Technical Note

Basic Laboratory Manual: Analysis of Animal Feed and Physical Evaluation

Muluken Getachew Alemu * and Abebe Mosenhe Wudu

Posted Date: 6 November 2024

doi: 10.20944/preprints202411.0421.v1

Keywords: Animal feed analysis; crude protein; dry matter; Kjeldahl method; Soxhlet extraction; physical evaluation

 doi:10.20944/preprints202411.0421.v1

Technical Note

Basic Laboratory Manual: Analysis of Animal Feed and Physical Evaluation

Muluken Getachew and Abebe Moseneh

Senior Technical Assistant, Department of Animal Science, Wollo University, Ethiopia
* Correspondence: mulukengetachew38@gmail.com

Abstract: This laboratory manual provides essential protocols and procedures for analyzing animal feed in the Animal Nutrition Laboratory at Wollo University. It is tailored to assist researchers, technical assistants, and students in evaluating feed quality to optimize ruminant nutrition, especially under arid conditions where feed costs impact livestock productivity. The manual covers safety guidelines, sample preparation, and detailed procedures for determining moisture, dry matter, crude protein, crude fiber, ether extract, and ash content in animal feed samples. Special emphasis is placed on using the Kjeldahl method for protein determination and the Soxhlet apparatus for fat extraction. Additionally, the manual includes methods for producing urea molasses blocks, a valuable supplementary feed. Physical evaluation techniques such as assessing color, texture, odor, and mold presence are highlighted for rapid feed quality assessment, ensuring safe and effective diets.

Keywords: animal feed analysis; crude protein; dry matter; Kjeldahl method; Soxhlet extraction; physical evaluation

---

Laboratory Safety

- Always wear proper personal protection equipment (PPE) for the task you are carrying out (e.g. lab coat or coveralls, safety glasses or prescription glasses with side shields, gloves, face shield, respirator, aspirator, ear buds, etc.); see below for further details.
- Always know the hazardous properties of materials being use
- Always wash hands thoroughly before leaving the laboratory
- Never smoke in the building
- Never eat, drink, store food or apply cosmetics in laboratories
- Never perform unauthorized experiments
- Never engage in pranks, practical jokes or other acts of mischief
- Do not block access to emergency exits and emergency equipment
- Cell phones should not be used in the laboratory as they may become contaminated.
- Headphones are not allowed in the laboratory as they interfere with communication.
- Mouth pipetting is prohibited. Always attach and use a rubber suction bulb for transferring the solution if using a pipette, or use a mechanical pipetting device.

1. Introduction

The primary barrier to increasing revenue from small-scale ruminant production in arid regions is thought to be feed costs, which have a significant impact on animal nutrition and livestock productivity. Ethiopian's livestock scientists are searching for alternative feed resources to be included in well balanced diets that can lead to improvements in flock/herd productivity and in meat and milk quality. Careful laboratory testing is required to determine the nutritional content of suggested low-cost diets and how they affect product quality. Therefore, Wollo University Animal Nutrition Laboratory analyzes the quality of feeds.

Nutrition Laboratory is similar to food laboratory. It contains chemical and glassware, feed analysis equipment. Hence, such systems evoke certain instructions and directions, which should strictly be followed by personnel, students, staff members, beginners and researchers are encouraged

 doi:10.20944/preprints202411.0421.v1

to prioritize safety and security measures in order to protect themselves and safeguard laboratory equipment.

This manual covers some analyses conducted in the Animal Nutrition Laboratory at Wollo University, as well as the equipment needed. Feed analyses conducted in the laboratory include basic nutritional analyses such as moisture content, dry matter, crude protein, crude fiber, crude fat, ODM (organic dry matter), crude fat, For these analyses, the laboratory is equipped with Kjeldahl nitrogen analyzer, fiber analyzer, Soxhlet, sample shaker, balances, and centrifuges.

