Protein
Proteins are high molecular weight compounds containing carbon, hydrogen, nitrogen and in some cases sulphur. The building blocks of protein are called amino acids. There are some twenty three different amino acids and it is these (not protein per se) which are required by animals in their diet for their development and growth. A number of them cannot be produced in the body and these are known as essential amino acids. Examples are Lysine, Methionine, Tryptophan, Threonine and Cystine. The amount of essential amino acids available in meals is therefore an important consideration in animal feed formulation.

Determining nitrogen
The amount of nitrogen in the muscle tissue of animals is relatively constant at around 16 percent of the crude protein level. The crude protein level of meat and bone meal can therefore be measured by determining the nitrogen content and multiplying it by the factor 6.25 (100 + 16 = 6.25)

There are two primary reference methods that can be used to determine nitrogen content in meals; Kjeldahl and Dumas Combustion.

The Kjeldahl method involves high temperature digestion of the sample with concentrated sulfuric acid. Two additional steps, distillation and titration are performed to obtain the nitrogen result which is then converted to percentage crude protein using the factor 6.25.

Our preferred method is the Dumas system which determines the nitrogen content by combustion. In the presence of oxygen, a sample is combusted to produce nitrogen oxides and other gases. The use of a catalyst allows the nitrogen oxides to be reduced to elemental nitrogen. The amount of nitrogen present in the sample is measured using a thermal conductivity cell and will be displayed as a percentage of nitrogen and/or protein. Side products produced from combustion are removed through absorption. Again, Crude protein is calculated by multiplying the nitrogen content by the factor 6.25.
Meat and Bone Meal (MBM)

Why do we need to determine protein?
The Crude Protein test is important in the trading of rendered meals as a basic measure of quality and commercial value. As rendered meals are produced from natural raw materials which can be variable, close monitoring of their protein levels is important to both producers and end users. Feed manufacturers rely on minimum crude protein levels to be maintained for meals used in their formulations.
Moisture

Moisture is a term used to describe the amount of water remaining in meat and bone meal after it has been processed. Moisture is determined by measuring the loss in weight upon heating a sample in a drying oven at 135˚C for a given period of time. Moisture is expressed as a percentage:

**Why do we determine moisture?**

The moisture component of MBM after processing is ideally in the range 3 to 6 percent. Levels below 2 percent may indicate overcooking.

High levels of moisture are detrimental to the quality of the meal and a maximum limit of 10 percent is set. High moisture will depress the crude protein level of the meal and increase the potential for oxidation of the fat component. It will also affect the free flowing physical characteristics of the meal and increase the possibility of micro organism growth.
Fat

The fat content of meat and bone meal is the amount of fat remaining in the product after processing. Fat levels will vary from plant to plant depending upon raw material input and processing conditions. They normally range from around 8 to 13 percent with a maximum of 15 percent.

The analysis of the fat content of meat and bone meal is usually carried out by the Soxhlett Extraction method using petroleum ether solvent. The method can be performed automatically using an instrument such as a Soxtec 2050. Samples are loaded into the instrument and petroleum ether is dispensed into each of them. The Soxtec then performs four steps during which the samples are extracted, rinsed and the used solvent recovered. The samples are dried and weighed and the fat content calculated and expressed as a percentage.

Why do we determine fat?
Fat provides a valuable source of energy in animal feed. High levels of fat however can affect the free flowing characteristics of the meal causing handling problems which may include caking in bins and chutes. Excessive fat may also lead to problems with oxidative stability.
Ash

Ash is the residue remaining after incineration of a sample of meal at 600 degrees Celsius, and it reflects the ratio of bone to soft tissue in the raw material. It has an inverse relationship to the crude protein content – as ash increases, protein will decrease.

Ash is mainly bone residue and is therefore rich in calcium and phosphorus in the ratio of approximately 2:1. It also contains lesser amounts of other minerals.

