CHAPTER ONE

1.0 INTRODUCTION

Rapid technological progress in poultry sector has resulted in a remarkable reduction in the cost of production and marketing of poultry products. Falling retail prices of poultry products relative to the cost of other animal products due to this improved technology has resulted in increased consumption of poultry products worldwide (Vocke, 1991). Poultry population of the Sudan was estimated to be (47,194,000) head of poultry all over the country. In Khartoum State the area of the study is said to be (13,403,096) head of poultry (Ministry of Agriculture, Animal Resources and Irrigation, 2015) (M.A.A.R.I). Also the actual poultry meat production was found to be (50,000,000 Kg) and egg production were estimated to be (40,000,000) egg tray. Egg and poultry meat per capita in Sudan were 1 kg and 1 kg/year respectively (M.A.A.R.I., 2017). The poultry stock is commonly fed on diets with high nutrients density to suit its simple and limited digestive system. These nutrients must be supplied in optimum proportions that promote their efficient utilization for the various body processes of maintenance and production. Poultry sector suffered from several problems including the deficiency of nutritional levels which lead to decreased production. It is very important to study the actual nutritional needs of poultry sector in Sudan due to its reasonable share in the national economic, but it still
needs attention to develop the structure and components of poultry feed to meet the specification outlined by Sudanese Standards and Metrological Organization (SSMO, 2015). Improvement of poultry feed quality depends on developing poultry feed industry in order to increase the efficiency of nutrients utilization of the feed ingredients. The quality of poultry feeds is based on the quality of its constituents and the raw material used to formulate the ration. Feed must supply all nutrients in adequate quantity and with high digestibility. Sudan is currently the only Arab country that is self-satisfied in its feed production, with the exception of concentrates, premixes, some feed additives and toxin binders (Freiji, 2008). The nutrient requirements for poultry are affected by many factors including: breed, age, sex, production stage, temperature, housing system, health status and purpose of production. Cereal grains are added primarily as a source of metabolizable energy while soybean, sesame and groundnut cakes represent the protein requirement. Feed additives used in poultry diets includes: mycotoxin binders, shell enhancers and some prebiotics or probiotics in order to improve health and growth National Research Council (NRC, 1994). Nutritionists have developed feeding standards for determining the daily nutritive requirements of poultry. There is difference, of course, between broiler diets and the diets of the laying hens. It is time to change this situation by making a new system to develop the concepts of poultry farming and feed manufacturing and
establish technical and economical solutions by making manufacturing units produce well balanced rations to meet the nutrients requirements and update the standards and measurements of poultry feeds and their inputs if required. The objective of this study was to obtain reliable data on chemical composition and nutritional value of poultry feeds available in the local markets in order to advise farmers for feed uses, and help feed millers to improve the quality of their products. Also the study aimed to assist in updating Sudanese specifications and standards outlined by the (SSMO).
2.1 Poultry Feed and Feeding:
Feed represents the major cost of poultry production, constituting up to
70 percent of the total cost, about 95 percent is used to meet energy and
protein requirements, about 3 to 4 percent for major mineral, trace
mineral and vitamin requirements, and 1 to 2 percent for various feed
additives. Poultry diets are formulated from a mixture of ingredients,
including cereal grains, cereal by-products, fats, plant protein sources,
animal byproducts, vitamin and mineral supplements, crystalline amino
acids and feed additives. These are assembled on a least-cost basis,
taking into consideration their nutrient contents as well as their unit
prices (Ravindran, 2012).

2.2 Poultry Feed Resources
The feed resources can be divided into two main categories as
conventional and nonconventional feed resources. Conventional feed
sources are those traditionally used, whereas, those non-conventional
once are not commonly and traditionally used as chicken feeds (Younas
and Yaqoob, 2005). Conventional feed resources are facing a problem of
competition with human foods. Gura (2008) stated that the recent feed
price increment may upset many of the plans to further development of
industrial livestock/poultry productions.
In Sudan poultry feed resources is met through local production. The principal raw materials required are oil cake, wheat bran, cereals, limestone, vitamins, minerals and salt. All the raw materials are locally available, except concentrate and vitamins which have been imported. Proteins, largely of vegetable origin, encourage the normal development of broiler and pullets which help them to lay eggs longer. According to Wilson and Beyer (2000) the profit from poultry production can be attained by minimizing feed cost which accounts for more than half of the total cost of production. Feed cost accounts to 60-70% of the total animal production cost. Any attempt to improve commercial poultry production and increase its efficiency therefore, needs to focus on better utilization of available feed resources. Knowledge of nutritional characteristics of these feeds and their optimal levels of inclusion in rations and optimum combination of ingredients composed locally available materials are very useful (Kamalzadeh et al., 2008). In tropical countries like Sudan where cereal grains are staple diet of human, the use of agro-industrial by-products as an alternate source of protein, partially or/and fully enables cheaper egg and meat production which in turn decreases consumption of cereal grains by animal (Meseret et al., 2012). The industrial sector in Sudan is still playing a minor role in the national economy. Nevertheless, at present, industries especially those depending on agricultural raw materials are undergoing significant revolution particularly owing to the current free market economic
A variety of oil crops are known to grow in Sudan. Among the major oil crops, for instance cotton and groundnut seed are produced annually (Solomon, 1992) and used mainly by the oil processing industries. Currently, residues of oil extraction industries are providing a large quantity of oil seed cakes and meals to poultry farmers at a relatively reasonable cost.