This laboratory manual serves as a primary resource for researchers, collaborators, and technicians in the Animal Nutrition Laboratory who visit Wollo University for training or joint research endeavors.

2. Feed Analysis

Sample Preparation

The process of preparing samples ensures that they are uniformly prepared for all of the nutritional analyses. The two most important processes are drying and grinding. The preparation of the sample is done in accordance with the requested analyses and sample type. Samples that are wet upon receipt are dried overnight at 60 °C in an air-circulation oven to produce air-dried samples that are prepared for grinding. Using a grinding machine, feed samples are ground to a particle size of 1 mm. Samples that have been dried and ground are kept sealed and out of direct sunlight. To prevent insect damage, caution must be used.

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

2.1. Determination of Moisture in Feedstuffs

The amount of free water that is present in any feedstock is referred to as moisture. Any feedstock sample can be kept free of moisture by placing it in an oven. "Dry Matter" refers to the amount that is left over after this process.

2.1.1. Apparatus and Equipment

- Metal Tong
- Heat resistant gloves
- Spatula
- Permanent markers
- Hot air oven
- Petri-dish
- Desiccators
- Balance machine

2.1.2. Procedure

Step 1. Petri-dish preparation

Place the clean glass petri-dish (120 mm in diameter) in oven and dried in 105^0c for 20 minutes. Keep the lid opened and separated. Take out the petri-dish from the oven and put into the desiccator to cool.

Step 2. Sample preparation

 doi:10.20944/preprints202411.0421.v1

The petri-dish is ready to use in the analysis of moisture. Calibration status of the balance should check before weighting. Use petri-dish of 120mm in diameter to take 10g of sample.

Step 3. Drying on hot air oven

Place the petri-dish with sample inside the hot air oven carefully. Close the door tightly. Set the temperature at 130^0c for 2hr. after 2hr open and put the petri-dish from oven into desiccator.

Step 4. Final weight

Now take the final weight of the dish containing dried sample. Clean the balance after measuring.

Step 5. Calculation

$$Moisture = \frac{Ws - (W1 - W2)}{Ws}$$

W_s= weight of sample
W_1= weight of dish
W_2= weight of dish after drying

2.2. Determination of Dry Matter (DM)

Dry matter is the portion of forages that have been dehydrated. Dry matter content is the foundation for all nutritional analyses. The Animal Oxygen Analysis and Chemistry (AOAC) method for determining the moisture content of animal feed has been modified for the Wollo University Animal Nutrition Laboratory to work differently.

2.2.1. Equipment

- Silica crucibles
- Desiccators
- Hot air oven
- Balance machine

2.2.2. Procedure

- ✓ Dried and grinding samples
- ✓ Dry empty crucibles or container overnight at 105 °C
- ✓ Cool samples in desiccators to room temperature
- ✓ Measure oven-dry crucible (W_t)
- ✓ Add approximately 2g of ground sample; record weight (Ws)
- ✓ Dry overnight at 105 °C for 24hr
- ✓ Allow the desiccators to cool down to room temperature
- ✓ Weight oven dry crucible and sample = (W0)

$$\%DM = \frac{W0 - Wt}{Ws} x100$$

2.3. Determination of Ash

2.3.1. Equipment

- Sensitive balance (Figure 2)
- Muffle furnace (550 °C) (Figure 6)
- Desiccator (Figure 2)
- Porcelain or silica crucibles (Figure 5)

 doi:10.20944/preprints202411.0421.v1

Figure 1. Grinding Machine.

Figure 2. Balance machine and Dissector.

 doi:10.20944/preprints202411.0421.v1

Figure 3. Heat sensitive glove, spatula, and metal tong.

Figure 4. Hot air oven and Petri-dish.

Figure 5. Silica crucibles.

 doi:10.20944/preprints202411.0421.v1

Figure 6. Muffle furnace.

