PRACTICAL MANUAL
FOR
ANIMAL NUTRITION
PAPER I
(UNIT- I & II)

Name- Roll no.- Batch -

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FOREWORD

I am glad to see the Laboratory Manual ***PRINCIPLES OF ANIMAL NUTRITION & FEED TECHNOLOGY, APPLIED RUMINANT NUTRITION****-I isprepared by* ***Prof. R. N. Dhore, Prof .M.A. Akbar, Professor, Dr. Reenu Saini and Dr. Alok Ranjan Assistant Professor, Dept. of Animal Nutrition****. It is appreciable to note that the manual covers the practical syllabus (MSVE-2016) of B.V.Sc.&A.H. course as per the standards laid down by Veterinary Council of India.*

Prof. R. N. Dhore, Prof .M.A. Akbar, Professor, Dr. Reenu Saini and Dr. Alok Ranjan have devoted keenly to prepare this manual with their excellent knowledge and expertise in the field of Animal Nutrition. They have covered all the aspects like objectives, outline and description, material and methods and observation to be taken care off.

Definitely this manual will be helpful for smooth and effective conduction of practicals and ensure a handbook for students for entire life in the profession.

I congratulate Prof. R. N. Dhore, Prof .M.A. Akbar, Professor, Dr. Reenu Saini and Dr. Alok Ranjan for their strenuous efforts and excellent presentation of this manual.

Dean
Mahatma Jyotiba Fule College of
Veterinary & Animal Science, Chomu, Jaipur

PREFACE

This Manual has been prepared for the undergraduate students of B.V.Sc. & A.H. in accordance with the syllabus (MSVE-2016) designed by the Veterinary Council of India. The efforts have been made to make the manuscript worthy, realistic and easily understandable for the students, teachers and personnel of feed industry, Extension workers of department of Animal Nutrition for applied use at field condition. We hope this manual will serve very useful tool to the undergraduate and graduate students of Veterinary Science who are undergoing courses in Animal Nutrition.

It's our pleasure to thank Dean Sir, M.J.F College of veterinary and Animal Sciences, Chomu, Jaipur for providing necessary facilities, cheerful environment and rendering all helps in preparing this course manual.

Computer operator and typist Mr. Ashutosh Sharma worked hard for very existence of this manual so I acknowledge his efforts.

Course Incharge

Dept. of Animal Nutrition

PRACTICAL MANUAL
ANIMAL NUTRITION

CERTIFICATE

This is to certify that this manual contains bonafide practical work of Mr./Ms. ………………………………………………. bearing Roll No. ……….. Student of the Mahatma Jyotiba Fule College of Veterinary & Animal Science Chomu, Jaipur, student in the Second Year B.V.Sc. and A.H. degree,course no. ANN (Unit – I & II) during the academic Session 2020-2021.

Date: Signature of course teacher (Assistant Professor)

Signature of External Examiner Signature of Head ofDepartment

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Exercise1. General precaution while working in nutrition research laboratory

1. Laboratory should have efficient ventilation and exhaust fan and it should be neat and clean.

2. Reagent and Chemicals should be kept in properly labeled bottles on the shelf and these should be kept in a systematic way giving a very good look to the visitor.

3. Near the glassware washing sink, chromic acid prepared according to the following formula, should be kept in 1 liter capacity cylinder. Pipettes after using should be kept in this chromic acid solution at least for overnight and then should be washed.

Preparation of chromic acid -

Weight 60 grams potassium dichromate on physical balance and transfer to a 1000 ml pyrex beaker , add 300 ml ordinary water , mix it thoroughly using glass Rod, heat and solution to boil and dissolve potassium dichromate. Allowed to cool the solution and then add slowly commercial sulphuric acid (about 460 ml mixed thoroughly using glass rod)

4. Everyday used glassware should be washed using any detergent powder and then rinsed with glass distilled water and these should be kept in an oven for overnight at 100*C .next day remove the glassware and transfer to a wooden almirah specified for it.

