two reagents are used in proximate analysis in addition to previously mentioned standard solutions.

A. PREPARATION OF REAGENTS

1. Preparation of 1.25 % (w/v) H_2SO_4 solution

Calculations

To prepare 1.25 % (w/V) H_2SO_4 solution, 12.5 g of H_2SO_4 (100%) is to be added to distilled water to make up the volume of 1000 ml.

$$\text{Volume of } H_2SO_4 \text{ to be taken} = \frac{12.5 \times 100}{1.84 \times 97} = 7 \text{ ml}$$

Procedure

Add 7.0 ml of concentrated H_2SO_4 (specific gravity 1.84 and 97% concentration) in a 1000 ml volumetric flask half-filled with distilled water. Shake well and add distilled water to make volume up to the mark.

2. Preparation of 1.25% of NaOH solution

Add 13.16 g of NaOH (95% NaOH) in one litre distilled water and shake well.

B. PREPARATION OF INDICATORS

1. **Methyl red:** It is a standard solution in 50% absolute alcohol.
2. **Methyl Orange:** 0.5% solution in distilled water.
3. **Methylene blue:** Dissolve one standard tablet of Methylene blue (BDH) in 200ml of previously boiled water. Keep in a coloured bottle in a cool and dark place.
4. **Methyl violet or Gentian violet:** It is a 1% solution in 50% absolute alcohol.

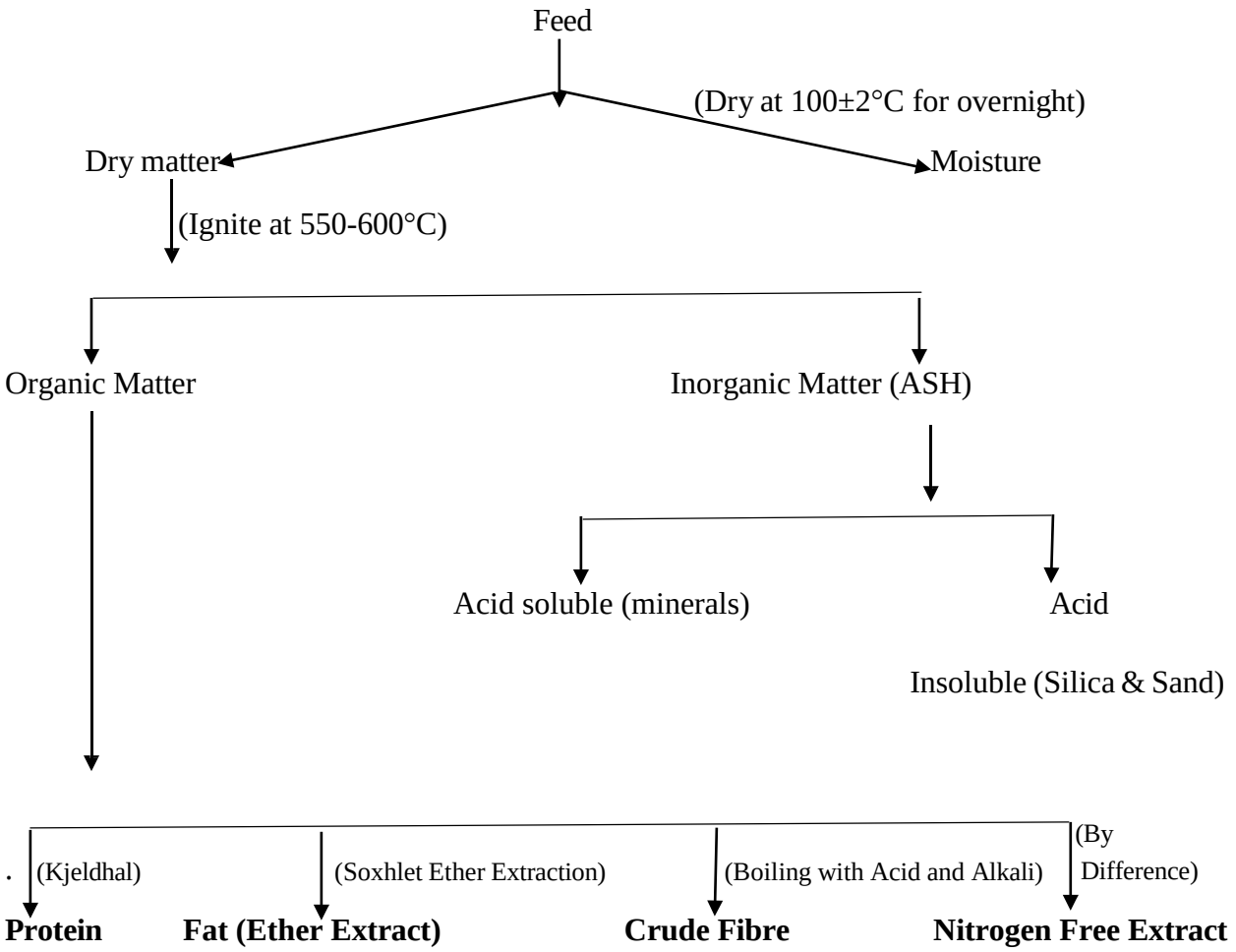
5. **Phenolpthalein:** It is a 1% solution in absolute alcohol.
6. **Bromo-cresol (green or purple):** It is a 0.1% solution in alcohol.

