FEED ANALYSIS PRACTICALS BY PROXIMATE METHOD

PRACTICAL- I

Title: - FEED SAMPLE COLLECTION AND DETERMINATION OF MOISTURE/DM CONTENT

Introduction

Following the standard procedure collecting and preparing a sample is essential for accurate analysis on a given lots of food/feed. We cannot analyze the whole material to determine its chemical composition. So, representative samples from the whole material must be taken following variable procedures. Generally, feeds are categorized in to two main groups namely, moisture and dry matter. The moisture content is the water part; where as the dry matter is composed of organic and inorganic components of a feed. The moisture content of feeds varies considerably and affect the dry matter concentration. Therefore, before running any further nutritional analysis, it must be expressed on the basis of dry matter, in order to avoid error due to variation in moisture content. This is done by putting the sample in an oven; until all the moisture is removed. The length of stay in an oven and the level of temperature to be applied may vary according the type of feed to be treated.

Objective: To be familiar with the collection of **Representative Samples** from the total material/ FEED SAMPLE /available and the methods of moisture/Dry matter/ percentage determination

Materials and methods

Sickle, tray, triple beam balance, oven, mill, plastic bags were used, Grass samples that grow around the office and class rooms were collected using sickle at the height similar to what would be grazed or mowed from many randomly selected places. The samples collected were brought to the nutrition laboratory, chopped and mixed thoroughly to make it a representative of the whole area. Then empty tray was weighed and labeled. Then after the sample along with the tray was weighted and put in to an oven for drying. After about 14 hours, the 1st reading was taken. The second and the third readings were

taken with in one and half hour intervals. After obtaining constant weight the samples were ground and preserved for further chemical analysis with a plastic bag. The parameters analyzed were;

► Moisture % = $\frac{\text{loss in wt (moisture content)}}{\text{Original sample wt}} \times 100$

► Dm % = $\frac{\text{Dry wt of sample}}{\text{Original sample wt}} \times 100$

Result

Table 1. Determination of dry matter weight of grass samples

Tray		Tray + Fresh wt(g)	Fresh wt Alone(g)	Try + oven Dry wt(g)	Dry wt Alone(g)	Moisture content	% Moisture Content (%)	dry Matter (%)
No	weight							
10	760.9	1073.9	313	899	138.1	174.9	55.88	44.12

Conclusion

With regard to the two results, there exists a 1.03 % difference in DM & moisture percentage between my samples and the other sample used as a replication done by my colleague. These results seem to be good as it is practically difficult to obtain similar results from two different samples. As observed in the results the DM percentage of the samples increased so much because all grass samples were nearly drying due to moisture stress.

FEED ANALYSIS PRACTICALS BY PROXIMATE METHOD

PRACTICAL- II

Title: - **CRUDE ASH DETERMINATION**

Introduction

Feeds are composed of moisture, organic and inorganic matter. After removing the moisture content of the sample using an oven the remaining portions are organic and inorganic components. The inorganic matter of the sample is obtained by burning the sample in a muffle furnace at a temperature of 550^0C for about 3 hours. By doing so, the organic matter disappears in the form of water and CO_2.Then the remaining portion is the inorganic matter known as ash. This ash contains different minerals that are required by the animals' body for normal growth and production. In addition to the component minerals, the ash contains impurities like silicates which are not components of the sample and there are cases where by some minerals volatilized during burning. Due to such losses and additions the residue is termed as crude ash.

Objective: To **be acquainted with the methods of determination of ash contents of grass samples**

Materials and methods

Crucible, sensitive balance, desiccators, muffle furnace, spatula, and spoon were used. Two empty crucibles from the desiccators were labeled and weighed. Then powdered grass sample weighing about one gram was added in the crucibles and the weights registered. The Crucibles along with the sample were put in to a muffle furnace adjusted at a temperature of 550^0c and left for 3 hours. After 3 hors the crucibles were taken out of the furnace and allowed to cool in desiccators. After cooling, the crucibles were weighed and the amount of ash was obtained by difference.

Results and discussion

Table 2. **Crude ash determination**

Crucible No	Weight of			Crucible with		Percent		Remark
	Crucible	Sample	ash	Sample	ash	CF	ash	
28	17.9747	1.0012	0.1176	18.9759	18.0923	88.25	11.75	
44	17.1170	1.008	0.1175	18.1178	17.2345	88.26	11.74	

There are many factors that influence the mineral content of plants. Some of these factors are soil type, stage of growth and species of plants. Natural grass land includes large numbers of species of plant legumes, herbs and grasses. While collecting the sample it was difficult to selectively cut same grass species. as they are growing mixed. As a result, deferent legumes and herb species were included in the sample. Since legumes are rich in their mineral content than grasses, this condition may increase the ash content of the sample. But on the other hand, as the plant gets matured it decreased in its mineral content. The basis for saying this is that, the moisture content of pasture at early growth stage lunges from 75-85% but in the case of the sample tested the moisture content was about 55% indicating the sample being matured and this implies that the mineral content to be reduced and may intern decrease total ash content.