5. Freshly procured chemicals should be arranged in an alphabetical order in the Steel almirah and these should be entered in the subsidiary register of respective laboratory.

6. Systematic breakage record register should be maintained where is worker should mention the items broken and sign.

7. Workers should put on well drill laboratory coats will analyzing any material in the laboratory. While using commercial acid, it would be e advisable to use acid proof hand gloves from the safety point of views.

8. While working in kjeldhal distillation room, every worker should use fume protecting face mask which are manufactured by several companies in India.

9. Distilled water containing bottle should always b corked to prevent it from the contamination of atmospheric ammonia. Distilled water used in nitrogen or ammonia estimation work should be free from ammonia and it should be checked using Tashiro's indicator. Ammonia free distilled water always give rose pink color when tested with a drop of Tashiro's indicator otherwise green color will appear in the case of ammonia contaminated water.

10. Workers should remember by heart that water should never be added in the acid. Always acid is added in water.

11. During summer season, Ammonia bottle should be opened after keeping it in the ice for 2 hour. Otherwise there may be chances of injuries to the worker.

12. Analytical balance Pan and platform should always cleaned using camel hair brush, before starting the job of weighing. Adjustment of balance should be checked before starting the weighing.

13. Every instrument should have log book .The workers try to record the timings of use of instrument in the log book and sign.

14. Workers should be honest and sincere will recording the final results of analysis. Every sample should be analyzed in duplicate and final record should be range with ± 0.5 -0.8 errors.

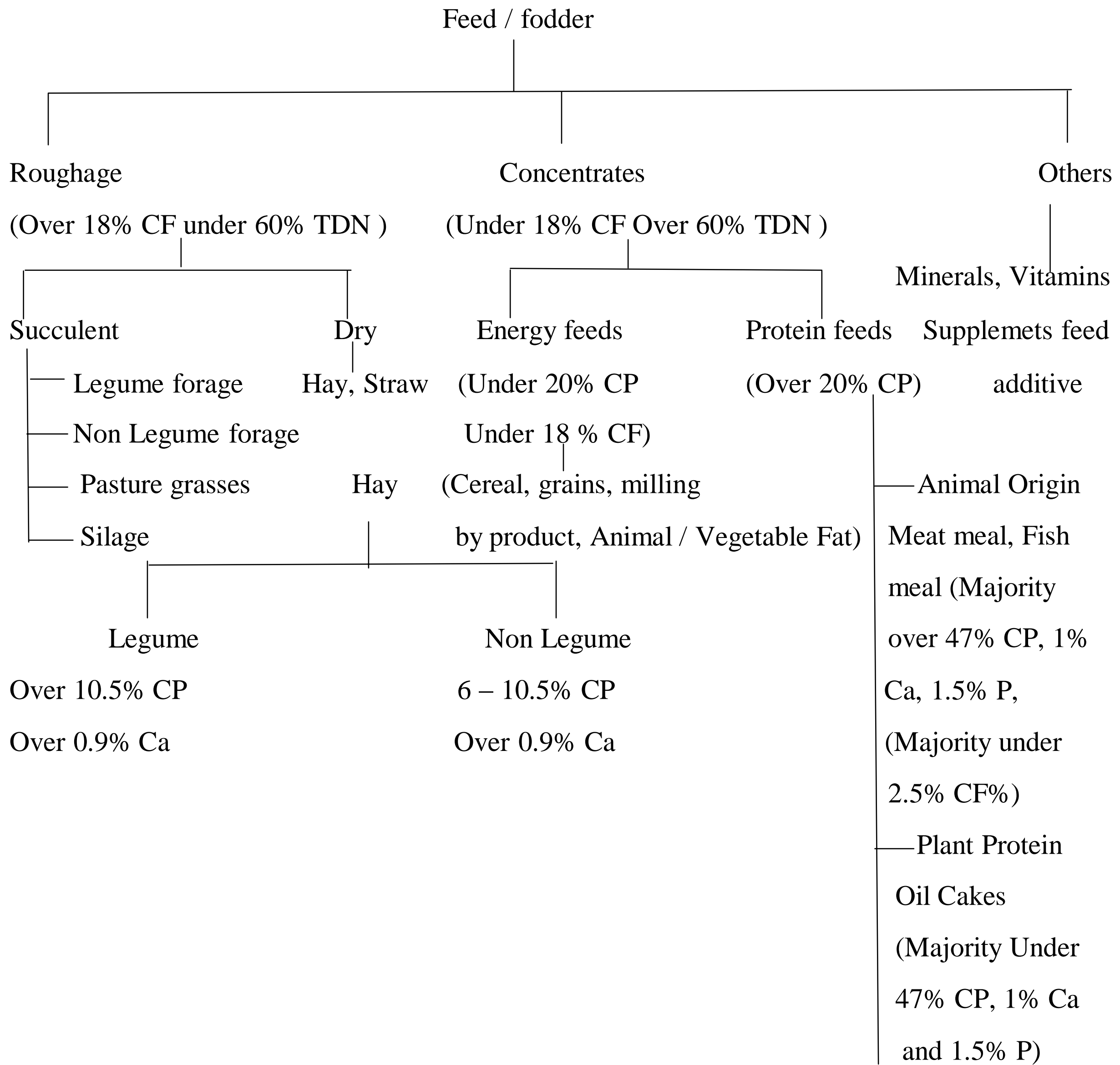
15. Workers should never be allowed to smoke in the laboratory because there are chances of catching fire due to inflammable chemicals.