EXERCISE

1. Prepare 1 litre of 1.25% solution of H_2SO_4.
2. Prepare 1 litre of 1.25% solution of NaOH.

5.WEENDE'S SYSTEM OF ANALYSIS

- The system consists of determination of water, ash crude fat (ether extract), crude protein and crude fiber.
- The proximate analysis scheme for routine description of animal feedstuffs devised by Henneberg and Stochmann (1865) of the Weende experiment station in Germany. It is often referred to as the Weende's system and was principally devised to separate carbohydrates into two fractions: crude fiber and nitrogen free extract (NFE).
- Proximate system has some failings, which prevent it from being an extremely valuable predictive aid, and considerable research has been conducted in recent years to refine it.
- Nitrogen free extract (NFE) is not determined actually. It is calculated.
- NFE represents the soluble fraction of carbohydrate consisting of sugar, starch, and glycogen and to some event hemicelluloses.



Limitation

- NFE is not estimate by lab method, but by formula. Crude fiber value is not perfect because some hemicelluloses dissolve in acid and alkali boiling.
- Ash gives no indication of chemical element in it.
- Method not included vitamin content.

Exercise Question

- Find out the proximate principle of given sample.

6. ESTIMATION OF DRY MATTER IN FEED SAMPLES

Dry matter or, more specifically, moisture determination is an important analysis, in determination of concentration of other nutrients that is usually expressed on a dry matter basis (as a percentage of the dry matter).

Common methods of dry matter analysis

Method	Occasions for use
100˚C drying	Most mixed feeds, hays, range grasses with 85 to 99% DM
Freeze drying	High- moisture, fermented feeds
Saponification	High-moisture, fermented feeds
Vaccum drying	Meat or tissue samples
Toluene distillation	Silages

Note: High- moisture feeds usually contain volatile nutrients that can be lost with 100°C oven drying.

Apparatus

- Tong
- Desiccators
- Weighing balance
- Spatula
- Porcelain crucible / Petridish
- Hot air oven

Procedure

- Take a clean dry petridish and find out its weight. Take about 5 to l0g of feed, place it in the petridish and cover the petridish. Find out the weight of Petridish with the feed.

- The temperature of hot air oven is to be kept at 105°C to 110°C.
- Place the petridish in the hot air oven partially opening the lid at 105°C to 110°C for 12 h or overnight
- Cool in desiccator, and record weight with dried feed.
- Again, place the petridish in hot air oven; continue the drying for another 30 minutes.
- Due to loss of water, there will be a reduction in the weight of substance. This is taken as moisture content of the feed.

Calculation

- Weight of petri dish = A g
- Weight of petri dish + Feed sample = B g
- Weight of petri dish + Dried material = C g

$$\%\,\text{Moisture in feed} = \frac{B - C}{B - A} \times 100$$

- Dry matter content of feed (%) = 100 – % of moisture

Exercise question

- Find out the moisture content of Neem leaves

7. ESTIMATION OF TOTAL ASH IN FEED SAMPLES

Ash will contain many contaminating materials like sand or soil, which may inflate a sample's ash value. Generally, Ashing is a preparatory step for further analysis of specific minerals by spectrophotometric or atomic absorption techniques.

Principle

Ash is inorganic ignition of biological material at 500-600°C in a muffle furnace. Organic matter is oxidized & inorganic matter remains.

Materials

- Silica crucible
- Muffle furnace

Procedure

- Take the weight of a clean dry silica crucible.
- Place about 2 to 5 g of the sample and weigh this to find out accurate weight of the sample taken. After drying of sample in hot air oven and Cool in desiccators.
- Place pans plus samples in muffle furnace and ash at 500°C for 3hrs.
- Cool in muffle for at least 8 hrs. Then in desiccators.
- Weigh the silica crucible with ash.