2.3.2. Procedure

- Ignite dry matter samples overnight at 550 °C for 2:30hr in muffle furnace
- Allow the desiccators to cool down to room temperature
- Weigh ignited crucible and sample (Wa)
- Weight oven dry crucible and sample = (W_0)
- Weight oven-dry crucible (W_t)

$$\%\text{Ash} = \frac{\text{Wa} - \text{Wt}}{\text{Wo} - \text{Wt}} x100$$

2.3.3. Precaution

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

2.4. Determination of Organic Dry Matter (ODM)

Organic dry matter of feedstuff can be calculated by using the following formula adopted from AOAC.

$$\%\text{ODM} = 100 - \%Ash$$

2.5. Determination of Crude Protein

It is every nitrogenous substance found in the feedstock sample. True protein and non-true protein (non-protein nitrogen), like urea, are included in it. When it comes to farm animals' nutrition, crude protein is regarded as a significant component. The Kjeldahl method is used to calculate total nitrogen, or crude protein.

2.5.1. Equipment

- Digestion rack
- Balance machine (Figure 2)
- Spatula (Figure 3)
- Acid proof glove (Figure 3)
- Funnel
- Kjeldahl flask
- Mixer machine
- Dropper
- Pipette
- Sample shaker

 doi:10.20944/preprints202411.0421.v1

- Conical flask
- Volumetric flask
- Measuring cylinder
- Hot plate with magnetic stirrer

2.5.2. Chemicals/Reagent Preparation

1. Catalyst (potassium sulphate + copper sulphate + selenium sulphate

$KSo_4 + CuSo_4 + SeO_2$

Ratio 5 3 1

Procedure

- Clean everything what you need to prepare the catalyst
- Use clean and separated spatula for weighting different reagent/chemicals
- Transfer into same mixer chamber to mix catalysts
- Close the mixer chamber tightly with lid
- Mix all the tree chemicals using the mixer machine

2. Sulfuric Acid (concentrated 95-98%)

3. 40% Sodium Hydroxide solution

Procedure

- Take weight of 40g of NaOH pellet
- Transfer the weighted NaOH into the flask and shake slightly to mix
- Take 80ml of distilled water into the flask
- Label the flask with 40% NaOH, the wait too dissolve all the pellets and cool at room temperature
- After cooling, add water to make the final 100ml volume

4. 4% Boric Acid solution preparation

Procedure

- Weight 4g of boric acid powder
- Transfer the boric acid powder into 40ml of some hot distilled water
- Stir with a clean glass rod to dissolve boric acid well. Tur off the hot plate and cool the boric acid solution
- Label a 100ml volumetric flask with 4% boric acid solution
- Take the cooled boric acid solution into the volumetric flask
- Add distilled water to volume up to 100ml, and then rotate the flask to mix 4% of boric acid solution.

5. 0.1N hydrochloric acid (standardized)

Procedure

Step 1. Phenolphthalein indicator preparation

- Dissolve 2g phenolphthalein indicator powder into 100ml of ethanol and mix well by shaking

Step 2. Dilute 0.83ml of HCl (concentrated) with distilled water to make the total volume of 100ml.

- Label a 100ml volumetric flask with 0.1N HCl
- Drop 80ml of distilled water into 100ml volumetric flask
- Pipette 0.83ml of concentrated HCl (37%) into the flask
- Add distilled water enough to make 100ml of the final volume
- Shake the flask to mix the HCl with distilled water

Step 3. Standardized newly prepared 0.1N HCl with standard 0.1N NaOH solution and find the actual normality.