2.3 Components of Poultry Feeds

Poultry diets are made primarily from a mixture of several feedstuffs such as cereal grains, soya bean meal, animal by-product meals, fats and vitamin and mineral premixes (Alimon and Hair-Bejo, 1995). A poultry diet is expected to contain three essential nutrients of protein, vitamins, and minerals as well as provides adequate metabolizable energy (ME). The most easily available sources of energy are the carbohydrates contained in common grains, grain by-products and plants generally (Dateh, 2013). The important and basic components of a laying hen and broiler diets include energy, carbohydrates, protein and amino acids, fat, and vitamins and minerals. Not only must all of these nutrient sources be present in the diet, but they must also be present in certain amounts (Depersio, 2011). Most of the carbohydrate in poultry diets is provided by cereal grains. Suitable quantities of fat may be added to increase dietary energy concentrations and palatability. Protein is essential in all animal life. Proteins make up a large part of the muscle, skin, beak, feathers, cartilage and internal organs of animals and are needed for
growth, egg production, reproduction, production of antibodies to fight diseases. The dietary requirement for protein is actually a requirement for amino acids. Specific amino acids must be provided in proper amounts and in some definite ratios to others. An under supply of a single essential amino acid will inhibit the responses to those in adequate supply (Fanatico, 2010). In any protein, the limiting amino acid is the one which is below the standard. For poultry, methionine is usually the first limiting amino acid and lysine the second limiting amino acid. Since protein is not stored in the body to any considerable extent, any protein consumed above the birds’ requirement is oxidized for energy. However, since protein sources are expensive and uneconomic for energy provision protein levels are usually stated in precise terms to be closer to the minimum requirement than other nutrients. Protein sources can be of a plant origin such as soya and groundnut cake or of an animal origin, such as fish meal. Some sources of minerals include Oyster shell and limestone which are both rich in calcium (Dateh, 2013). Bone meal is a very good source of both calcium and phosphorus amongst others. Common salt can satisfy the birds’ sodium and chloride requirements. However trace mineral requirements are usually met by supplementation via the vitamin/mineral premix (Scheideler, 2009).

2.4 Nutrient Requirements for Poultry

Chinrasri (2004) and Laohakaset (1997) defined nutrient requirement as the amount of nutrients needed by animals to maintain their activities,
maximize growth and feed utilization efficiency, improve laying capacity and hatchability. Poultry diets must be formulated to provide all of the bird’s nutrient requirements if optimum growth and production is to be achieved.

2.4.1 Carbohydrate
Soluble carbohydrates consist of starch and sugars, which are easily digested, and are the main source of energy in animal feeds (Kellems and Church, 2010). Digestibility of starch and sugars is high and animals are able to utilize them well. Excess energy is stored as body fat (Lukuyu et al., 2009). In cereal grains, starch and sugars comprise up to 80%, serving as the main source of energy for maintenance and production. Crude Fiber (CF) consists of cellulose, hemi-cellulose and lignin and is found in the fibrous parts of plant material (Lukuyu et al., 2009). Care must be taken with swine and poultry, as these are capable of digesting very little of the fibrous component in feedstuffs (Kellems and Church, 2010). Soluble carbohydrates Starches: Cereals, cereal byproducts.

2.4.2 Fats
Fats are high in energy and provide about 2.25 times more energy than the same amount of carbohydrates, thus acting as a useful source of stored energy. Fat soluble vitamins are present in the lipid content of feeds, and therefore must be present in the feed, however excess fat
lowers feed intake. Essential fatty acids within the oil fraction of feed ingredients are necessary for chick growth and egg production with an adequate fat content. Stored fat also acts as a thermal insulator therefore maintaining body temperature (McDonald et al., 2011).

2.4.3 Protein
Proteins are one of the most important feed components and are an essential nutrient. Proteins are necessary for several functions within the animal body, and are a major constituent of most body tissues. Proteins are composed of amino acids which contain nitrogen (N), Protein is required every day for growth and development, maintenance, and production. Protein requirements tend to decline with age, nonetheless, requirements for amino acids are generally high. Essential amino acids (e.g. lysine and methionine) cannot be adequately synthesized within the body and deficiencies limit the synthesis of proteins, which is why pre-mixes are commonly used in compounded feeds. (Lukuyu et al., 2009; Parr, 1988). Main sources are oilseed by-products fish meal.

2.4.4 Minerals
Mineral components should be considered individually during feed formulation, as these are important in body and tissue structure, digestion and absorption of nutrients and egg shell development. They can be classified into macro and micro-elements. Micro-elements are typically supplied as pre-mixes to be included in the ration, whilst the most important limiting macro-elements such as phosphorous (P),
calcium Ca and sodium (Na), are typically supplied through the inclusion of inorganic material (Parr, 1988). Although the exact role that every mineral plays in an animal’s metabolism is not clear, it is known that deficiencies of certain minerals cause symptoms which are relieved by adding the element to the diet (McDonald et al., 2011). Minerals are commonly referred to as Ash, which is the inorganic material which remains after burning a feed sample (Lukuyu et al., 2009). Bone meal (P, Ca) Cereal grain (P) Fish meal (Ca) Limestone (Ca) Common salt (Na) Dicalcium phosphate (Ca,P) are the common sources of minerals.

2.4.5 Vitamins
The natural dietary supply of vitamins provided by the raw materials must be considered before supplementing (Lonsdale, 1989). Although vitamins are required in relatively small quantities, they are very important in maintaining good animal health, especially because costs are low in relations to the consequences of a deficiency (such as a disordered metabolism and eventually disease) (McDonald et al., 2011) whilst non ruminants rely solely on feed for the supply of fat-soluble vitamins, meaning that vitamin supplementation is essential in non-ruminant feeding (Parr, 1988). Cereals, Oilseed by-products Manufactured pre-mixes are the main sources.
2.5 Recommendations for Energy and Nutrients of Layers