Observation

- Weight of empty silica crucible = W_1 gm
- Weight of silica crucible + feed sample = W_2 gm
- Weight of silica crucible + ash = W_3 gm

Calculation

$$\text{Total ash \% (DMB)} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

Precaution

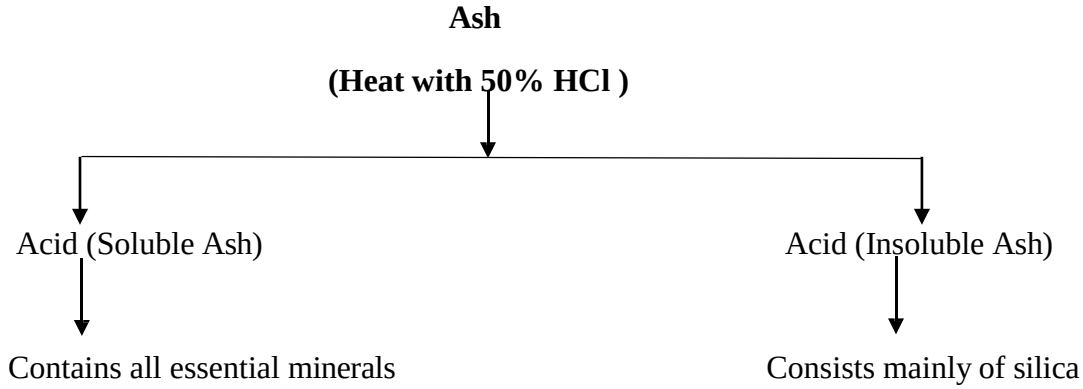
- The ash is highly hygroscopic and thus weighing should be done quickly.

Exercise question

- What fraction of feed is represented by Total Ash?

8.ESTIMATION OF ACID INSOLUBLE ASH IN FEED SAMPLES

Introduction



Significance of insoluble ash in feed sample

- The insoluble fraction mainly consists of silica and other impurities.
- The presence of high insoluble fraction is measure of impurity and adulteration.
- In case of certain feed like rice bran, the high value of total ash is due to the large fraction of insoluble ash, mainly due to the presence of sand.

Object

- Preparation of soluble fraction of the total ash for estimation of minerals.
- To determine the percentage of insoluble ash in the feed.

Principle

The total ash is treated with dilute acids to obtain the soluble fraction of the ash that is separated from the insoluble residue by means of filtration. The filter paper carrying insoluble ash is ignited and weighed for finding the insoluble ash.

Reagents

- Nitric acid (HNO_3)
- Hydrochloric acid (HCI)

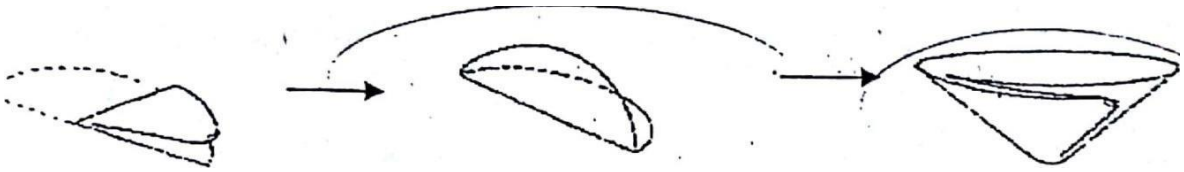
Apparatus

- Muffle furnace

- Weighing balance
- Desiccator
- Beaker
- Crucible
- Whatsman filter paper

Procedure

- Add 20 ml of 50% HCL to the ash and heat the contents in water bath for 10 min.
- After heating transfer the contents to 250 ml beaker along with washing till the crucible is free of acid.
- Boil the contents for 30 min. and filter through Whatsman filter paper.
- Make the volume filtrate to250 ml by adding distilled water.
- Keep this prepared solution of acid soluble ash as a solution for taking aliquate for estimation of calcium and phosphorus.



Calculation

- Weight of crucible + insoluble ash = Z
- Weight of empty crucible = X
- Weight of insoluble ash = Z-X
- Weight of feed taken for ashing = Y-X (Previous exercise)
- Weight of feed taken for ashing + Weight of empty crucible= Y
- **% of insoluble ash =** $\frac{Z - X}{Y \quad X} \times 100$

Exercise question

- Why should you obtain the soluble fraction of total ash?
- What is the significance of insoluble ash in a feed sample?