- Take 0.1N standard NaOH solution into burette
- Take the initial burette reading
- Measure 20ml of newly prepared HCl solution and take into conical flask
- Add 3-4 drops of phenolphthalein indicator into conical flask
- Titrate it with standard 0.1 NaOH solution
- Take final burette reading after the color changed

 doi:10.20944/preprints202411.0421.v1

Step 4. Calculation for standardization of 0.1N HCl

- Burette reading of NaOH (V2)
- Normality of NaOH (N2)
- Volume of prepared HCl (V1)
- Normality of HCl (N1)

$$N_1 = \frac{V_2\ X\ N_2}{N_1}$$

Rewrite the actual normality of HCl from the calculation of standardization

6. Methyl red indicator

- Dissolve 100mg Methyl red indicator powder into 100ml of methanol and mix well by shaking

Determination of total nitrogen (crude protein) using the Kjeldahl method

2.5.3. Procedures

1. Digestion

- Label the kjeldahls flask with the sample number
- Take the weighted sample into the flask
- Again, weight 3g of catalyst
- Take the catalyst into the flask to mix sample
- Take 20ml of concentrated H_2SO_4 and pour the acid into the sample flask
- Shake the flask gently to mix the acid with sample and catalyst
- Place the flask on digestion unit carefully
- Turn on the digester power and set the temperature at 230^0 c and water circulation open
- After 2hr clean green color solution indicates the end of digestion
- Turn off the digester and wait to cool the flask
- Now, the digested sample diluted with distilled water
- Add 20ml of distilled water into the flask, mix and pour the digested sample into 100ml volumetric flask
- Add enough water to make the final volume of 100ml

2. Distillation

- Measure 30ml of 4% boric acid and pour into a conical flask
- Place the flask on the distillate collection unit
- Take 10ml of digested sample to transfer into distillation flask
- Now, add 50ml of 40% NaOH
- Add another 50ml of distilled water
- Run the distillation at 200^0c for 1hr
- Turn off the distillation after collect approximately 100ml of distillated

3. Titration

- Take 0.1N HCl into burette
- Note the initial burette reading
- Add few drops of methyl red indicator into the conical flask and mix well
- Start titration-adding 0.1N HCl
- Place a white background at bottom of the flask to transparence colors
- Start titration adding 0.1N HCl
- Stop the titration if the color is changed into orange
- Note final reading of burette

4. Calculation

Finally calculate nitrogen and crude protein

$$N\% = \frac{V_1 + n_1\ x\ F_1\ x\ M_{wn}}{W_s\ x\ 10}$$

 doi:10.20944/preprints202411.0421.v1

$$\text{Crude protein } \% = N\% \; x \; Factor \; x \; F_2$$

Where,

V_1= Volume of 0.1N HCl (final burette reading –initial burette reading)

n_1= Normality of HCl

F_1= Acid factor

F_2= Dilution Factor

Mwn=Molecular weight of nitrogen =14.007

2.6. Determination of Crude Fat or Ether Extract

Crude fat or ether 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. Lipids are a group of materials that are insoluble in water but soluble in ether, chloroform, and benzene. The ether extraction procedure itself is quite simple and usually involves a reflux apparatus in which the ether is boiled, condensed, and allowed to pass through the feed sample.

2.6.1. Apparatus and Equipment

- Soxhlet apparatus
- Soxhlet extractor
- Filter paper
- Measuring cylinder (Figure 8)
- Thimbles

Figure 7. Funnel and Kjeldahl digester unit.