16. Fire extinguishing equipment should always be present in laboratory so as to take care of the accidental firing.

17. Filter paper after you should be thrown away in the waste paper basket .floor of the laboratory should be neat and clean. Working place table should also be cleaned daily.

Exercise 2. Familiarization of various feed stuff, 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%

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. Maiz e (*Zea mays*)
3. Bajra (*Pennisetum typhoideum*)
4. Bar ley (*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*)

Exercise 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

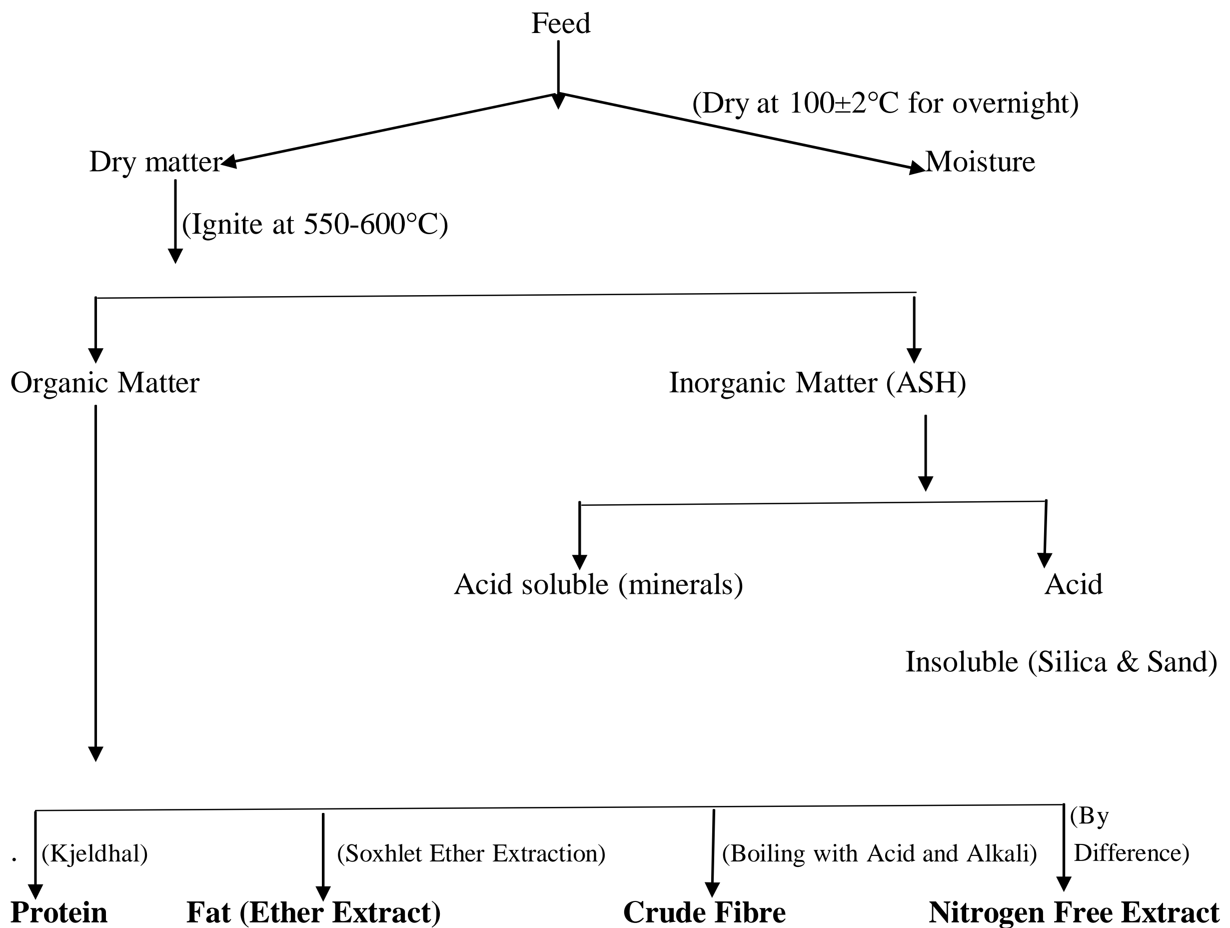
A portion of the samples received in the laboratory may be subjected for moisture estimation. Take the rest of sample is dried in a hot air oven at 60°C, ground in mill to about 1mm particle size, mixed thoroughly and transferred into a clean dry air tight container for further analysis. In case of silages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