9.ESTIMATION OF CRUDE PROTEIN IN FEED SAMPLES

Kjeldhal method

Principle

In a feed sample organic nitrogen when digested with concentrates sulphuric acid in the presence of a catalyst (K_2SO_4 & $CuSO_4$ in 9.5:0.5 ratios). NH_3 liberated by making the solution alkaline in form of $(NH_4)_2SO_4$. This solution when distilled into a known volume of standard acid through chemical trapping reactions (ammonium borate) which is than back titrated protein content is obtained by multiplying % nitrogen value with factor 6.25.

- Digestion mixture /catalyst made up of K_2SO_4 and $CuSO_4$ in 9.5:0.5 ratios: The K_2SO_4 is added to raise the boiling point of effective oxidation of material and is added as a catalyst to speed up the reaction.

Apparatus required

- Kjeldahal flask
- Tilt measure
- Beaker
- Distillation apparatus
- Burette
- Volumetric flask
- Electric hot plate/electric heater

Chemicals

- Concentrate H_2SO_4
- Saturated NaOH 40%
- Tesirose indicator (methyl blue and methyl orange)
- N/10 HC1

Procedure

It is divided in 3 parts

- Digestion
- Distillation
- Titration

Digestion

- Take 2-3 gm of dried feed, sample in a dry kjeldahl flask.
- Add 20-30 ml concentrate H_2SO_4 (10 m1/gm feed)
- Add 2.5 gm digestion mixture.
- Place the flask in the digestion electric heater and heat till the solution become clean transparent blue.
- Off the electric switch, remove the flask from the heater and cool it.

Distillation

- Add 5-10 ml distilled water into the Kjeldahl flask and transfer the solution into a 100 m1 (as for desire) volumetric flask.
- 3-5m1 rinsing of such type should be followed by addition of distilled water into volumetric flask, make the volume 100 m1. This is called as aliquate.
- Take 10 m1 (desire volume of aliquate) of aliquate in another Kjeldahl flask distillation assembly.
- Add saturated NaOH solution, 20-40 m1 (40% NaOH) in a just a sufficient volume of NaOH makes the solution alkaline.
- Set the conical flask contain 2% boric acid (Tashiro's indicator mixture) in the last end of the condenser.
- Now switch on the heater to allow the distillation of 10-15minutes.
- At the end of distillation nitrogen trapped in the form of $(NH_4)_2SO_4$. Nitrogen is released in the form of ammonia and trapped by 2% boric acid solution by cooling NH_3 gas by condenser.

Titration

- Remove the conical flask after washing the tip of condenser with distilled water into the flask.
- For the back titration, boric acid solution containing NH_3 the titrated standard N/10 HCl or H_2SO_4 (ammonium borate — green color). Titration complete when violet or pink color appear.
- The cud point is reach to change the color green to pink/violet.
- Record the volume of solution used in titration to calculate the volume of, standard H_2SO_4 used ammonia approx.

Calculation

$$\% N_2 \text{ (nitrogen)} = \frac{V \times 0.0014 \times d}{W} \times 100$$

% Crude Protein (CP) = % Nitrogen X 6.25

NOTE- plant protein contains 16% nitrogen (100/16 = (6.25)

Where

V= Volume of standard solution of acid used (N/10 H_2SO_4)

0.0014 =Conversion factor

d =--- Dilution factor= 100/10=10

W= Weight of sample in gm.

Exercise question

- What is the role of catalyst during digestion? • Give examples of animal protein or vegetable protein sources.

10. ESTIMATION OF ETHER EXTRACT IN FEED SAMPLES

Soxhlet method

Crude fat or ether extract is estimated by extracting the feed sample using continues evaporation and condensation of fat solvent like petroleum ether, diethyl ether, benzene, hexane etc. In special made extraction apparatus that is Soxhlate apparatus. Diethyl ether extract include all fraction of which is soluble in ether is simple fat and neutral fat compound. Fatty acids, esters, chlorophyll, steroids, carotenoids, waxes, and fat soluble vitamins.

Apparatus

- Soxhlate apparatus
  a) Condenser
  b) Extraction tube
  c) Oil flask
- Thimble
- Desiccators
- Analytical balance

Reagents

- Petroleum ether or any other solvent

Procedure

- Weight a clean and dry oil flask.
- Weight of dry sample in aluminum scoop and transfer it in to an extraction.
- Thimble made up What's man filter paper.
- Place the thimble carefully in extraction tube and fit soxhlate oil flask to the extraction. Pour sufficient petroleum ether in to the extractor along its sidewall until it begin to siphon.
- Again poured ether to fill the extractor through about and half (1/2).
- Connect the condenser through extractor**.**
- Switch on the heating unit and allow the extraction to proceed at rapid rate.