 doi:10.20944/preprints202411.0421.v1

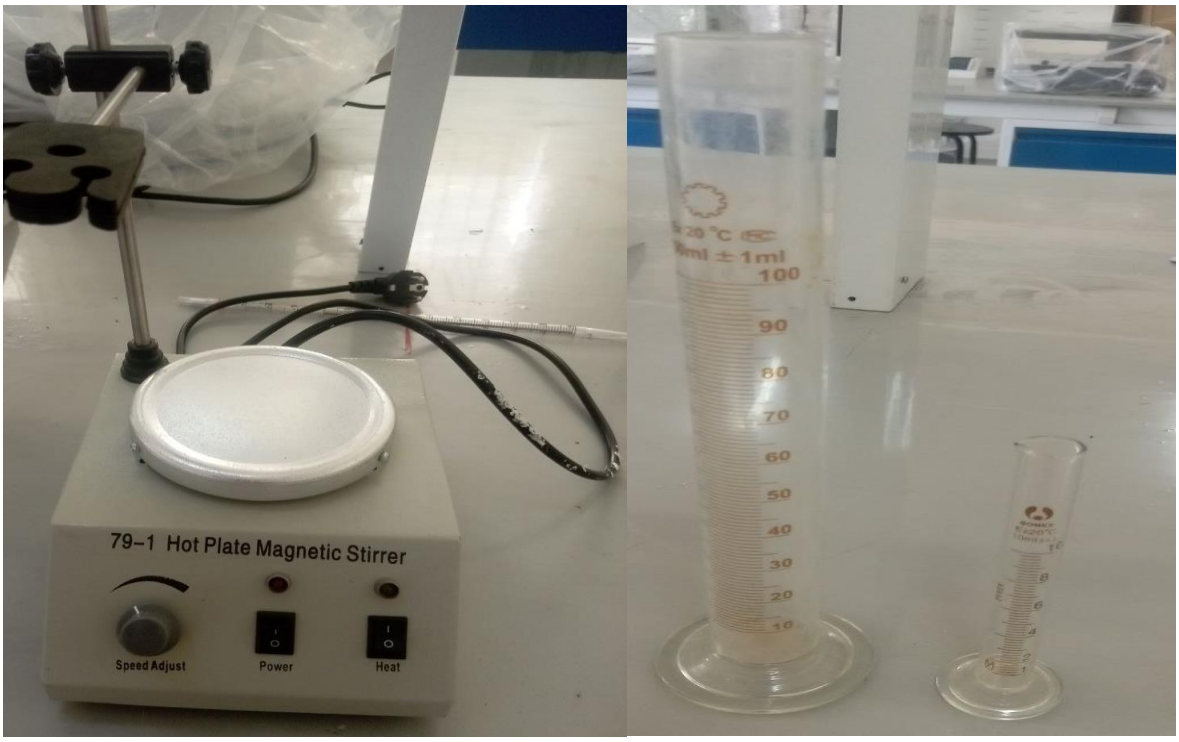

Figure 8. Hot plate with magnetic stirrer and measuring cylinder.

Figure 9. Soxhlet appatus and extractor.

2.6.2. Chemical/Reagent

- n-hexane 95%

2.6.3. Procedure

Step 1. Thimble Preparation

- Gather everything you need to make a thimble with filter paper

 doi:10.20944/preprints202411.0421.v1

- Make a thimble with filter paper
- Place the thimble on balance machine
- Take weight of a thimbles

Step 2. Sample Preparation

- Grind the sample if it is solid
- Put about 4.5g of sample into the thimble.
- Take note weight of the sample
- Place small amount of cotton into thimble in a way that covers the sample
- Fold the thimble to enclose the sample
- Take a cellulose thimble (sample holder)
- Label the thimble contain sample with sample number and put inside the cellulose thimble
- Take a cleaned and dried flat bottom flask
- Take a weight of the flask placing in a balance machine

Step 3. Fat extraction

- Set up soxhlet extraction unit placing the sample in it
- Add sufficient amount of n-hexane
- Run the water through the condenser of soxhlet extractor
- Turn on the power and active for 6hr
- Take out the sample from thimble
- Rotate the flask to evaporate the excess n-hexane

Step 4. Taking final weight

- Place the flask inside the oven to remove moisture and hexane
- Set the temperature at 1100c for 30 minutes
- Take out the dried flask and place in desiccator to cool for 20 minutes
- Measure the final weight of flask after cooling