2.5.1 Energy Requirement

Energy in poultry feed is expressed world-wide (in Germany since the early 1960s) in terms of apparent metabolisable energy. Contents of components and complete diets and recommendations for daily intake are commonly expressed in kJ or MJ (occasionally still in kcal). The daily energy needs are the sum of requirements for maintenance and for production. The maintenance requirements are primarily determined by metabolic body mass of the hens. Additional factors are activity, ambient temperature, condition of feather cover and genotype. The energy requirements for production are primarily determined by daily egg mass output, body mass increase between sexual maturity and mature weight and regrowth of feathers. All recommendations for laying hens in conventional cages, with the exception of the NRC (1975) figures, are in close agreement. The latter assume higher energy requirements for maintenance, which accounts for 60 % of total energy needs, while only 40 % are used for production. Additional energy will also be needed for dissipation of body heat in case the house temperature exceeds the thermo-neutral optimum. This would be a frequent problem in subtropical and tropical regions, occasionally also during hot summers in moderate climate zones like central Europe additional energy is also needed if the ambient temperature drops below 15 °C. NRC (1975) assumed that laying hens can adjust their daily energy
intake by increased feed consumption, provided a minimum of 9.6 MJ/kg feed is assured. Although hens tend to adjust their feed intake to some degree on the basis of energy content. Adequate energy supply at high ambient temperatures is always a challenge. With increasing temperature, laying hens reduce their daily feed intake and thereby energy and nutrient intake. In older literature it has been suggested to increase energy density at high temperature to compensate for reduced feed intake. At high temperature, when the daily intake is already low, the hens will reduce their intake less in response to increased energy concentration of feed. The energy concentration of layer diets can be increased by added fat or oil, which has the additional advantage of improved feed structure and reduced metabolic heat production compared to other feed components (NRC 1994)

2.5.2 Crude Protein and Amino Acids Requirement
The protein and amino acid (AA) requirements for laying hens have been the subject of extensive research in the past. Other estimates of requirements were derived from metabolic studies and performance trials. The AA requirements published by Gesellschaft für Ernährungsphysiologie (GfE, 1999) are in the range of other recommendations. With the exception of tryptophan, the NRC (1994) listed the lowest levels for all AA, while Arbeitsgemeinschaft für Wirkstoffe in der Tierernährung (AWT, 2000) advocates a higher lysine level than other sources. The calculations can also be based on the
concept of ideal proteins as described by Gramzow (2001) and others. From the known relationship to other AA, the requirements for all other AA can then be derived Lemme (2009). The levels listed by GfE and NRC (1994) are lower than those from other sources and take no safety limit into account. For application in practice, about 10 % higher levels should be used (e.g. 6.9 g instead of 6.3 g lysine/kg feed with 11.4 MJ/kg). Results of a recent trial Halle et al. (2005) comparing recommended levels with 15 % higher or lower AA levels. In this trial, higher concentrations did not improve performance, but lower levels of lysine and methionine had significant negative effects on egg output and feed conversion ratio. In the past, recommendations were usually expressed in terms of total amino acids. More recently, AWT (2000) and (Lemme, 2009) suggested to focus on true digestible AA for layers, which differs from the concept of standardized prececal (ileal) digestible amino acids (Kluth and Rodehutscord, 2009).

2.5.3 Macro-Elements Requirement

To calculate adequate phosphorus requirement is difficult, because the digestibility of phytate-P from plants and phytase concentration in plants vary considerably. The requirement recommendations for this element are currently expressed in terms of available P (aP) or non-phytate-P (NPP), but this is not satisfactory (GfE 1999 and 2004); a new system is suggested, based on “usable” phosphorus (World’s Poultry Science Association) (WPSA ,1985) and GfE (1999). Differences in the Ca
recommendations result from the assumed utilization: GfE assumed 55 % (at peak production), WPSA 50 % (on average), and modern phase feeding assumes only 40 % toward the end of the laying period. The recommendations for the contents of macro-elements in complete layer rations based on the results of factorial experiments or trials focused on the response to increasing dosage of given elements. Leeson and Summers (2005) present recommendations for specified hen age, energy content of feed and daily feed intake. The rather high Ca levels quoted by these authors are partly explained by the high energy level of typical feed formulation in the USA, with corresponding lower feed intake. The NRC (1994) recommendations vary with feed intake, while PolnischeAkademie der Wissenschaften (PAN, 2005) take strain of layer and rate of lay into account in addition to feed intake. In agreement with WPSA (1984) recommendations, both sources recommend increased Ca levels as the hens get older. The recommended levels for phosphorus appear excessive and are probably due to the uncertainties discussed above. In a recent trial, Kozlowsli and Jeroch (2011) demonstrated that much lower levels of non-phytate-P are adequate, provided the feed contains sufficient phytase. As an added benefit, the hens would excrete less P.

The most important trace elements in layer rations are iron, copper, zinc, manganese, iodine and selenium. The recommended levels are exclusively derived from dose-effect feeding trials and show
considerable variation. With the exception of Fe, the NRC (1975) values are probably too low under commercial conditions. The GfE (1999) advocates levels of trace elements, which are optimal for the most productive and most efficient individual layers under commercial conditions”. Some authors recommend higher levels in breeder rations than in layer feed, but GfE considers the recommendations adequate for parent stock as well. The scientific support for such claims is, however, limited and perhaps outdated. Additions of trace elements in feed supplements follow recommendations. However, in designing feed supplements, the trace elements contained in components are often ignored, and this may lead to overconsumption and excessive levels in excreta. Questions regarding the use of organic vs. inorganic compounds of trace elements have recently been discussed by Schenkel (2008). It has been demonstrated that some organic compounds of trace elements (especially Se) have a higher bio-availability than inorganic compounds in poultry as well. This means that lower levels in daily intake can reduce levels in excreta without sacrificing productivity and health. Experimental results for organic compounds of Zn-, Mn- and Cu are still inconclusive.

2.5.4 Vitamins Requirement
Balanced poultry feed requires feed additives for most vitamins. The GfE and NRC recommendations are based on dose-response feeding experiments. In some experiments, the effects of different dosage were
not only related to egg production, but also to contents in liver and egg yolk as well as biochemical parameters. It should be pointed out that the recommendations are based on feeding experiments many years ago, when the rate of production was much lower and feed conversion ratio (FCR) higher, the vitamin A intake per unit egg mass is reduced by about one third due to higher production, if the feed formulation follows the NRC recommendations (2930 IE/kg feed). According to Leeson (2007) the NRC figures are not adequate for today’s highly efficient layers. Other authors recommend much higher vitamin levels than NRC (1994) and GfE (1999), especially for fat soluble vitamins. In feed formulation, vitamins contained in components are usually ignored. This is justified for vitamins A, D3 und B12 because today’s commercial rations contain only plant components, which may contain only low concentrations of β-carotene. Other vitamins are contained in sufficient, sometimes even excessive, concentration in feed components. The recommendations of Whitehead (1998) take the contents of B vitamins in components into account.

