Laboratory Exercise 4
ANSC 3400 - Animal Nutrition

Proximate Analysis, Digestibility and TDN

Name____________________________
Section_______

Before beginning this exercise, read pages 36-38 (Methods for Assessing Nutrient Content of Feeds), 43-45 (Estimating Nutritional Value of a Feed), and 141-144 (Laboratory Analysis Methods) in your nutrition text. Part of understanding nutrition, particularly the applied animal feeding aspects of nutrition, includes having a modest understanding of laboratory analyses that are commonly utilized. Each procedure has its value, but each also has its limitations.

A. Read about sampling procedures. Why do we have to sample? How do we get a representative sample? Answer here:

B. Chemical procedures usually are accurate in measuring the quantity of a nutrient in a feed. What do chemical procedures not tell us about a nutrient? Why do we sometimes also use biological procedures? Answer here:

Feeds are analyzed by a number of methods. Some of them are new, and some that are still used are very old. Proximate analysis has been used for a very long time, but is still the starting point for most feeds analysis.

Proximate analysis analyzes for the following:

<table>
<thead>
<tr>
<th>Category</th>
<th>Abbreviation</th>
<th>Approximate contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>H₂O</td>
<td>Water (100 - %H₂O = %DM¹)</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>CP</td>
<td>Protein and NPN² compounds</td>
</tr>
<tr>
<td>Ether Extract</td>
<td>EE</td>
<td>Fat</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>CF</td>
<td>Cellulose &amp; all fibrous carbohydrates + lignin (hopefully)</td>
</tr>
<tr>
<td>Ash</td>
<td>Ash</td>
<td>Total minerals</td>
</tr>
<tr>
<td>Nitrogen Free Extract</td>
<td>NFE</td>
<td>Starches, sugars &amp; all soluble carbohydrates (hopefully)</td>
</tr>
</tbody>
</table>

¹ DM = Dry matter
² NPN = Nonprotein nitrogen (Nitrogen that is in compounds other than protein)

These are determined according to the following methods:

1. **Water**
   1. Weigh an original sample of the feed
   2. Place in an oven at 90-95 degrees C
   3. Remove, cool and weigh the sample. Repeat 1, 2, and 3 until constant weight is obtained, or follow a previously established protocol that has determined the proper length of drying time.
   4. Calculate water lost by difference between the final sample weight and the original sample weight.
   5. Calculate % water by dividing by the original sample weight X 100.
2. **Crude Protein (Kjeldahl method)**
   1. Weigh an original sample of the feed.
   2. Place in a Kjeldahl flash and boil with sulfuric acid, converting the N to (NH4)2SO4.
   3. Cool, dilute, neutralize with sodium hydroxide, distill and titrate the distillate with a known concentration of acid.
   4. Multiply the amount of Nitrogen determined by this procedure by 6.25 to calculate Crude Protein.
      - The word “crude” is used because the Kjeldahl method measures total nitrogen in the sample, and this value then is multiplied times 6.25 to get protein. Most N is in true protein; however, some N is in compounds that are not part of the protein. (This is called NPN.) Examples of NPN include urea, some B-vitamins that have N in their structure, nucleic acids, free amino acids.)
      - Other, newer methods, such as spectrophotometric ones, sometimes replace the wet chemistry methods of Kjeldahl, but the principle of N X 6.25 remains the same.
   5. Determine the % CP by dividing the quantity of CP by the original sample weight X 100.

3. **Ether extract (total fat or lipids in a sample)**
   1. Weigh an original sample of the feed.
   2. Place in an oven to remove water, remove and weigh again.
   3. Reflux the sample with ether to remove the fat.
   4. Dry and weigh the remaining, moisture-free and fat-free sample.
   5. Calculate the amount of fat lost by subtracting the last weight from the weight after water removal.
   6. Calculate the % fat in the feed by dividing the fat lost by the original sample weight X 100.

4. **Crude fiber**
   1. Weigh an original sample of the feed.
   2. Boil the sample in acid, then rinse the residue to remove everything soluble.
   3. Boil the sample in alkali, then rinse the residue to remove everything soluble.
   4. Dry the sample and weigh it. **The remaining material is the crude fiber + some ash.**
   5. Determine the amount of ash in the sample by placing what remains in an ashing oven and burn at 600 degrees C. What remains is ash. Weigh this and subtract it from the crude fiber + ash weight.
   6. Determine the % CF by dividing the crude fiber weight by the original sample weight X 100.