Exercise 4: Weende's system of analysis

- The system consist 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 determines 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.

Exercise 5: Estimation of dry matter

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 pctridish 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 Empty Crucible = A g
- Weight of dish + Feed sample = B g
- Weight of 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

Exercise 6: Estimation of Total Ash

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 remain.

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_2gm
- Weight of silica crucible + ash = W_3gm

Calculation

$$\text{Total ash \% (DMB)} = \frac{(W3 - W1)}{(W2 - W1)} 100$$

Precaution

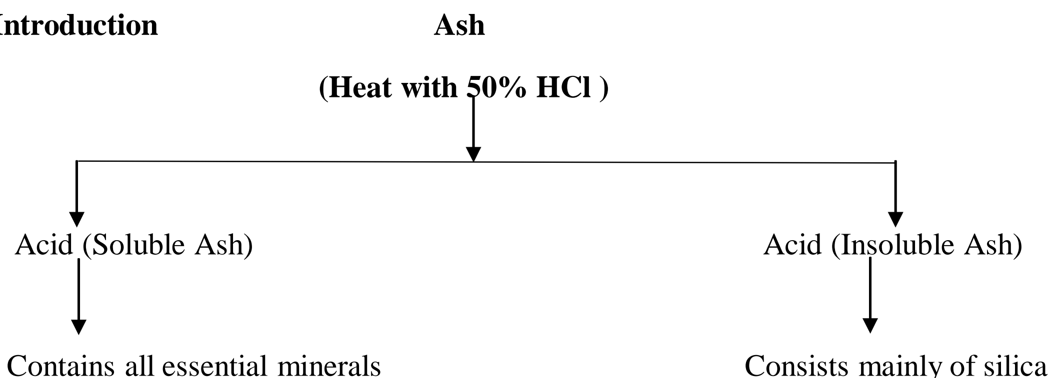
- The ash is highly hygroscopic and thus weighing should be done quickly.