2.6 Recommendations for Energy and Nutrients of Broiler

Feed is the most important input for poultry production and the availability of low-priced, high quality feeds is critical for the expansion of the poultry industry and quality Food and Agriculture Organization of the United Nations (FAO, 2003; Ismoyowati and Sumarmono, 2011). Diets for broilers are formulated to meet the requirements for those
indispensable amino acids (AA) that may limit meat production, namely lysine, methionine, tryptophan, and total sulfur amino acids (Perez-Bonilla et al., 2012). According to NRC (1994), diets based on corn and soybean meal with 15.0% CP can satisfy the amino acids requirements. However, several commercial guidelines for broilers (Lohmann, 2010; Cobb, 2011) recommend CP levels varying from 22 to 23% for the first part of the starting period. The reasons behind this practice are unknown but might be related to the interest to maximize meat production and reduce the possibility of a nonconventional indispensable amino acids (Arginine, leucine, Tryptophan, Valine) limiting body weight. However, an excess of CP in the diet increases nitrogen load to the environment (Latshaw and Zhao, 2011) and often results in increased feed cost. According to Perez-Bonilla et al. (2011) supplemental fat affects productive performance, but the effects depend on the amount and type of fat used in the diet (Grobas et al., 1999). In addition, supplemental fat might improve the digestibility of other components and feed efficiency (Bouvarel et al., 2010). Poultry can derive energy from simple carbohydrates, fat and protein. They cannot digest and utilize some complex carbohydrates, such as fiber, so feed formulation should use a system based on available energy. Metabolizable energy (ME) is the conventional measure of the available energy content of feed ingredients and the requirements of poultry. This takes account of energy losses in the faeces and urine. Metabolizable energy
requirements of commercial broiler depend on environmental temperature; it increases when the environment is cold or hot (Sakomura et al., 2005). Therefore, under heat stress situations, increasing energy levels in the diet of commercial broiler by the inclusion of oil may compensate the low feed intake and supply the higher energy requirements (Almeida et al., 2012). Minerals are the inorganic parts of feeds or tissues and are divided into macro (major) minerals and micro (minor) minerals. Minerals are required for skeletal formation, as cofactors of enzymes, and for maintenance of osmotic balance within the body. Macro minerals that are required in the diet of a broiler include calcium, chlorine, magnesium, phosphorus, potassium, and sodium. Two macro minerals that are particularly important in the diet of a broiler are calcium and phosphorus. The amounts of calcium required in the diet for broiler, as recommended by the NRC (1994), are 1.2 and 0.9% respectively. Micro minerals required in the diet include copper, iodine, iron, manganese, selenium, and zinc. Fat-soluble vitamins that are essential in the diet of a broiler include A, D3, E, and K. Water-soluble requirements include B12, biotin, choline, folacin, niacin, pantothenic acid, pyridoxine, riboflavin, and thiamin. Fats and oils are feed sources high in energy and can be added to a poultry diet to provide energy, and in turn improve productivity and efficiency. This is because oxidation of fats is an efficient way to obtain energy (NRC, 1994). A feed enriched in fat and incorporating a minimum of insoluble fiber is recommended.
After the onset of rearing a slightly lower energy level, richer in cellulose, will allow a good energy efficiency to be obtained (expressed in kcal) and plumage to be maintained (ISA, 2009).

2.7 Quality of Formulated Poultry Feeds

The efficiency of feed utilization in the livestock and poultry birds and the development of feed industry are dependent upon the quality of feeds. The quality of compounded animal feeds is based on the quality of its constituents i.e the raw material (cereals by products, oil seed meals and agro-industrial by products), used to formulate the ratio (Uppal et al., 2008). In Sudan, ingredients and processed feeds vary in nutritive value and there is no regular quality control mechanism in the country. Since a laying hen or broiler draw upon the nutrients provided in their diets to produce eggs or meat, the quality and formulation of the diet is of most importance to a producer, especially considering that 65 to 75% of the cost to produce eggs or meat is due to feed costs (Bell and Weaver, 2002; Hinrech and Steinfield, 2007). Due to this, it has become increasingly important for producers to find a balance between feeding their birds on least-cost basis as well as feeding the appropriate amounts of nutrients in the diet as the hens need them throughout their production cycle (Depersio, 2011). For maximum growth and good health, intensively reared poultry need a balanced array of nutrients in their diet. The nutrients required by birds vary according to species, age and the purpose of production whether the birds are kept for meat or egg
production. Formulation of balanced diets is fundamental to economical poultry production, and this process depends on knowledge of nutrient requirements of poultry”. In essence, there are three main factors that egg and broiler meat producer must be concerned with and they are; 1) the cost of feed, 2) the amount and quality of the meat, and 3) the profit made (NRC, 1994).
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area.

This study was carried out in Khartoum State, capital of Sudan. Broiler starter, broiler finisher and layer feeds samples were collected from six poultry feed factories (A, B, C, D, E, F) during 2013 to evaluate the quality of compound feeds in Sudan.

3.2 Feed Sampling Protocol

The following protocol provides details of the feed sampling, which was developed following internationally accredited scientific guidelines for feed sampling (FAO, 2011).

3.3 Procedure for Samples Collection

Several increment (hand grab) samples were taken from the open bag and combined in a clean container. Once again, the quartering technique was used to reach the desired size of the composite sample. Because settling of feed is common, handfuls were taken from the lower and upper end of the sack. In some factories feed is packed in 50kg bags, therefore, a probe was used in order to avoid damaging the bag.

The compound feeds selected for sampling included broiler starter, broiler finisher and layer feeds. In each production site, one sample of
each compounded feed was collected. Upon collection, the samples were placed in a polyethylene press-seal bags, of a size so that they are almost completely filled by the sample; the air was then removed by squeezing and sealing tightly. Samples were accurately labeled with a unique code using a permanent marker pen immediately after collection. In a separate log book, specific details of the samples were recorded.