Another factor expected to influence the mineral content of the sample was dust particles that landed on the plant parts as samples are collected along the road side.

Conclusion

Since the samples were a mixture of grasses, some species of legumes and herbs, it is difficult to tell confidently that the result obtained is with large or low amounts of minerals as the case may be in pure species or one type of plants.

FEED ANALYSIS PRACTICALS BY PROXIMATE METHOD

PRACTICAL- III

Title: - DETERMINATION OF CRUDE PROTEIN

Introduction

Proteins are complex organic compounds of high molecular weight. In common with carbohydrates & fats, they contain C, H, and O_2. In addition, they all contain nitrogen and generally sulfur. Some proteins also contain the elements P, Fe, Zn & Cu. proteins are found in all living cells, where they are intimately connected with all phases of activity that constitute the life of the cell and tissues, plant and many micro organisms are able to synthesis protein from simple nitrogenous compounds such as nitrates. Animals cannot synthesis the amino group and in order to build up body protein they must have a dietary source of amino acids. There are some that can be synthesized in the animal body.

The protein to be determined is said to be crude protein, since considerable variety of nitrogenous compounds, which are not classed as protein, occur in plants and animals. In plant analysis these compounds have been frequently classed together as non-protein nitrogenous compounds that distinguish them from true proteins determined in routine chemical analysis. Amino acids form the main parts of non-protein nitrogen fractions in plants and other compounds are nitrogenous lipids, amines, amides, purines, pyrimidines, nitrate and alkaloids and vitamin B complex containing nitrogen in their structure.

The protein content of a sample is determined by digesting the sample in a concentrated sulfuric acid to yield ammonium salt which up on treatment with sodium hydroxide and steam distillation ammonia released. The ammonia released is collected in a flask containing boric acid with indicator and finally the amount of nitrogen is determined by titrating the ammonia with a 0.1N HCl solution.

Objective: **To be acquainted with the methods of protein content determination of a given sample**

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Materials and methods

Materials and chemicals used were digestion and distillation tubes, Digestion unit, distillation units, electronic analytical balance, Erlenmeyer flask, filter paper, measuring cylinder, Automatic dispenser, shaker, heater, glove, Conc. H_2SO_4, 0.1N HC1, 50% NaOH, 4% Boric acid with Bromocresol green & Methyl Red indicator solution & catalyst tablet.

From the grass sample preserved in a plastic bag for chemical analysis, two samples weighting, 0.5004 & 0.5003g, as a replication were weighed using filter paper as a container with electronic analytical balance found in dairy laboratory. The samples were given treatment numbers 5 &13 respectively. The samples weighed were put in to digesting tubes and by wearing plastic gloves, 6ml of 97% concentrated Sulfuric acid from the automatic dispenser. Half catalyst tablet was added and thoroughly shacked until no particle adheres to the bottom part of the tube. Then the tubes were put on a heater in the hood to be heated for two hours with increasing heat gradually. After the samples were completely digested, they were removed from the heater. 20ml water was not added immediately it cooled at once, since we left the lab for lunch, and by the time we came back we found the solution being solidified, so to liquefy the sample, first 10ml of distilled H_2O was added on each sample and put on a heater again till it liquefies. After that the other 10ml H_2O was added then the solutions were transferred in to a distillation tube & taken to the dairy laboratory where the distilling apparatus is found. The samples were shacked using electric shaker. The time was adjusted at 4 minutes. 50ml NaOH was added to the distilling tube. The Erlenmeyer flask containing boric acid with indicators was set on the other side of the apparatus to collect the ammonia produced, and the distillation process started. When the adjusted time reached, it gave sound and the Erlenmeyer flask was taken out from the distillation apparatus and brought to the nutrition lab, to be filtrated with the 0.1NHCl solution. During the process of distillation, the sample assigned by treatment No 5, some portion of it was over flown due to some technical problems while setting the distillation tube with the distillation

apparatus, and it was tried to be re-poured the flown sample again in to the distillation tube.

Results and discussion.

By using the formula % N = $\frac{14.01 \times \text{titration value} \times \text{normality of acid}}{\text{Sample wt.} \times 10} \times 100$

The nitrogen content of the sample is obtained this nitrogen is multiplied by 6.25 to get the crude protein percentage and table is constructed for the result.

Table 3. **Crude protein determination**.