Exercise question

- What fraction of feed is represented by Total Ash?

Exercise 7: Estimation of acid insoluble ash

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 (HNO3)
- Hydrocloric acid (HCI)

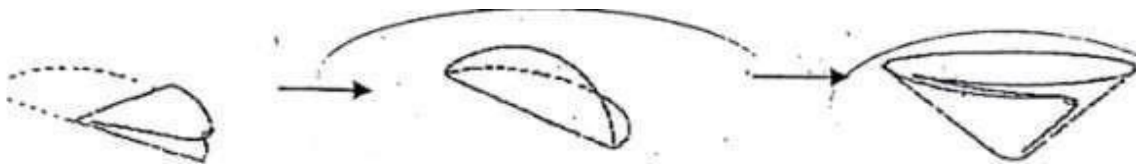
Apparatus

- Muffle furnace
- Weighing balance

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

Exercise question

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

Exercise 8: Estimation of crude protein

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
- Burrette
- 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 (10m1/gm feed)
- Add 2.5gm 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 100m1 (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 100m1. This is called as aliquate.
- Take 10m1 (desire volume of aliquate) of aliquate in another kjeldahl flask distillation assembly.
- Add saturated NaOH solution, 20-40m1 (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 beater 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 H2SO4)

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.

•

Exercise 9: Estimation of ether extract

Soxhlate method

Crude fat or ether is estimated by extracting the feed sample using continues evaporation and condensation of fat solvent like petroleum ether, diethyle 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 anyother solvent

Procedure

- Weight a clean and dryoil flask
- Weight of drysample in aluminium scoop and transfer it in to an extraction.
- Thimble made up whatsman filter paper.
- Place the thimble carefully in extraction tube and fit soxhlate oil flask to the extraction. Pour sufficient petroleumother in to the extractor along its sidewalluntil it beginto siphon.
- Again poured other to fill the extractor throughabout and half (1/2).
- Connect the condenser through extractor**.**
- Switch onthe heating unit and allow the extraction to proceed at rapid rate.

- Runequipment for about 6-8 hrs.
- After extraction is over switchoff 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 takingout 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 dying transfer the oil flask in to the desiccators for cooling to min temperature.
- Weight the oil flask to constant weight and record the weight of flask and fat after dying.
- Simultaneously dry the thimble in hot air oven to constant weight and preserve the sample for crude fibre estimation.

Observation

- Weight of sample = WI gm
- Weight ofoil flask (empty) = W2gm
- Weight ofoil flask and fat after extraction = W3gm

Calculation

Weight of crude fat = W_1

- % Ether Extract $= \dfrac{W_3 - W_2 gm}{W_1} \ldots 100$

Exercise question

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

Exercise 10: 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 2gm moisture and fat free sample is taken and transfer it into the spoutless beaker and add 200m1 of 1.25 % w/v H_2SO_4 solution to the beaker and place it on heat and allow to reflex for 1hr.
- Shake the content after every 5mint.
- After boiling for 1 hour remove the beaker fromhot plate and filter through the muslin cloth.

- Washthe residue fromtap water till free from acid and transfer the material to the same beaker,
- Add 200m1 of 1.25% w/v NaOH solutionagain reflex content for 1 hr.
- Transfer the residue into crucible and placed into in 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 cooland weight again.
- The loss ofweight due to ignition is the weight of crude fibre.

Calculation

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

Where -

- W_1= Weight ofcrucible + Residue before ashing
- W_2 = Weight of crucible + After ashing
- W = Weight of drysample
- Precautions
- Keep the volume constant during boiling.
- Avoid foaming byheat adjustment.