5. **Ash** (This needs to be done even though ash was determined in the previous step, because that value will not be accurate for the feedstuff because of the steps in which that sample was boiled in acid and alkali).
   1. Weigh an original sample of the feed.
   2. Place the sample in an ashing oven at 600 degrees C.
   3. Remove, cool and weigh the sample. What remains is ash.
   4. Determine the % ash by dividing the quantity of ash by the original sample weight X 100.

6. **NFE**
   1. NFE is determined by difference.
   2. 100 - Water - CP - EE - CF - Ash = NFE
   3. The value will already be a % figure if all the other values were expressed as %.

**Problems with Proximate Analysis Short-falls and Development of the Van Soest Detergent System**

The proximate analysis system serves as a very useful purpose in determining the nutritive value of feeds. However, there are some definite limitations of this system, especially with respect to both the crude fiber and NFE fractions. The material that was dissolved by the solvents (NFE) was assumed to be digestible and the residue (crude fiber) was assumed to be indigestible. Studies showed that in some cases crude fiber was more digestible than was NFE. Low digestibility of the NFE fraction arises from the fact that nearly all of the fibrous
hemicellulose and a variable amount of the lignin are dissolved in the weak acid and alkali. Both fractions have been shown to be relatively indigestible. Thus, predicting nutritive value from the proximate analysis figures became a major problem, especially with the more fibrous feeds.

Van Soest developed an extraction scheme that better describes the fiber fraction of feeds, particularly roughages. One fraction, acid detergent fiber (ADF), represents the amount of residue (primarily cellulose, lignin and variable amounts of silica) left after boiling a feed sample in an acid detergent solution. The ADF value is an indicator of relative digestibility and is used to predict the energy content (TDN, NE) of forages. Another fraction, neutral detergent fiber (NDF) is the insoluble fraction containing all plant cell wall components (cellulose, hemicellulose, lignin) left after boiling a feed sample in a neutral detergent solution. NDF is of low digestibility, but can be broken down somewhat by the digestive tract microorganisms. The NDF value is used to predict ruminant feed intake.

BEGIN LAB EXERCISE HERE

After reading the text book and the above information about proximate analysis, read and answer the following. Answers are given on the last page. Show your work in the space provided. When completed, show your work and answers to Dr. Schmidt to receive your homework which will be due the next Monday (Feb. 21). Be prepared to answer some questions about what you have learned (or should have learned!) when completing this exercise.

1. Proximate analysis subdivides feed components so that feeds may be compared on a nutrient content basis. The first component of interest is water.

The complete analysis is called the _______________ analysis.

2. The dry matter of feeds varies from 6 to 90% of total weight. This means 94 to 10% of the weight was water. The water is driven off a known weight of feed by heating at 105°C until no more weight is lost. If 2 grams are lost when 10 g of wet corn is dried, the wet corn was ________% dry matter.

3. Feeds and foods over 15% water content are subject to mold and bacterial degradation. Yet some foods are preserved by pickling (acid addition), by adding sugar or by sterilization. Mold decomposition might be expected if food contain less than _________% dry matter.

4. Water is a diluent of food nutrients. For example, if corn at 75% dry matter sells for $1.50 per bushel, corn containing 10% moisture (90% dry matter) is worth __________ per bushel.

5. Minerals in foods are classed in the proximate principle called ash. To determine ash, a weighed food sample is heated to 600°C for 2 hours and the residue weight is the ash. For example, if 15 g of Wheaties is heated to 600°C for 2 hours, only 0.6 g remains. Therefore, the ash content of Wheaties is ________%.

6. Ash content represents a grand total of all minerals. Since animals require specific amounts of specific minerals, ash (is) (is not) a good index of nutritive value of minerals in a food.
7. A third nutrient includes all substances soluble in ether and is thereby called "ether extract". This fraction will consist of triglycerides (fats), sterols, and pigments. If from 1 g of pork loin chop 0.42 g can be extracted with ether, this pork chop is ________% fat.

8. Fats are added to feeds to suppress dust and also increase the energy content, but if unsaturated fats are added at high levels, they may become rancid. Lard rancidity is probably a result of the presence of ________________.

9. A fourth fraction, crude protein, is estimated from nitrogen content of the feed. Proteins in feeds generally will contain about 16% nitrogen. This means every gram of nitrogen found in feed represents 6.25 (or $\frac{100}{16}$) grams of protein.

As an example, beef steak contains about 4% nitrogen. This means that steak is about ________% crude protein. Why do we use the word “crude?” (See page 2.)

10. Crude protein values reflect quantitatively the nitrogen content of feeds in general. But for animal nutrition, specific amounts of essential amino acids, not of protein, are required. Therefore, as an index of protein quality, crude protein is (good) (poor).