How Is It Done?
A sample of the meal is accurately weighed into a crucible and placed in a muffle furnace where it is incinerated at 600 Degrees Celsius. The residue is weighed and the result expressed as a percentage of the meal.
Crude Fibre

Crude fibre is synonymous with "roughage" in animal feed and is referred to as consisting of structural carbohydrates. Its components are mainly cellulose, hemicelluloses and lignin. These materials are insoluble in diluted acids and alkalis and have poor digestibility in animal feeds. In rendered meat and bone meal, crude fibre consists mainly of the cell walls of plant material which remains undigested in the animal.

How do we determine crude fibre?
A defatted sample of the MBM is weighed into a special fibre cap and the fibre is extracted by initially boiling it in diluted sulphuric acid for 30 minutes. Any acid soluble material will be digested and can then be removed. The residue is washed with boiling deionised water to remove any excess sulphuric acid and any soluble matter. This process is then repeated using dilute sodium hydroxide followed again by washing. The residue is then rinsed with acetone to remove any excess water and placed in an oven at 100˚C to dry. Finally, the fibre cap plus residue is incinerated at 600˚C with weighing before and after incineration. An empty capsule is also incinerated as a blank for use in the calculations. Crude fibre content is then calculated as a percentage of the meal.

Why do we determine crude fibre content?
Crude fibre in MBM is generally very low at less than 3 percent, and as such does not impact adversely on its utilization in baby pig or fish diets.
Pepsin Digestibility

Digestibility is the amount of feed material which, when consumed by an animal is absorbed into the body and is hence of nutritional value.

How do we determine pepsin digestibility content?
A portion of the sample is initially tested for crude protein and fat content and the results used in final calculations.

A defatted sample of the MBM is then digested with a pepsin solution of known concentration under constant agitation for 16 hours. Any digestible protein will have been taken up in the pepsin solution. The insoluble residue is then filtered and tested for protein. The difference between the digestible and indigestible protein is calculated and expressed as a percentage pepsin digestibility.

Why do we determine pepsin digestibility content?
In vivo testing of feed ingredients involving tightly monitored feeding trials on live animals is both expensive and time consuming and is not suitable for routine analysis of large numbers of samples. The pepsin digestibility test provides a relatively inexpensive and quick way of evaluating the digestibility of feed raw materials such as meat and bone meal using in vitro techniques. The correlation of the pepsin digestibility test results with in vivo trials on MBM is variable. However, researchers have found that reducing the pepsin concentration in the standard in vitro test from 0.2% to 0.002% increased the accuracy of the test as a predictor of in vivo MBM quality tests.
Screen tests

Screen tests are conducted to determine whether meal has been ground uniformly and to the required degree of fineness. Meal which is too course or inconsistent in its grind can lead to processing problems for feed and pet food manufacturers.

How do we screen?
A sample of the meal is passed through a series of mesh screens under controlled conditions. The particles retained on each screen are weighed and the results are calculated and expressed as a % of total sample. The general standard for meat and bone meal is that 98 percent should pass through a 2.00mm mesh screen.
Meat and bone meal is a valuable source of available calcium, phosphorus and trace minerals for pig and poultry rations. The ratio of calcium to phosphorus is approximately 2:1, consistent with that of bone. In general meat and bone meal is sold on the basis of minimum 8 percent calcium and 4 percent phosphorus, but actual levels will vary from one renderer to another depending upon raw material input.

**How do we determine calcium and phosphorus content?**

A sample of the meal is reduced to ash in a muffle furnace to remove the organic material. The ash is then digested with a mixture of hydrochloric and nitric acids to liberate the Ca\(^{2+}\) and P\(^{3-}\) ions. This is then diluted to exactly 100ml and a measured quantity is removed for the calcium test. A separate aliquot is taken for the phosphorus test (see below).

**Calcium:** The solution is buffered to maintain its acidity, and ammonium oxalate is added resulting in the binding of the Ca\(^{2+}\) ions as calcium oxalate.

After filtering and washing away any excess free oxalate, the solution is acidified with sulphuric acid and titrated with potassium permanganate to determine the moles of oxalate and calculate the calcium content. One mole of oxalate equals one mole of calcium.