3.4 **Proximate Chemical Analysis of Samples**

The feed samples were subjected to proximate analysis as the standard procedures of Association of official analytical chemists (AOAC, 1990) in triplicate (Appendex1). The analytical parameters measured were dry matter (DM%), crude protein (CP%), crude fat (fat%) crude fiber (CF%). Aflatoxin (ppb) and total mineral content (% and/or mg/kg). Metabolizable energy (ME) (kcal.kg⁻¹) was calculated according to the method described by Lodhi et al. (1976). Aflatoxin determination was carried out by (ELIZA) technique and the total mineral content was measured by ICP Spectrometer.

3.5 **Statistical analysis**

Microsoft Excel (2013) was used to produce simple descriptive statistics with the data generated from the feeds chemical analysis. GenStat 17th Edition was used to analyze data relating to product quality.
CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Chemical Composition of Layer Feed

The proximate composition of all layer feeds has been presented in Table (1). The CF%, Fat%, DM% and aflatoxin (ppb) in all feeds (A, B, C, D, E) were within the acceptable ranges stated by NRC (1994) and SSMO (2015), (3.5-4.5%, 2.9-4.5%, 94.31-94.79% and 7.6-18.3 ppb respectively) this suggests that all feeds analyzed are appropriate, low level of aflatoxin reflected the quality and storage conditions of raw materials used in formulating these feeds, or may be due to the fact that high DM contents control the growth of mould in feeds, thereby reducing deterioration of feeds (Kaijage et al., 2014). Higher CP content than the required levels were observed in feed D (20.98%). On the other hand, all other feeds CP% A (17.9), B (18.6), C (18.0) and E (17.86) almost within the recommended ranges of (NRC,1994 and SSMO,2015). These differences may be due to the type of agro-industrial by-products used in these feeds. The metabolizable energy (Kcal/Kg) content varied between low 2679 Kcal/Kg (feed D) to very high 3127.9 Kcal/Kg (feed B) than the required levels recorded by (NRC,1994 and SSMO,2015) these variations could be due to the differences in ingredients used as sources of energy in these feeds and/or the rate of inclusions of these ingredients different factories my
formulate their rations according to the nutrient requirements of each production stage, hybrid strain or season of production (Leeson and Summer, 2001). High levels of ash above the recommendations of NRC (1994) and SSMO (2015) were recorded in all layer feeds (10.33 to 20.85%) this result may indicate a high mineral content but it was not, so it may be due to the presence of undesirable materials (Rao and Xiang, 2009).

4.2 Mineral Content of Layer Feed

The results of mineral content of layer feeds (Table 2) showed that the Ca (3.0 -3.5%), Mg (150 – 210 mg/kg), Mn (11.6 - 13.74 mg/kg) and Na (0.05 – 0.18 %) content are generally low in all feeds, on the contrary K (0.52 – 0.6 %) and Zn (55.26- 92.45%) are high than the required recommendations of NRC (1994) and SSMO (2015). On the other hand, Fe content of feed D (62.4mg/kg) and feed E (69.1mg/kg) slightly higher than the required level (60mg/kg) except feed A, B and C feeds showed low values (50.34mg/kg , 24.5mg/kg and 41.4mg/kg). These variations in mineral contents of feeds might be attributed to dietary mineral contents and/or sources or feed additives used in feeds. On the other hand, some factories have followed the practice of adjusting the dietary levels of minerals in order to maintain a constant intake of these nutrients as temperature and thus feed intake levels vary.
### Table (1) Chemical Composition of Layer feed

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<tr>
<th>Treatments Parameters</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tr>
<td>CP%</td>
<td>17.90(^b)±0.08</td>
<td>18.6(^b)±0.06</td>
<td>18.0(^b)±0.08</td>
<td>20.98(^a)±0.19</td>
<td>17.86(^b)±0.16</td>
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<tr>
<td>*ME(kcal/kg)</td>
<td>2916.4(^c)±3.7</td>
<td>3127.9(^a)±2.5</td>
<td>2912.4(^c)±3.4</td>
<td>2679.2(^c)±2.02</td>
<td>2954.4(^b)±14.9</td>
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<tr>
<td>CF%</td>
<td>4.5(^a)±0.03</td>
<td>3.8(^b)±0.05</td>
<td>4.4(^a)±0.07</td>
<td>3.7(^b)±0.03</td>
<td>3.5(^b)±0.24</td>
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<td>Fat%</td>
<td>3.2(^b)±0.15</td>
<td>4.5(^a)±0.011</td>
<td>3.5(^b)±0.05</td>
<td>3.4(^b)±0.009</td>
<td>2.9(^c)±0.04</td>
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<td>DM%</td>
<td>94.9±0.05</td>
<td>94.9±0.05</td>
<td>94.79±0.09</td>
<td>94.31±0.02</td>
<td>94.46±0.10</td>
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<tr>
<td>Ash%</td>
<td>13.43(^b)±0.03</td>
<td>10.33(^c)±0.04</td>
<td>14.83(^b)±0.07</td>
<td>20.85(^a)±0.015</td>
<td>14.06(^b)±0.10</td>
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<tr>
<td>Aflatoxin(ppb)</td>
<td>12.6(^b)±0.4</td>
<td>18.3(^a)±0.02</td>
<td>7.6(^d)±0.1</td>
<td>11.64(^b)±0.05</td>
<td>8.45(^c)±0.19</td>
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*ME was calculated according to the equation of Lodhi et al (1976)
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<th>Treatments Parameters</th>
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<td><strong>Means ±SE</strong></td>
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<td>C</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>Ca%</td>
<td>3.1b±0.06</td>
<td>3.07 c±0.04</td>
<td>3.4a±0.11</td>
<td>3.0b±0.09</td>
<td>3.5a±0.09</td>
<td></td>
</tr>
<tr>
<td>Fe(mg/kg)</td>
<td>50.34c±0.08</td>
<td>24.5e±0.2</td>
<td>41.4d±0.28</td>
<td>62.4b±0.26</td>
<td>69.1a±0.06</td>
<td></td>
</tr>
<tr>
<td>Mg(mg/kg)</td>
<td>216.7c±12</td>
<td>150d±5.8</td>
<td>246.7a±3.3</td>
<td>210c±5.8</td>
<td>226.7b±8.8</td>
<td></td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>12.7 a±0.024</td>
<td>11.6b±0.029</td>
<td>13.3a±0.052</td>
<td>11.82b±0.028</td>
<td>13.74a±0.028</td>
<td></td>
</tr>
<tr>
<td>Na%</td>
<td>0.12b±0.003</td>
<td>0.05d±0.006</td>
<td>0.12b±0.003</td>
<td>0.11c±0.003</td>
<td>0.19a±0.003</td>
<td></td>
</tr>
<tr>
<td>K%</td>
<td>0.55a±0.01</td>
<td>0.53a±0.02</td>
<td>0.53a±0.009</td>
<td>0.52a±0.006</td>
<td>0.49b±0.006</td>
<td></td>
</tr>
<tr>
<td>Zn%</td>
<td>92.45a±0.23</td>
<td>55.26d±0.17</td>
<td>79.64c±0.22</td>
<td>90.56a±0.1</td>
<td>85.41b±0.1</td>
<td></td>
</tr>
</tbody>
</table>
4.3 Chemical Composition of Broiler Starter Feed