Step 5. Calculation

$$\%\text{Crude Fat} = \frac{W_2 - W_1}{W_s} x100$$

Where

W_1= weight of flask
W_2= weight of flask and fat
W_s= weight of sample

2.7. Determination of Crude Fiber

2.7.1. Reagents

- Sulfuric acid solution, 0.255N, 1.25 g of H2SO4/100 mL
- Sodium hydroxide solution, 0.313N, 1.25 g of NaOH/100 mL, free of Na2CO3 (concentrations of these solutions must be checked by titration)
- Alcohol - Methanol, isopropyl alcohol, 95% ethanol, reagent ethanol
- Bumping chips or granules - antifoam agent (decaling)

2.7.2. Apparatus

- Digestion apparatus
- Ashing dishes
- Desiccator
- Filtering device
- Suction filter: to accommodate filtering devices. Attach suction flask to trap in line with aspirator or other source of vacuum with valve to break vacuum.

 doi:10.20944/preprints202411.0421.v1

2.8. Determination of NFE (Nitrogen Free Extract)

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.

NFE on as feed basis = 100 – (Moisture+ Crude protein +Ether extract + Crude fiber + Total ash)

NFE on dry matter basis = 100 – (Crude protein + Ether extract + Crude fiber + Total ash)

3. Urea Molasses Blocks (UMB)

UMMB is composed of various ingredients, each of which adds something unique to the mixture. Typically, it consists of molasses, urea, cement, wheat bran, protein-rich byproducts, water, and salt that are combined and processed into a block shape. Molasses provides energy and minerals like sulfur. It increases its intake by the animal. Urea is a non-protein nitrogen source, which is essential to improve the digestibility of the feed by providing fermentable nitrogen. Cereal bran is the most common fibrous feed used and provides energy and helps hold the block together. Noug seed cake is added to supply protein and it is a bypass protein source and provides immediate function for the animal. Salt is added to the blocks to supply minerals and to control the rate of consumption. To make the block, cement is used. It makes the block hard and provides calcium.

3.1. Preparation of the Ingredients

The weight of the block to be made determines the amount of each ingredient to be mixed. Using the following proportion, UMB can be produced by thoroughly mixing the exact quantities of the components;

- Molasses (**34%)**
- Urea (**10%)**
- Cement (**15%)**
- Wheat bran (**25%)**
- Noug seed cake (**13%)** and
- Common salt (**3%)**.

3.2. Apparatus

- Molding instrument
- Ingredients
- Mixing equipment
- Weighing scales.

3.3. Procedure

1. Collect the following ingredients and prepare based on the required nutrient block. First, all of the ingredients are weighed out and placed in sacks, plastic bags, or buckets.

- Molasses
- Urea
- Cement
- wheat bran
- Noug seed cake
- Common salt

2. The cement and water are mixed in the tank by hand or by using a wooden paddle
3. The salt, molasses and urea are then added and similarly mixed
4. Finally the bran is added quite slowly as all the ingredients are mixed together
5. The mixed ingredient will shoveled into the moulds where it is tamped to displace the air
6. After moulding, the blocks are usually left for 24 hours before being placed in storage.

Precautions While Supplementing Urea Molasses Block

It is essential to note the following while supplementing Urea Molasses Block

➢ Feed to ruminants only (sheep, goats and cattle).

 doi:10.20944/preprints202411.0421.v1

- Do not feed to monogastrics, (i.e., horses, donkeys, or pigs).
- Do not feed to young ruminants less than six months of age (kids, lambs)
- Blocks should be used as a supplement and not as the basic ration
- A minimum of coarse forage in the rumen is essential
- Never give blocks to an emaciated animal with an empty stomach. There is the risk of poisoning due to excessive consumption
- The amount of blocks fed to sheep and goats should be limited to 100 grams/day while for cattle it should be limited to 700 grams/day.
- The blocks should never be supplied in ground form or dissolved in water as this can result in over consumption
- Supply sufficient amount of water *ad lib*