**Phosphorus:** To the aliquot drawn from the original solution for the phosphorus test, 20 ml of Molybdovanadate is added. This binds to the phosphate forming a complex, the concentration of which can be measured by its colour intensity and read on a Spectrophotometer.
Peroxide Value

Peroxide value is used as an indicator of the extent of oxidation of the fat component in MBM. Oxidation occurs at the unsaturated bonds in the fatty acid chains of the fat component.

How is it determined?
The fat is firstly extracted from the MBM with the use of petroleum spirit. The PV is determined by firstly dissolving a weighed sample of tallow in a solvent of acetic acid and iso-octane.

Potassium iodide is then added and the peroxides react with it to liberate iodine. The reaction is then stopped by diluting the mixture with deionised water and the amount of liberated iodine is measured by titrating with sodium thiosulphate. The amount of iodide liberated is expressed as milli-equivalents (meq) of peroxide per 1000g of sample.

Why is it determined?
High peroxide value is undesirable in meat and bone meal and may be the result of inadequate raw material handling, processing, storage or transport conditions.
Wool and Hair

The wool and hair content in MBM is of little nutritional value for monogastric animals such as pigs and poultry. The protein keratin in wool and hair is indigestible unless the rendering process includes a pressure cycle capable of hydrolyzing keratin. A high content of wool and hair in MBM will lower its pepsin digestibility. The maximum level of wool and hair in MBM is set at 1 percent.

How is wool and hair content determined?
A sample of MBM is sieved and the wool and hair remaining is collected, washed with acetone, dried, and weighed. The results are expressed as % of total weight of sample.
Minerals including sodium chloride are of nutritional and functional importance in animal feed, however their levels need to be known and/or controlled to ensure optimum usage and avoid dietary problems. The maximum sodium chloride content of meat and bone meal is 1 percent.

How is salt content determined?
CIS uses the Volhard method to determine the salt content of meat and bone meal.

The sample is initially treated with excess silver nitrate (AgNO3) then concentrated nitric acid (HNO3) and placed on a hotplate to boil. It is important that AgNO3 then HNO3 is added to ensure complete precipitation of AgCl. If the HNO3 is added first, chlorides are lost by volatilization as HCl.

An excess volume of AgNO3 solution is added so that all the Cl- present will react. Concentrated potassium permanganate (KMnO4) is then added to oxidise any organic matter that has not been removed by HNO3. This is then further boiled, cooled and diluted before being back-titrated with ammonium thiocyanate (NH4SCN) solution using ferric ammonium sulphate (NH4Fe(SO4)2 ) solution as an indicator.

The titrate remains pale yellow as excess (unreacted) Ag+ react with -SCN to form AgSCN precipitate. Once all Ag+ ions have reacted, the slightest excess of -SCN reacts with Fe3+ ions to form a dark complex. The concentration of Cl- is determined by subtracting the titration findings of the moles of Ag+ that reacted with –SCN from the total moles of AgNO3 added to the solution.
Free Fatty Acid (FFA)

The fat content of meat and bone meal is relatively high with levels ranging up to as much as 15 percent.
Fat is subject to quality deterioration due to various reactions including hydrolysis and oxidation which in animal feed may lead to reduced nutritional value and palatability problems.

The FFA test is a measure of the hydrolytic rancidity in fats. The majority of fats are triglycerides, a combination of glycerol with three fatty acids attached. However one or more of the fatty acid can be detached from the glycerol through hydrolysis. The detached fatty acid is called free fatty acid (FFA). The hydrolysis of triglycerides in rendering is accelerated by high moisture, heat and enzymes produced by bacteria present in raw material and finished product.

How do we determine FFA?
FFA is obtained by direct titration with standard alkali (sodium hydroxide). The fat is firstly extracted from the MBM with the use of petroleum spirit. Next the recovered MBM fat dissolved in neutralized isopropanol with the aid of a hotplate. The amount of FFA in the sample is determined from the volume of standard alkali required to neutralise the fat. Example of a free fatty acid reacting with a standard alkali (sodium hydroxide):
CH3(CH2)14CO2H (free fatty acid) + NaOH → CH3(CH2)14CO2Na + H2O