- Run equipment for about 6-8 hrs.
- After extraction is over switch off the heater.
- After some times when vaporization of ether stop carefully remove the thimble in such way that no sample material is scattered out.
- After taking out the thimble reunite the assembly.
- Switch on the heater to collect ether as much as possible.
- The level of ether and extractor must be below the siphon end to prevent the re-siphon the ether in to oil flask.
- This collected ether may be used subsequently.
- After recovery of solvent oil flask the oil flask is taken out the assembly and transfer to the hot air oven. Keep the oil flask in to the oven for complete evaporation of solvent at 100°C.
- After drying transfer the oil flask in to the desiccators for cooling to room temperature.
- Weight the oil flask to constant weight and record the weight of flask and fat after drying.
- Simultaneously dry the thimble in hot air oven to constant weight and preserve the sample for crude fibre estimation.

Observation

- Weight of sample = W_1 gm
- Weight of oil flask (empty) = W_2 gm
- Weight of oil flask and fat after extraction = W_3 gm

Calculation

Weight of crude fat (W_4) = $W_3 - W_2$

- % Ether Extract $= \frac{W_3 - W_2}{W_1} \times 100$

Exercise question

- Why the estimation is called as estimation of crude fat?
- What vitamins are found in fat fraction of feeds?

11. ESTIMATION OF CRUDE FIBRE IN A FEED / FAECES SAMPLE

Objective

Estimation of crude fiber in a feed / faeces sample. Crude fiber includes which are undigested by monogastric animal. eg- pig, poultry etc. it consists of cellulose, hemicelluloses and highly variable proportion of lignin along with minerals.

Principle

The estimation is based on treating the moisture and fat free sample successively with dilute (1.25%) acid or alkali (1.25%).

Apparatus require

- Spoutless beaker (1 liter capacity)
- Round bottom condenser
- Measuring cylinder
- Buchkner's funnel
- Hot air oven
- Hot plate
- Muffle furnace
- Silica glass crucible
- Muslin cloth
- Desiccators

Reagent

- 1.25% W/V H_2SO_4
- 1.25% W/V NaOH

Procedure

- Weight about 2 gm moisture and fat free sample is taken and transfer it into the spoutless beaker and add 200 m1 of 1.25 % w/v H_2SO_4 solution to the beaker and place it on heat and allow to boil for 1 hour.
- Shake the content after every 5 mint.
- After boiling for l hour remove the beaker from hot plate and filter through the muslin cloth.

- Wash the residue from tap water till free from acid and transfer the material into the same beaker.
- Add 200 m1 of 1.25% w/v NaOH solution again boil content for 1 hr.
- Transfer the residue into crucible and placed into hot air oven @ 100±5°C to dry to a constant weight.
- Ignite the residue in the muffle furnace at 550-600°C for 4-6 hrs. Then cool and weight again.
- The loss of weight due to ignition is the weight of crude fibre.

Calculation

$$\% \text{ Crude Fiber} = \frac{W_1 - W_2}{W} \ldots\ldots\ldots\ldots\ldots\ldots 100$$

Where -

- W_1= Weight of crucible + Residue before ashing
- W_2 = Weight of crucible + After ashing
- W = Weight of dry sample
- Precautions
- Keep the volume constant during boiling.
- Avoid foaming by heat adjustment.

Exercise question

- What fraction of carbohydrates does the crude fibre represent

12. DETERMINATION OF NFE (NITROGEN FREE EXTRACT) IN FEED SAMPLES

Introduction

- Nitrogen free extract (NFE) represents the soluble carbohydrate fraction of the feed.
- In the Weende's system of analysis NFE is not estimated but calculated.

Objective

- To calculate the nitrogen free extract content of the feed.

Principle

- Nitrogen free extract (NFE) represents the soluble carbohydrate fraction of the feed. In the Weende's system of analysis NFE is not estimated but calculated.

Procedure

- NFE on as feed basis = 100 – (Moisture + Crude protein +Ether extract + Crude fibre + Total ash)
- NFE on dry matter basis = 100 – (Crude protein +Ether extract + Crude fibre + Total ash)

Exercise

- Calculate the NFE of fish meal containing crude protein 46%, ether extract 5%, crude fibre 1% and total ash 13%.

13. DETERMINATION OF CALCIUM IN FEED SAMPLES

Objective

To determine the percentage of calcium in the given feed sample.

Principle

Calcium is precipitated in acidic medium as insoluble calcium oxalate by adding saturated ammonium oxalate solution. The precipitation is dissolve in dilute H_2SO_4 heated and the oxalic acid thus released is titrated against standard $KMnO_4$ solution. In warm condition (70°C) to get calcium content of sample.