4. Physical Evaluation of Feedstuffs

Feedstuffs must be physically inspected in order to be evaluated for quality and suitability for use in animal diets. Examining the feed's color, texture, odor, foreign material content, and mold contamination are all part of this assessment. Physical evaluation of feed is a quick, practical method to assess feed quality based on visible characteristics such as color, texture, odor, and the presence of foreign material or mold. These attributes can reveal essential information about the freshness, safety, and potential nutrient value of the feed. For instance, a green color in forages often indicates higher nutritional content, while a musty odor or dark coloration may signal spoilage or mold contamination, which can be harmful to livestock. Evaluating texture and particle size also helps determine digestibility, especially in ruminants. This initial, hands-on assessment is crucial for selecting feeds that are safe and beneficial, ensuring they meet animals' dietary needs.

1. Color

A feedstuff's color can reveal information about its nutritional value, maturity, and freshness. A rich nutrient profile is suggested by a green forage, which has a higher chlorophyll content, whereas oxidation or spoiling may be indicated by a brown or dark color.

2. Texture

Texture is related to the feed's physical consistency and particle size. Coarse forages might be harder to digest than finer ones, and excessively dusty feed might make animals eat less. For ruminants, texture is particularly crucial because digestion and rumination are impacted by particle size.

3. Odor

High-quality feed usually has a pleasant, fresh smell, whereas musty or sour smells could be signs of fermentation, mold growth, or spoiling. Additionally, odor can be used to identify problems such as fermentation in silage or rancidity in fats.

4. Presence of Foreign Material

Physical evaluation entails locating any non-feed material that could be dangerous and lower the quality of the feed, such as rocks, soil, plastic, or weeds. Eliminating foreign objects is crucial to protecting animals from possible harm.

5. Mold and Fungal Growth

Feed containing mold has the potential to be hazardous.

Reference

AOAC (Association of Official Analytical Chemists). 1995. Animal Feed: Sample preparation (950.02). Official methods of analysis, 16th edition.

AOAC (Association of Official Analytical Chemists). 1995. Ash of Animal Feed (942.05). Official methods of analysis, 16th edition.

AOAC (Association of Official Analytical Chemists). 1995. Fat (Crude) or Ether Extract in Animal Feed (920.39). Official methods of analysis, 16th Edition.

AOAC (Association of Official Analytical Chemists). 1995. Fiber (Acid Detergent) and Lignin in Animal Feed (973.18). Official methods of analysis, 16th edition.

 doi:10.20944/preprints202411.0421.v1

AOAC (Association of Official Analytical Chemists). 1995. Moisture in Animal Feed (930.15). Official methods of analysis, 16th edition.

AOAC (Association of Official Analytical Chemists). 1995. Laboratory Safety. Appendix B. Official methods of analysis, 18th edition.

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis, 15th edition. Arlington, VA.

AOAC (Association of Official Analytical Chemists). 2000. AOAC Official Method 970.26. Official methods of analysis, 17th edition.

AOAC (Association of Official Analytical Chemists). 2000. AOAC Official Method 972.16. Official methods of analysis, 17th edition. Gaithersburg, MD, USA.

AOAC (Association of Official Analytical Chemists). 2000. Official Method 947.05. Official methods of analysis, 17th edition. Gaithersburg, MD, USA.

Kunju, P.J.G. (1986). "Urea molasses block lick: A feed supplement for ruminants" Proceedings of the International Workshop, Kandy, Sri Lanka p261.

L.O. GarcÃa and J.I.R. Restrepo. 1995. Multi-nutrient block handbook. Food and agriculture organization of the United Nations Rome, FAO Economic and Social Development Series No.3/45

Van Soest, P. J. 1975. Physio-chemical aspects of fibre digestion. In: I. W. McDonald, and AC. I. Warner (Ed.), Digestion and Metabolism in the Ruminant. The Univ. New England Publ. Unit, Armidale, Australia.

Zaklouta M., Hilali M., Nefzaoui A. and Haylani M. 2011. Animal nutrition and product quality laboratory manual. ICARDA, Aleppo, Syria. viii + 92 pp.