As seen in Table (3).CP content in all broiler starter feeds almost similar to the recommended level (23%) stated by (NRC, 1994 and SSMO, 2015) except feed E recorded lower value (18.3%). Slightly higher ME content than the required levels were reported in feed A (3280.2 Kcal/Kg) and feed F (3282.7 kcal/kg). On the contrary lower value was recorded in feed E (2914 kcal/kg). Feed B (3197 kcal/kg), C (3176.1 kcal/kg), and D (3154.2 kcal/kg) satisfy ME requirement (3100 - 3202.6 kcal/kg) stated by (NRC, 1994 and SSMO 2015) this result may indicated that some factories may add fats in their feeds to stimulates feed and energy consumption at high ambient temperature (Fuller and Rendon, 1977). Low levels of CF content were recorded in all feeds (3.7% feed F to 5.5% D feed) compared to the maximum recommended level (5%) mentioned by (NRC, 1994 and SSMO, 2015). Feed A, D and F reported higher fat content (7.5% 6.6% and 6.83%) lower values were recorded in feed B (4.2%), C (4.8%) and E (2.96%). The DM content of all feeds were within the acceptable ranges (93.18- 95.0%) stated by (NRC, 1994 and SSMO, 2015), on the other hand, ash content ranges between (5.76% Feed C to 6.0% Feed B except feed E reported higher percent (14.6%) than the recommended level (8) stated by (SSMO 2015). High levels of aflatoxin (ppb) than the permitted level (≤20) were noticed in feed A (49.15) followed by C (35.63ppb) and D (35.31ppb), on the contrary too low levels of aflatoxin 5.7 ppb and 6.89
ppb were reported in B and F feeds. This variation might be due to the type and/or storage conditions of cakes used in these feeds.

4.4 Mineral Content of Broiler Starter Feed

As shown in table (4). The Ca% range between 0.83 feed C to 0.88 % feed A which is almost within the acceptable ranges stated by (NRC, 1994 and SSMO 2015). On the contrary feed E recorded higher Ca content (3.4%) this might be due to laboratory error. Generally Zn and K content in all feeds were higher, meanwhile Fe, Mg and Na were lower, it has been known for some time that K requirement of growing chickens increased with increased temperature, therefore, dietary K levels should be increased for birds reared in heat stressed environment to maximize gain in weight of broiler (Huston, 1978 and Smith and Teeter, 1987). This scientific observation might be considered by the imported super concentrate companies.

4.5 Chemical Composition of Broiler Finisher Feed

As presented in table (5) Almost feed A, C, and E CP% content fall within the acceptable ranges (18 -20%) recommended by (NRC, 1994 and SSMO, 2015), meanwhile, feed B(23.16%), D (22.10%), and F (21.89%) recorded slightly higher CP than that outlined by both organizations, these variations may be due to the sources of protein used in these feeds (meat or cakes). ME (kcal/kg) in all feeds were met the recommendations of (NRC, 1994 and SSMO, 2015) except feed E which
resulted in lower ME content (2747 Kcal/kg) this result based on the assumption that different factories may used different varieties of sorghum or add fats as a source of energy in their rations. The proximate analysis of feeds revealed that CF and Ash content in all feeds recorded lower values than the recommended levels stated by (NRC, 1994 and SSMO, 2015). Feed A (6.7%), D (6.7%), E (5.6%) and F (6.6%) had slightly higher fat content than the required range (2-5%) stated by (SSMO 2015), mean while feed B (3.5%) and C (4.6%) had acceptable fat content, . DM% in all feeds were within the ranges (90-95%) stated by (NRC, 1994 and SSMO, 2015). Feed A, B, C and D reported higher Aflatoxin content than the permitted level (20 ppb) (NRC 1994 and SSMO 2015). On the contrary feed E and F recorded lower values (19.0 and 10.32 ppb), This result reflect the quality of raw materials (Cakes and cereals) and their storage condition.
# Table (3) Chemical Composition of Broiler Starter feed

<table>
<thead>
<tr>
<th>Treatments Parameters</th>
<th>Means ±SE</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>CP%</td>
<td>23.92±0.04</td>
<td>23.0±0.02</td>
<td>23.05±0.01</td>
<td>23.48±0.18</td>
<td>18.3±0.2</td>
<td>23.32±0.3</td>
</tr>
<tr>
<td>*ME(kcal/kg)</td>
<td>3280.2±2.5</td>
<td>3197±2.1</td>
<td>3176.1±1.1</td>
<td>3154.2±1.1</td>
<td>3141±8.6</td>
<td>3282±2.1</td>
</tr>
<tr>
<td>CF%</td>
<td>4.24±0.06</td>
<td>4.49±0.01</td>
<td>4.32±0.03</td>
<td>5.5±0.1</td>
<td>4.4±0.1</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td>Fat%</td>
<td>7.5±0.006</td>
<td>4.2±0.14</td>
<td>4.8±0.04</td>
<td>6.60±0.03</td>
<td>2.96±0.006</td>
<td>6.83±1.5</td>
</tr>
<tr>
<td>DM%</td>
<td>94.52±0.16</td>
<td>94.98±0.02</td>
<td>93.18±0.05</td>
<td>94.78±0.01</td>
<td>95±0.1</td>
<td>94.78±0.1</td>
</tr>
<tr>
<td>Ash%</td>
<td>5.99±0.01</td>
<td>6.0±0.03</td>
<td>5.76±0.03</td>
<td>7.05±0.10</td>
<td>14.60±0.14</td>
<td>5.9±0.1</td>
</tr>
<tr>
<td>Aflatoxin(ppb)</td>
<td>49.15±0.15</td>
<td>5.7±0.08</td>
<td>35.63±0.07</td>
<td>35.31±0.12</td>
<td>6.89±0.006</td>
<td>14.4±0.1</td>
</tr>
</tbody>
</table>