11. A fifth proximate principle is crude fiber. This is the residue which remains after a series of acid and base washings and represents the less digestible carbohydrates, primarily cellulose and lignin. If from 10 g of corn, 0.4 g organic residue remains following acid and base washing, the crude fiber content of corn was ________%.

12. Crude fiber contributes bulk to the diet but is generally low in digestibility. The sixth fraction, called nitrogen free extract (NFE), is the more soluble and digestible carbohydrate fraction. NFE content is determined by difference, so is 100 minus the percentages of each of the other five components, namely ____________________, ____________________, ____________________, ____________________, and ____________________.

(Check 1, 5, 7, 9 and 11 for help)

13. The carbohydrates are found in two fractions, the more digestible in the ________________ fraction and the less digestible in the ________________ fraction.

14. As an example, if corn grain contains 85% dry matter, 4% ash, 4% ether extract, 1.44% nitrogen (careful) and 5% fiber, it has ________% nitrogen free extract.
A 100 g feed sample was dried in an oven at 100°C for 24 hours. After oven-drying, the sample weighed 89 gm. A 5 gm oven-dried sample was analyzed for crude protein content by the Kjeldahl method. The sample contained 3.24% nitrogen. A second 5 gm oven-dried sample was extracted with ether. After extraction, the sample weighed 4.831 gm. To determine the crude fiber concentration, a 2.5 g sample was boiled in dilute sulfuric acid followed by boiling in dilute sodium hydroxide. After boiling in acid and alkali, the dried sample weighed 2.442 gm. Mineral concentration was determined by heating a 1 gm sample in an oven at 600° C until it is reduced to ash. The ash weighed 0.0225 g.

Calculate the percent moisture, crude protein, crude fat, crude fiber and mineral content on a dry matter and as-fed (air-dry) basis.

A. Percent dry matter (89.0%)

B. Percent crude protein – (20.25% DM basis)  (18.02% As-fed basis)

C. Crude fat – (3.38% DM basis)  (3.00% As-fed basis)

D. Crude fiber – (2.32% DM basis)  (2.06% As-fed basis)

E. Mineral content – (2.25% DM basis)  (2.00% As-fed basis)

This Concludes Discussion of Proximate Analysis.
II. EVALUATION OF FEEDS

Coefficients of digestibility

Chemical analysis is the starting point for determining the nutritive value of a feedstuff; however the gross composition does not tell anything about the value of the feedstuff in livestock diets. A digestion trial is conducted in combination with the proximate analysis to determine the nutritive value of a feedstuff. Two common methods for conducting a digestion trial are:

1. Total feed consumption and total fecal collection.
   a. Animals are fed a given amount of feed or fed at a constant rate over a 4 to 5-day period.
   b. All fecal material is collected, weighed, and a sample taken.
   c. Feed and fecal material are oven-dried to determine the dry matter content.
   d. A proximate analysis of the feed and feces is conducted.

2. Marker method
   a. Animals are fed a diet containing a physiological inert material that does not contain the element under investigation and that will not diffuse (carmine, ferric oxide, chromic oxide).
   b. Sample of feed and fecal material is collected, oven-dried, and a proximate analysis is conducted.
   c. Concentration of the marker (inert material) in the feed and fecal material is determined.
   d. In this method, neither the total feed consumed nor the total fecal output is required.

The value of feed nutrients to an animal is assessed by digestibility or nutrient retention trials. **Apparent digestibility** is the disappearance during passage through the gastrointestinal tract. It is calculated by the following formula:

\[
\text{Apparent digestibility (%) } = \frac{\text{Quantity of nutrient eaten} - \text{Quantity of nutrient in feces}}{\text{Quantity of nutrient eaten}} \times 100
\]

or, more precisely

\[
\text{Apparent digestibility (%) } = \frac{[\text{wt. of feed } \times \% \text{ nutr. in feed} - \text{wt. of feces } \times \% \text{ nutr. in feces}]}{\text{wt. of feed } \times \% \text{ nutr. in feed.}} \times 100
\]

16. If 20 pounds of dry matter is consumed by a steer and 20 pounds of feces at 20% dry matter is produced, the apparent digestibility of dry matter is __________ %.

17. If the feed dry matter is 2% nitrogen and dried feces from the previous questions has 3% nitrogen, the digestibility of crude protein is __________ %.

(Check 9 for help)
18. Availability of energy from feeds considers the relative caloric value of nutrients in the proximate analysis. Carbohydrates and protein yield 4 kcal metabolizable energy for every gram digested and ether extract yields 9 kcal per gram. Water and minerals contribute no energy. On a weight basis compared to digested carbohydrate, such as starch, digested carbohydrate, protein and fat provide energy at a ratio of 1:1: ____.