Exercise question

- What fraction ofcarbohydrates does the crude fibre represent

Exercise 11: Determination of NFE (Nitrogen Free Extract)

Introduction

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

Objective

To calculate the nitrogen free extract content ofthe feed.

Principle

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

Procedure

- NFE on as feed basis = 100 – (Moisture + Crude protein +Ether extract + Crude fibre + Totalash)
- NFE on drymatter basis = 100 – (Crude protein +Ether extract + Crude fibre + Totalash)

Exercise

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

Exercise 12: Determination of calcium

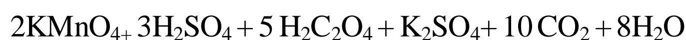
Objective

To determine the percentage ofcalcium 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(100m1)
- Burette
- Pipette
- Hot plate
- Funnel
- Bottle
- Whatsman filter paper(no.40)

Reagent

- Concentrate HCI
- Ammoniumoxalate 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 aliquate 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 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 N83 solution [1:4] drop by droptill 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 whatsmann 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

Mlof0.1NKMnO4usedX 0.002X 10X 100

- **Ca%=………………………………………………………..**

Gmofsampletakenforashing

Exercise question

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

Exercise 13. Determination of phosphorus in feed samples

Objective

To determine the percentage ofphosphorus 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/10H_2SO_4
- Phenolphthalein indicator

Procedure

- Take 25 ml of aliquate 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.
- Keptbeakerovernight to allowthe precipitate to settledown.
- Filterthesupernatantto thewhatsmanfilterpaper 40 and 42 thenwashtheprecipitate 2-3 timeswith 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.
- Addtwodrops ofPhenolphthaleinindicatorto thebeaker.
- ExcessofNaOHsolutionistitratedwithN/10H_2SO_4solution.

Calculation

Mlof0.1NaOHusedX 0.0001347X 10X 100

P%=…………………………………………………………..

Gmofsampletakenforashing

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)

Exercisequestion

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

Exercise 14: 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 VonSoest 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- 15m1)
- Berzelius beaker without spout (1000m1)

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) Amylase

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

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

%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

Same as used for NDF

- Refluxingapparatus with6 heatingunit condenser
- Filteringdevice
- Goochcrucible withcourseporosity(50m1)
- Berzelius beaker withoutspout (1000m1)
- Vacuumsystem

Reagent

- Acid detergent solution

Composition= 1N H_2SO_4 + cetyltrimethylammonium bromide (20gm)

- Acetone

Procedure

- Weight 0.5gmair dried sampleground to passthrough 1mmscreenandplace in to a Berzeliusbeaker.
- Add 100m1 AD solution to beaker
- Place beaker on reflux condensing

- Heatingunit & start beating
- Reflux for 60 min from the onset of boiling
- After 60 min of boiling switchoff the heater and take outthe beaker fromrefluxing 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 repeatedlyuntil 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_3g

Calculation

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

Exercise 15: 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.

Exercise-16

Calculation of nutritive value of different feed stuffs in terms of DCP, TDN, NR and SE

Principle: The quality of feed and fodder are based on the presence of nutrients and their nutritional worth in terms of digestible crude protein (DCP), total digestible nutrients (TDN), nutritive ratio (NR) and starch equivalent (SE).

Objective:

1. To evaluate nutritional value of feed and fodder.
2. The value to be used for formulation of ration for various categories of animal performing different type of work.

Procedure:

In general the most common practice of evaluation of food nutrients is firstly find out the digestive coefficient through digestion trials in terms of protein (DCP) and energy (TDN, SE).