*ME was calculated according to the equation of Lodhi et al (1976)
### Table (4). Mineral Content of Broiler Starter feed

<table>
<thead>
<tr>
<th>Treatments Parameters</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca%</td>
<td>0.88 ± 0.01</td>
<td>0.84 ± 0.008</td>
<td>0.83 ± 0.006</td>
<td>0.84 ± 0.003</td>
<td>3.4 ± 0.09</td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>50.5 ± 0.15</td>
<td>28.23 ± 0.28</td>
<td>51.3 ± 0.09</td>
<td>50.8 ± 0.15</td>
<td>35.2 ± 0.15</td>
</tr>
<tr>
<td>Mg (mg/kg)</td>
<td>230 ± 5.77</td>
<td>176.7 ± 3.33</td>
<td>216.7 ± 3.33</td>
<td>220 ± 5.77</td>
<td>260 ± 5.77</td>
</tr>
<tr>
<td>Mn(mg/kg)</td>
<td>108.67 ± 0.88</td>
<td>75.83 ± 0.18</td>
<td>150.3 ± 0.33</td>
<td>99.29 ± 0.07</td>
<td>141.1 ± 0.59</td>
</tr>
<tr>
<td>Na%</td>
<td>0.13 ± 0.003</td>
<td>0.09 ± 0.003</td>
<td>0.20 ± 0.003</td>
<td>0.13 ± 0.003</td>
<td>0.12 ± 0.006</td>
</tr>
<tr>
<td>K%</td>
<td>0.56 ± 0.003</td>
<td>0.57 ± 0.003</td>
<td>0.60 ± 0.006</td>
<td>0.60 ± 0.007</td>
<td>0.53 ± 0.003</td>
</tr>
<tr>
<td>Zn%</td>
<td>70.9 ± 0.06</td>
<td>21.8 ± 0.03</td>
<td>33.63 ± 0.09</td>
<td>73.9 ± 0.03</td>
<td>85.42 ± 0.02</td>
</tr>
</tbody>
</table>
### Table (5). Chemical Composition of Broiler Finisher feed

<table>
<thead>
<tr>
<th>Treatments Parameters</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP%</td>
<td>20.0c±0.11</td>
<td>23.16 a±0.04</td>
<td>20.30c±0.10</td>
<td>22.10b±0.03</td>
<td>20.20c±0.10</td>
</tr>
<tr>
<td>*ME(kcal/kg)</td>
<td>3349.21a±3.5</td>
<td>3189.3e±5.4</td>
<td>3206c±7.3</td>
<td>3274.9d±3.9</td>
<td>2747.2f±4.1</td>
</tr>
<tr>
<td>CF%</td>
<td>3.6c±3.5</td>
<td>3.4c±0.13</td>
<td>4.2a±0.13</td>
<td>4.0b±0.10</td>
<td>4.0b±0.10</td>
</tr>
<tr>
<td>Fat%</td>
<td>6.7a±0.01</td>
<td>3.5c±0.02</td>
<td>4.7b±0.03</td>
<td>6.7a±0.07</td>
<td>4.6b±0.11</td>
</tr>
<tr>
<td>DM%</td>
<td>94.48a±0.03</td>
<td>94.05b±0.02</td>
<td>93.46c±0.03</td>
<td>94.04b±0.06</td>
<td>94.23d±0.08</td>
</tr>
<tr>
<td>Ash%</td>
<td>5.1b±0.03</td>
<td>5.96a±0.003</td>
<td>5.85a±0.01</td>
<td>5.79a±0.01</td>
<td>2.62 c±0.17</td>
</tr>
<tr>
<td>Aflatoxin(ppb)</td>
<td>24.71d±0.03</td>
<td>27.22b±0.01</td>
<td>43.59a±0.04</td>
<td>26.42c±0.05</td>
<td>19.0e±0.01</td>
</tr>
</tbody>
</table>

*ME was calculated according to the equation of Lodhi et al (1976)
4.6 Mineral Content of Broiler Finisher Feed

Higher levels of K (0.49 – 0.6% ), Na (0.14- 0.21% ), Mn (107.5 -141.7 mg/kg) and Zn (73.86 – 105.5 mg/kg) (Table 6) were recorded in all broiler finisher feeds except B and C feeds showed lower values of Zn (0.22 mg/kg and 0.49 mg/kg %) than the recommended levels stated by (NRC,1994 and SSMO, 2015). On the other hand, feed D recorded acceptable level of Na (0.12%). On the contrary low levels of Fe (14.12 - 45.33 mg/kg ) and Mg(136 - 233mg/kg) were noticed in all feeds except feed F showed an acceptable level of Fe(80.03mg/kg). Fe content might be attributed to the soil. Fe content and irrigation water Fe in which botanical source of feed harvested. Ca contents almost satisfy the requirements ( 0.83 to 0.91%) except D and E feeds contained very low levels of Ca (0.24% and 0.49%). Respectively however, the feeds factories might be considered the water soluble minerals or minerals and vitamins additives which common used in broiler farms.
Table (6). Mineral Content of Broiler Finisher feed