The calcium is determined volumetrically by titrating the solution with standard $KMnO_4$.

$2KMnO_4 + 3H_2SO_4 + 5\,H_2C_2O_4 + K_2SO_4 + 10\,CO_2 + 8H_2O$

Apparatus

- Beaker(100 m1)
- Burette
- Pipette
- Hot plate
- Funnel
- Bottle
- What's man filter paper (no.40)

Reagent

- Concentrate HCI
- Ammonium oxalate solution (saturated)
- Concentrate H_2SO_4
- N/10 $KMnO_4$ solution [3.16 gm. $KMnO_4$ of per lit.)
- Concentrate NH_3 and dilute NH_3
- Methyl red indicator

Procedure

- Take 25m1 aliquot from stock solution of acid soluble ash in 250 ml beaker.

- Add 50 ml of distilled water to beaker and add 10 ml of saturated ammonium oxalate solution and 10 ml of concentrate HCI simultaneously to beaker.
- Add 2 drop of methyl red to the beaker.
- Now adjust the acidity of solution to pH 4.6 by adding drop by drop concentrate solution till a brown orange colour precipitate begins to appear.
- Add dilute NH_3 solution [1:4] drop by drop till a white ppt appears.
- Keep the contents of beaker over night to allow the precipitant to settle down. On the next day filter the supernatant through what's man filter No.40. Take care that maximum precipitate should remain in it.
- Wash the precipitate several times and hot distilled water then transfers filter paper along with precipitates to same beaker and acid 100 ml of hot distilled water.
- Add 10 ml of concentrate H_2SO_4 to dissolve the precipitate and heat the solution 60 -70°C.
- Titrate the solution against 0.1 N $KMnO_4$ until a stable pink colour appears.

Calculation

- **$Ca\% = \frac{\text{ml of 0.1 N } KMnO_4 \text{ used X 0.002 X 10 X 100}}{\text{gm of sample taken for ashing}}$**

Exercise question

- What is the Calcium content of the following:
    1. Bone meal:
    2. Mineral mixture for Poultry
    3. Fish meal

14. DETERMINATION OF PHOSPHORUS IN FEED SAMPLES

Objective

- To determine the percentage of phosphorus in the given feed sample

Principle

- Phosphorus is precipitate as yellow precipitate of phosphor – ammonium - molybdate by adding ammonium molybdate solution and concentrate HNO_3. Precipitate is washed and dissolved in a measured volume of 0.1N NaOH excess is titrated is back with 0.1 N H_2SO_4.

Apparatus

- Beaker, pipette, burette, funnel and whatsman filter paper 40, and 42

Reagents

- Ammonium molybdate solution
- Conc. HNO_3
- HNO_3 3%
- N/10 NaOH
- N/10 H_2SO_4
- Phenolphthalein indicator

Procedure

- Take 25 ml of aliquot in a beaker of 250 ml from stock solution of acid soluble ash.
- Add 10 ml cone. HNO_3 and 10 ml of freshly pipette saturated ammonium molybdate solution
- At this stage yellow precipitate of phosphor ammonium molybdate begins to appear.
- Kept beaker over night to allow the precipitate to settle down.
- Filter the supernatant to the whatsman filter paper 40 and 42 then wash the precipitate 2-3 times with 2% HNO_3 and several times with 3% KNO_3 solution until the precipitate become acid free.
- Transfer the filter paper which contains some precipitate to the same beaker and residues of precipitate should be dissolved in 25 or 50 ml of N/10 NaOH solution.
- Add two drops of Phenolphthalein indicator to the beaker.
- Excess of NaOH solution is titrated with N/10 H_2SO_4 solution.

Calculation

$$P\% = \frac{\text{ml of 0.1 NaOH used X 0.0001347 X 10 X 100}}{\text{Gm of sample taken for ashing}}$$

Here

0.0001347.7 = conversion factor

10 = dilution factor (250/25)

ml of N/10 NaOH used = (ml of N/10 NaOH taken to dissolve the precipitate) — (titrated volume)

Exercise question

- List out few ingredients that are rich sources of phosphorus?

15. DEMONSTRATION OF DETERGENT METHOD OF FORAGE ANALYSIS

Introduction –

Partition of carbohydrate of forage by weende's system of proximate analysis of CF is not realistic either chemically or nutritionally. This CF method estimate the proportion of CHO that resist digestion when boiled in dilute H_2SO_4 followed by subsequent boiling with dilute alkali which leads to error of estimation of the CF in feed. In addition it was also observed in some cases that CF was more digestible than NFE because lignin that part of NFE.