Trt. No	Type of Sample	Titration In (ml)	Weight in (g)			%Crude Protein	Remark
			Sample	N	CP		
5	Grass	0.5	0.5004	0.0007	0.0044	0.88	
13	Grass	1.0	0.5003	0.0014	0.0088	1.75	

As it is observed from the Table 3, the results, 0.88 and 1.75% CP for treatment No 5 &13 respectively were different from each other mainly because of the reason that treatment No 5 was subjected to sample loss due to certain technical problems in the distillation apparatus. So, the variations in the results were expected to happen. As it is seen from other students work some got as high as 19% and some others got as low as 2.6% CP. These variations among results could be due to the soil type, growth stage of samples, grass species, and largely due to experimental errors incurred while running the experiment, with the environment being constant for all samples collected. According to some literatures, grass pasture with grazing height contain 22.5% CP, grass hay with high digestibility contain 10.1 and those pasture grasses with low digestibility contain 9.2% crude protein. While collecting our samples we realized that some legumes like that of clover were mixed. So, it was expected to have high CP percentage, as legumes are riches in protein content, However, the result obtained was turned to have low CP content indicating the opposite of the real image of our assumption.

Conclusion

The result obtained being lower than expected and even much lower than those of experiments done by other students and still lower than the information obtained from literatures, it is not a dependable result, so it must be repeated using more samples as a replication, and it should not be used as reference material at this level.

FEED ANALYSIS PRACTICALS BY PROXIMATE METHOD

PRACTICAL- IV

Title: - DETERMINATIOM OF CRUDE FAT

Introduction:

Lipid or fats are a group of substances found in plants and animals tissue, insoluble in water but soluble in organic solvents such as benzene, ether and chlorophorm. They act as electron carriers, as substrate carriers in enzymatic reactions as component of biological membranes and as store of energy. In the proximate analysis, crude fat can be extracted using the above-mentioned organic solvents with soxhlet apparatus. The extract contains triglycerides, Diglycerides, Monoglycerides; in addition, it contains free fatty acids, waxes, cholesterol, chlorophyll, carotenoides, steroids and fat-soluble vitamins, nitrogen-containing substances such as phosphatides and sphingo lipids. Phosphatides and sphingo lipids are considered both in the crude fat and crude protein analysis. All the above-mentioned contribute to the inaccuracy of fat determination by proximate analysis method. That is why the term crude fat is used to represent fat in a sample.

Objective: - To Be Familiar with the Methods of Crude Fat Determination

Materials and Methods

Materials and reagents used were, electronic balance fitter paper, thread, pick up forceps, soxhlet or extractor, boiling flask, condensers, hot plate, oven, funnel, bottle, beaker dissector, piece of cloth, benzene, grass sample. Two samples weighing 1.005 and 1.0007g as a replication were taken by an electronic balance in dairy lab. Using a pre weighed filter paper as a container. And treatment Nos 3 & 4 were given respectively. Then the filter paper was wrapped and tied tightly with thread. Two boiling flasks from the desecrator were taken & weighed. The wrapped samples were put in to the soxhlet, boiling flasks were put onto the hot plate, then the apparatus was assembled.

Finally, benzene was added with the amount of 1 ½ times the capacity of the extractor using funnel through the condenser. Then the hot plate was set on and stayed for three-hrs on extraction. The next morning the wrapped sample was removed using pick up forceps and hot plate was set on again to evaporate the benzene that was found in the boiling flask, by detaching and pouring the benzene that was condensed in the extractor using piece of cloth for heat protection. After the content of the benzene reduced, the boiling flask containing fat residue was detached and put in to an oven adjusted at 105^0c for 1 ½ hrs. for further evaporation of moistures and benzene. Then the boiling flask was removed from the oven and put in to desiccators to be cooled. After cooling the flask along with the sample ether extract was weighed and the result was obtained by difference and table is formulated as shown below.

Results and Discussion

Per cent crude fat was calculated using the formula: -

$$\text{Crude fat percent} = \frac{\text{weight of crude fat}}{\text{Weight of sample}} \times 100$$

Table 4. **Crude fat determination**.

Trt. No	Type of Sample	Weight in (g)					% Ether extract	Remark
		Filter paper + sample	sample	Flask	Flask + EE	Ether extract		
3	grass	2.7332	1.005	60.8200	60.8507	0.0307	3.07	
4	grass	2.8428	1.007	60.9541	60.9938	0.0397	3.97	

As it is clearly observed from the table above that, the percent Ether extract in the two samples/ treatments showed great variation about 25.57per cent. The causes for these variations may arise from the inaccurate during weighing the sample, improper mixing and specially treatment No 3 was subjected to excessive heat that may damage certain components of the sample and may reduce the weight to some extent. However, treatment No 4 was relatively better than that of the former one in its heat treatment. When the average of our sample is compared with the results obtained by other students, some of them got above our results. The information obtained from some references indicated that an increase in the age of plants or plants with low digestibility showed a tendency of decreasing in their fat content.