19. This means that on an equal weight basis, fat provides 2 1/4 times the energy to an animal as protein and carbohydrate. For animals with a small capacity gastrointestinal tract, such as baby pigs or chicks, ______ should be added to the diet to increase "energy density" (or energy per pound of food).

20. These energy values of carbohydrates, proteins and fats (4, 4 and 9 kcal/g) are called Physiological Fuel Values and can be used to estimate the kilocalories (kcal) of metabolizable energy consumed. This is what we call "calories" in human nutrition. If a serving of milk (8 oz. or 240 g) contains 11.2 g of lactose (carbohydrate), 8.6 g of fat, and 7.9 g of protein, how many kcal of energy are in the serving?_______

** You will be expected to know the caloric values (Physiological Fuel Values) of carbohydrates, proteins and fats. **

21. Using the caloric equivalent of carbohydrate, protein and fat (4, 4, 9 kcal/g) or the starch equivalents of these nutrients (1, 1 and 2 1/4 times weight) the amount of energy digested by an animal may be calculated. The amount of starch equivalents digested from a feed is called Total Digestible Nutrient (TDN) content.

If pure corn starch is 85% digested, it has a TDN content of ______ %.  

22. If casein (pure protein) is 70% digested, its TDN content is ______ %.  

23. If lard (pure fat) is 80% digested, its TDN content is ______ _____% (Careful)! (See 20 for help)

24. If water and ash are 60% digestible, what is their TDN content? ________ %.

**The formula for calculating the total digestible nutrients of a feed is**

\[
\text{TDN} = \text{digestible carbohydrates} + \text{digestible protein} + (\text{digestible fat} \times 2.25)
\]

The value ranges from about 50% for roughages to about 80% for grains.

The above formula is the simplest for TDN. The more precise way of writing the formula for TDN is

\[
\text{TDN} = (\% \text{ crude fiber} \times \text{digestion coefficient}) + (\% \text{ NFE} \times \text{dig. coeff.}) + (\% \text{ crude protein} \times \text{dig. coeff.}) + (\% \text{ fat} \times \text{dig. coeff.} \times 2.25)
\]

See number 13. The carbohydrates include two components: crude fiber and NFE.
Example of TDN calculation

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Feed</th>
<th>Digestion coefficient, %</th>
<th>Digestible nutrient, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude fiber, %</td>
<td>17.89</td>
<td>73.6</td>
<td>13.17</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>18.40</td>
<td>79.2</td>
<td></td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>2.01</td>
<td>53.9 (x 2.25)</td>
<td></td>
</tr>
<tr>
<td>NFE, %</td>
<td>57.02</td>
<td>81.7</td>
<td>46.58</td>
</tr>
</tbody>
</table>

TDN content of feed = 76.76%

25. Corn has the following composition and digestibility for various nutrients:

<table>
<thead>
<tr>
<th>% in corn</th>
<th>Digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>15.0</td>
</tr>
<tr>
<td>Ash</td>
<td>5.0</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>1.44</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>6.0</td>
</tr>
<tr>
<td>Ether extract</td>
<td>4.0</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td></td>
</tr>
</tbody>
</table>

Calculate the NFE value, and then calculate the TDN of the corn. (Check 9 and 12 for help.)

Good. You now are an expert on Proximate Analysis, digestibility and TDN! If you do not feel like an expert, review your text book, notes, and laboratory exercises. This is important material, and you will be expected to know it.
ANSWERS

Proximate Analysis

1. Proximate
2. 80%
3. 85%
4. $1.80
5. 4%
6. is not
7. 42%
8. unsaturated fats
9. 25%
10. poor
11. 4%
12. water, ash, ether extract, crude protein, crude fiber
13. nitrogen free extract, crude fiber
14. 63%
15. Answers given with the problem

Apparent Digestibility & TDN

16. 80%
17. 70% Note that the same answer is obtained using either crude protein or nitrogen to do the calculations. Try it.
18. 2.25
19. fat
20. 154 kcal (153.8) Look on the labels of other foods at home and calculate the calories (kcal) per serving. See how close your calculated values come to the calorie value printed on the label.
21. 85%
22. 70%
23. 180%. TDN content can be over 100% since it compares available energy to a carbohydrate, and fat has a higher "energy density" (2.25 times higher than carbohydrate).
24. 0 See number 17. Water and ash contribute no energy, so they do not add to the TDN value.
25. Protein = 1.44 x 6.25 = 9%
   NFE = 100 - 15 - 5 - 9 - 6 - 4 = 61%
   TDN = 9(.85) + 6(.70) + 4(.80)(2.25) + 61(.95) = 77%