To overcome this limitation a rapid method of partitioning of feed CHO in to fraction based on nutritional availability was developed by Von-Soest and his associate in 1967. In dry matter of forage is divided in to cell content of neutral detergent soluble (NDS) and cell wall constituents of neutral detergent fibre (NDF), the first fraction that is cell content that is highly digestible and is related to cellular content consisting of soluble carbohydrate, protein, NPN, and other water soluble substances.

Whereas the second fraction correspond the plant cell wall constituents consisting, of plant cellulose, lignin, silica and fibre bound nitrogen.

Estimation of neutral detergent solution and cell content

Principal

Refluxing the sample with neutral detergent solution (NDS) followed by filtering and washing with hot water as well as acetone leads to solubilization of cell content and the remaining material after drying is neutral detergent fibre (NDF).

The method utilized detergent which complex with protein to render it soluble and utilizes a chelating agent EDTA to remove heavy metals.

Apparatus

- Refluxing apparatus with 6 heating condenser.
- Filtering device / solution pump.
- Gooch crucible coarse (capacity- 15 m1)
- Berzelius beaker without spout (1000 m1)

Reagent

- Neutral detergent solution

Composition

a. Distilled water 1 liter
b. Sodium lauryl sulphate -30gm
c. EDTA: solution salt -18.61 gm
d. Sodium borate dehydrate-6.81 gin
e. Disodium hydrogen phosphate -4.56gm 0 2
f. Ethoxy ethanol-10 ml
g. Acetone
h. Amylas

Preparation

- Dissolve sodium lauryl sulphate in distilled water and add 2-ethoxy ethanol.
- Place EDTA and borax in to a large beaker flask and add some distilled water and heat until dissolve.
- After heating add to the solution containing Na-lauryl sulphate and 2-ethoxy ethanol.
- Place disodium phosphate in a beaker add sortie distilled water and heat until dissolve mixed with the solution containing other ingredients.
- Adjust the pH between 6.9-7.1 using Na0H / HCI.

Procedure

- Weigh 0.5gm .sample, grind to pass a 1mm screen and add into 1 lb capacity Berzelius beaker (spoutless)
- Add 100 m1 NDS
- Place beaker on refluxing unite and start the heater
- Reflux for 60min for onset of boiling place weighed gooch crucible on the filtering device and rinsed with hot water.
- If the smooth filtration doesn't occur, add 1 -2ml amylase enzyme solution to crucible and add 30 ml boiling H_2O.
- Allow to stand the whole mixture for 5-10 mints.

Observation

- Weight of sample — W_1 g
- Weight of crucible W_2 g(empty)
- Weight of crucible + fibre W_3 g

$$\%NDF = \frac{(W_3 - W_2)}{W_1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 100$$

%Cell wall content = 100 - % NDF

Determination of ADF

The extraction of plant material with AD Solution by refluxing for 1 hr and filtration afterward gives acid detergent fibre.

Apparatus

- Refluxing apparatus with 6 heating unit condenser
- Filtering device
- Gooch crucible with course porosity (50m1)
- Berzelius beaker without spout (1000m1)
- Vacuum system

Reagent

- Acid detergent solution
- Composition= 1N H_2SO_4 + Cetyl trimethylammonium bromide (20gm)
- Acetone

Procedure

- Weight 0.5gm air dried sample ground to pass through 1mm screen and place in to a Berzelius beaker.
- Add 100m1 AD solution to beaker
- Place beaker on reflux condensing
- Heating unit & start beating
- Reflux for 60 min from the onset of boiling

- After 60 min of boiling switch off the heater and take out the beaker from refluxing unit.
- Weight clean dry Gooch crucible of 50 ml capacity.
- Take the weight of crucible (two) on filtering device and drain with hot water.
- Filter the content of beaker trough good crucible using vacuum system.
- Wash crucible twice with hot water and mince the inner side of the crucible with hot water same day.
- Wash the residue with acetone repeatedly until filter liquid is colorless.
- Remove crucible from filtering device and place in air for 15 min so that acetone may escape then place it in oven for overnight at 100 ± 5˚C for drying.
- After drying cool the crucible in the desiccator to room temperature and weighed to constant weight.

Observation

- Weight of dried sample = W_1 g
- Weight of Crucible = W_2 g
- Weight of crucible + residue after drying = W_3

Calculation

$$\%ADF = \frac{(W_3 - W_2)}{W_1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 100$$

16. QUALITATIVE DETERMINATION OF UNDESIRABLE CONSTITUENTS AND COMMON ADULTERANTS OF FEED

- The following physical characteristics of the feed ingredients should be considered for determination of feed quality.
- The ingredients should possess their characteristic color, odour and texture.
- Presence of adulterants.
- The ingredients should be free from insect, mite and mould infestation.
- There should not be any broken / damaged seeds /grain or any foreign material.
- The ingredients should not be damp. The dampness can be detected by pressing the ingredients in the hand.
- The raw ingredients may contain some undesirable constituents or adulterants. This will have an influence on the nutritive value of the feed or may cause harm to the animal.