Conclusion

Except the variation that exists between the two samples or treatments, the over all average of the result obtained was acceptable, so log as it was between the highest and lowest values as it was seen in the discussion part. Had it been with more replications, it would have been easier for us to identity the causes of variation between the two treatments. Therefore, it is advisable to use more replications.

Reference

- Edwards, R.A. et. al. 1981. Animal Nutrition. William clower Beccles Ltd., New York:

- Harris, L.E. and E.W. Crampton. 1969. Applied Animal Nutrition. W.H. Freeman and company, San Francisco;

FEED ANALYSIS PRACTICALS BY PROXIMATE METHOD

PRACTICAL -V

Title: CRUDE FIBER DETRMINATION

Introduction

What is crude fiber? Where does it come from? what is the objective of this test? As it is already known the word crude fiber refers to the residue of a feed that is insoluble after successive boiling with dilute alkali and dilute sulfuric acid. In other words, it is the portion of the total carbohydrate from the food that is resistant to the acid and alkali treatment. This portion of the carbohydrate constitutes cellulose, hemicelluloses and a portion of lignin.

In general, carbohydrates are groups of substances formed by photosynthesis in the plant and contain carbon, oxygen and hydrogen. These three elements join in a certain definite fashion and form complex substances what are known as polysaccharides. These polysaccharides include xylans, arabans and mannans, which are sometimes classed as hemi cellulose. Hemi cellulose together with cellulose are found as a principal part of the plant cell wall structure. Even though, lignin is not a carbohydrate, it forms a physical combination with cellulose and often referred to as lignocelluloses, and it is considered in nutrition with the carbohydrate.

Since the indigestible residues of feedstuff of plant origin are largely of crude fiber, the physical role of crude fiber in a ration should not be overlooked.

Objectives: To be acquainted with the method of determining the portion of crude fiber in a dry fat free grass sample.

Materials and methods

Materials and reagents used were, boiling flask, crucible, vacuum pump, oven, muffle furnace, electric balance, stove, desiccator, pieces of cloth, measuring cylinder, hot water, 1.25% H_2SO_4, 1.25% NaOH. The dry fat free grass sample from which ether was

extracted was added into a boiling flask and 200 ml of 1.25% H_2SO_4 was measured using measuring cylinder and poured in to the flask. The mixture was thoroughly shacked to mix the sample with the chemical uniformly. The flask was put on the stove and the soxhlet was fitted to allow the evaporating particles go through the apparatus. The flask was kept for half an hour on boiling. Using the vacuum pump, flask and gutch crucible, the liquid remained after boiling was removed from the sample. Hot water was added to remove the acid residue from the sample. The sample in the crucible was again put into the boiling flask. 200 ml of 1.25 NaOH was measured with measuring cylinder and poured in to the flask containing the sample. The flask was put on the stove and allowed to boil for half an hour. Using vacuum pump, the liquid was removed from the sample and the sample was retained in the crucible. The crucible along with the samples was put in the oven to remove the moisture and kept until constant weight.

The samples along with the crucible were transferred to the muffle furnace to be ignited. Here the samples were kept for long time in the muffle furnace due to power failure. Then the sample was taken out of the muffle and put in to the desiccators to be cooled. After cooling, the samples were weighed and the results were obtained by difference.

Results

Weight of crude fiber was calculated by the formula

► Crude fiber = $\frac{\text{weight of crude fiber}}{\text{Weight of original sample}} \times 100$

The results obtained were organized as indicated in Table 5.

Table 5. **Crude fiber determination**.

Trt No	Types of sample	Weight in grams					% CF	Remark
		Original sample	crucible	Fiber + Ash	fiber	Ash		
4	grass	1.0007	49.9057	0.2896	0.2685	0.0211	26.83	

Discussion

The constituent of the feed sample like protein, starch, ash, sugar, cellulose, hemicellulose and lignin were removed in the ether extraction process. These substances were attacked by the chemicals used in the following manner; protein was

partially extracted by the addition of dilute H_2SO_4 and complete extraction is accomplished by the addition of 1.25 % dilute NaOH. The starch and sugar are hydrolyzed and extracted by the addition of 1.25 % dilute H_2SO_4. Cellulose was slightly affected by both reagents. Hemicellulose variably extracted by the addition of dilute H_2SO_4 and extensively extracted by the dilute NaOH. lignin is slightly affected by dilute H_2SO_4 and highly extracted by the dilute NaOH. The final residue was ignited to obtain crude fiber by difference. It is clearly seen from the table above that our grass sample gave crude fiber content of 26.83 per cent.

Conclusion

So by directly relating our experiments to the information obtained from books, it is a good indication of the proportion of crude fiber in our fat free dry grass sample.

Reference

Crampton, E, W. and HARRIS. L.E. 1969. Applied Animal Nutrition
H.W. Freeman and company, San Francisco.