Calculation of digestible nutrients:

$$\%\ \text{Digestible Nutrient} = \frac{\%\ \text{nutrient in feed x}\%\ \text{digestibility coefficient of nutrient}}{100}$$

$$\%\text{DCP} = \frac{\%\ \text{CP in feed x}\ \%\ \text{digestibility coefficient of CP}}{100}$$

$$\%\text{DCF} = \frac{\%\ \text{CF in feed x}\ \%\ \text{digestibility coefficient of CF}}{100}$$

$$\%\text{DEE} = \frac{\%\ \text{EE in feed x}\ \%\ \text{digestibility coefficient of EE}}{100}$$

$$\%\text{DNFE} = \frac{\%\ \text{NFE in feed x}\ \%\ \text{digestibility coefficient of NFE}}{100}$$

Calculation of Total digestible nutrient (TDN):

$$TDN = \%DCP + \%DCF + \%DNFE + (\%DEE \times 2.25)$$

Digestible fat is multiplied by the factor 2.25 because of its higher energy value than carbohydrate and protein.

Calculation of Nutritive Ratio (NR):

It is the ratio of digestible protein to the sum of digestible carbohydrate and fat, the later being multiplied by 2.25.

$$\text{Nutritive ratio} = \frac{DCF + DNFE + DEE \times 2.25}{DCP}$$

$$\text{Nutritive ratio} = \frac{TDN - DCP}{DCP}$$

Calculation of Starch equivalent (SE):

Starch equivalent of a feed means that the amount of feed require to produce as much animal fat as is being produced by a unit of starch when fed in addition to maintenance. Fat producing power of each of digestible nutrient is calculated by using appropriate factors and upon addition the total producing power *i.e.* starch equivalent is known. Fat producing power of each nutrient as given by Kellner's is as follows.

Nutrient	Kellner's value (SE factor)
Digestible protein	0.94
Fat from roughages	1.90
Fat from cereal grains	2.10
Fat from oil cakes	2.40
Digestible carbohydrate and fibre	1

$$\% \text{ SE} = \%\text{DCP} \times 0.94 + \%\text{DCF} \times 1\ 53 + \%\text{DNFE} \times 1 + \%\text{DEE} \times (1.90\text{-}2.40)$$

When kellner tested feedstuffs instead of pure nutrients, he found that fat producing power was less than calculated from their content of digestible nutrients therefore kellner instituted two types of correction factors:

1. Correction factor for concentrates (The werticeit or value number): Thedigestible nutrients are multiplied by number ranges from 95 to 100.

   Corrected SE = Calculated SE x Value number/100

2. The fibre correction for roughages:Following correction factors are deducted from digestible nutrients for every 1% CF present in the original feed.

Feed stuff	**Correction factor**
Dry roughages unchopped	0.58
Dry roughages chopped	0.29
Green roughages with 4 to 16% CF	0.29-0.58

Exercise-17

Calculation of nutritive value of different feed stuffs in terms of DCP,TDN, NR and SE

Q. Calculate the DCP, TDN, NR and SE from following observations.

Chemical composition of green maize		Digestible coefficient
Nutrient	%	%
CP	1.5	82.22
CF	8.0	58.33
EE	0.5	53.33
NFE	13.0	59.00

(Fibre correction factor= 0.34)

Exercise-18

Calculation of nutritive value of different feed stuffs in terms of DCP,TDN, NR and SE

Q. Calculate the DCP, TDN, NR and SE from following observations.

Nutrient	% composition of feed	% composition of faeces
DM	87.0	27.0
CP	6.9	4.2
EE	1.9	0.9
CF	19.8	16.8
NFE	59.4	38.0

Feed Intake- 11 kg Faeces voided- 13 kg

(Fibre correction factor= 0.58)

Exercise-19

Calculation of nutritive value of different feed stuffs in terms of DCP,TDN, NR and SE

Q. Calculate the DCP, TDN, NR and SE from following observations.

Nutrient	% composition of feed	% composition of faeces
DM	90.0	25.0
CP	10.0	5.8
EE	2.5	1.5
CF	19.0	15.7
NFE	61.2	70.51

Feed Intake- 10 kg Faeces voided- 13 kg

(Fibre correction factor= 0.34)