The adulterants present in the feed can be widely grouped as-

1. **Accidental present adulterants**

   Most of the feed ingredients for livestock are agricultural or allied products / by-products. During the course of their processing many unwanted materials such as husk, cobs, hulls, stones, mud, pebbles, sand and weed seeds can get accidentally incorporated. These are called as accidental adulterants. The presence of these adulterants may increase the crude fibre / silica contents of the ingredient and thereby reduce the digestibility and nutritive value of the ingredient.

2. **Intentional added adulterants**

   As a fraudulent practice in order to make more profit the wholesale dealers/ retailers may intentionally add husk, cobs, hulls, stones, mud, pebbles, sand, weed seeds and also some chemical substances like urea to increase the weight or nutritive value by default. The chemical characteristics should also be considered to determine the soundness of the ingredients.

Objective

To test the presence of adulterants in the feed.

Procedure for detection of adulterants

Whenever raw ingredients are purchased a representative sample should be drawn out using the standard procedure. The sample is further screened for the presence of adulterants as follows:-

- A handful of the sample is taken and spread over a white paper on a table.
- It is examined under sufficient natural or artificial light.
- The ingredient is methodically counted and pushed to one side looking for the presence of adulterants which are also counted.
- Based on the count the proportionate presence of adulterants can be arrived at.
- To detect chemical adulterants sample has to be sent laboratory.

Test for Urea

Reagents

- Urease solution – Dissolve 0.2 g of urease powder in 50 ml of distilled water.
- Standard urea solution (0, 1, 2 …5%)
- Cresol red indicator (0.1%)

Procedure

- Weigh 10 g of test sample and add 100 ml of distilled water. Stir and filter with Whatsman No. 4
- Pipette 2 ml of standard solution and test sample into white porcelain spot plates.
- Add 2 – 3 drop of cresol red indicator and add 2 – 3 drops of urease solution.
- Let it stand for 3 – 5 minutes, if urea is present, it will form a deep red purple spreading like spider's web appearance, in contrast to the yellow color of the indictor.
- Compare the test sample with the standard urea sample. This test should be read within 10 – 12 minutes.

Test for salt

Reagents

- Silver nitrate solution (5%).
- Nitric acid solution (1.2)
- Ammonium hydroxide solution (1:1)
- Standard sodium chloride solution (0, 0.1, 0.2, 0.3)

Procedure

- Weigh 1 gm of sample and add 100 ml of distilled water. Stir and filter Whatsman No.4
- Pipette 1 ml of standard solution and 8 ml of nitric acid solution. Stir and add 1 ml of silver nitrate solution.

- Stir and compare the test sample with standard sample. This test should be read within 5 minutes.

Positive results

- Salt gives a white turbidity

Test for Magnesium sulphate

Reagents

- Solution A – Potassium hydroxide 1N.
- Solution B – dissolve 12.7 g of iodine and 40 g of potassium iodide in 25 ml of distilled water. Stir and then dilute to 100 ml.

Procedure

- Mix solution A with an excess amount of solution B to give a very dark brown colour mixture.
- Take a small part of the dark brown colour mixture and add 2-3 drops of solution A until it turns a pale yellow.
- Moisten the filter paper with this pale yellow solution and then sprinkle with the sample to be tested.

Positive Results

- Magnesium gives Yellow brown spots.
- Note: The mixture of solution A and B deteriorates very quickly and should be freshly mixed for each test.

Test for Hoof or horn

Reagents

- Glacial acetic acid (1:1)

Procedure

- For quick test, place 2-3 particles of amber colour lest sample into an evaporating dish.
- Add 5 ml of glacial acetic acid into the evaporating dish and let it stand for 60 minutes.
- If hoof and horn are present, the test particles will still remain hard and tough. Gelatin will be become soft and swollen.

Test for leather

Reagents

- Ammonium molybdate solution:– Dissolve 5 g of Ammonium Molybdate in 100 ml of distilled water and pour into 35 ml of concentrate nitric acid.

Procedure

- Pick up brown to black test sample particles and place in petridish.
- Add 3 – 5 drops of ammonium molybdate and let it stand for 5 – 10 minutes. Leather meal will give no colour change. Meat and bone meal gives a greenish yellow colour.