<table>
<thead>
<tr>
<th>Treatments Parameters</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Means ±SE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca%</td>
<td>0.9(^a)±0.004</td>
<td>0.91(^a)±0.006</td>
<td>0.83(^b)±0.003</td>
<td>0.24(^d)±0.004</td>
<td>0.49(^c)±0.007</td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>23.6(^e)±0.02</td>
<td>45.33(^b)±0.01</td>
<td>42.59(^c)±0.05</td>
<td>14.12(^l)±0.04</td>
<td>40.5(^d)±0.03</td>
</tr>
<tr>
<td>Mg (mg/kg)</td>
<td>143.3(^e)±3.3</td>
<td>206.7(^c)±3.3</td>
<td>216(^b)±3.3</td>
<td>136(^l)±3.3</td>
<td>150(^d)±5.77</td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>107.5(^e)±0.5</td>
<td>110.5(^d)±0.6</td>
<td>141.7(^a)±0.52</td>
<td>141.3(^a)±0.58</td>
<td>116.3(^c)±0.58</td>
</tr>
<tr>
<td>Na%</td>
<td>0.16(^b)±0.006</td>
<td>0.14(^c)±0.006</td>
<td>0.21(^a)±0.006</td>
<td>0.12(^d)±0.006</td>
<td>0.15(^c)±0.01</td>
</tr>
<tr>
<td>K%</td>
<td>0.57(^b)±0.003</td>
<td>0.60(^a)±0.006</td>
<td>0.52(^c)±0.003</td>
<td>0.60(^a)±0.006</td>
<td>0.49(^d)±0.006</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>62.6(^d)±0.06</td>
<td>24.87(^b)±0.3</td>
<td>35.3(^e)±0.1</td>
<td>101.2(^b)±0.2</td>
<td>73.86(^c)±0.1</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the obtained results of the present study, the following conclusions could be withdrawn:

1- The evaluation of broiler and layer feeds revealed an acceptable results of proximate analysis including some variations.

2- Most of the feeds nutrient composition agreed with NRC (1994) and to some degree with SSMO (2015) except in minerals and aflatoxin content of broiler feeds.

5.2 Recommendations

1- The variations observed among different poultry feeds compared to (NRC1994) and (SSMO 2015) specifications strongly indicates that confirmatory analyses should be conducted.

2- It is high time to update specifications of layer feeds by SSMO according to the production stage (period) and/or HD% egg production.

3- New techniques for quick feed analysis should be introduced Near Infra-Red Spectroscopy (NIR)

4- Further research concerning Amino acids should be conducted.
5-Necessity of establishment of feed-reference laboratories and efficient follow-up authorities.
REFERENCES


Appendix (1)

Proximate Chemical Analysis of Samples Procedures

1. Dry matter determination

Dry matter (DM) content, which is the non-moisture portion of the feed, was determined by heating a weighed sample of feed (3g per sample) in a drying oven at 103°C, until a constant weight is reached. The DM tests were performed by trained personnel at the Detasi Laboratory in Khartoum. Subsequent nutrient contents of feeds will be appropriately compared on a DM basis. Each step in the DM procedure is explained below:

1. Number the crucibles, rinse in water and dry the crucibles at 103 ± 2°C for at least 2 hours.

2. Place the crucibles in a desicator and immediately cover desicator and allow dishes to cool to room temperature. (Do not allow dishes to remain in the desicator for more than 2 hours).

iii. Weigh the empty crucibles to nearest 0.1 mg and record the weight on the work sheet as W1 (Appendix 5), removing each crucible one at a time from the desicator and keeping the desicator closed between dish removals.

3. Use tongs to handle crucibles and weigh about 3.0000g of sample into the tare weighed crucible to the nearest 0.1 mg. Record this as W2. Shake the dish gently to uniformly distribute the sample and expose it to the maximum surface area for drying.
4. Insert samples into a preheated oven at 103 ± 2 °C and dry for at least 2 hours, start timing once oven has reached the required temperature (dry to constant weight, may need to check this for various sample types once confirmed use that drying time).

5. Place samples in a desicator and close the desicator in order to allow cooling to room temperature. Do not allow samples to remain in the desicador for more than 2 hours.

6. Weigh the dish with the dried sample (recorded as W3), recording the weight to nearest 0.1 mg.

7. DM is then calculated as:

\[
\% \text{ DM} = \frac{(W3 - W1) \times 100}{(W2 - W1)}
\]

Where,

- W1 = weight of empty dish (g),
- W2 = weight of dish and sample (g), and
- W3 = weight of dish and sample after drying (g).

The dried portion was then returned to polyethylene press-seal freezer bags with the rest of the sample. The final step of sample preparation was that of reducing the sample size for proximate analysis analysis. Each sample was thoroughly mixed and the quartering technique was used to reduce each sample to two samples of 30g each. The duplicate samples were required as a risk mitigation in case any sample is lost or ruined during transport.
2. Crude Protein

Purpose and scope

Feed compounds and raw materials in which nitrogen is presented as protein.

Principle

The sample is mineralized by concentrated sulphuric acid in the presence of a catalyst. In this way, it is transformed into ammonium sulphate, the solution is distilled and ammonical nitrogen is caught in diluted acid and determined by the titration method.

Equipment

1. Desiccators
2. Grinding machine Retsch ZM 200, 12 teeth rotor and 1mm sieve
3. Analytical scale, Readability: 0,1 mg
4. Mineralization tubes
5. Mineralization equipment 390 – 420°C
6. Distilling equipment
7. Titration unit + dosimat

Reagents

1. Sulphuric acid, concentrated (98% pure)
2. Catalyst –Kjeltabs W (97,5% Na$_2$SO$_4$ + 1,5% CuSO$_4$·5H$_2$O + 1% Se)
3. Sodium hydroxide, 40% water solution 400 g NaOH in 1 liter (erlenmeyer)

4. Indicator solution dissolve 1 gr of bromecresol green and 0.7 gr of methyl red in 1 l of ethanol (96%).

5. Boric acid - solution mix 10 gr of boric acid in 1 l of distilled water, add 10 ml indicator

6. Hydrogen chloride 0.1000 M. The concentration must be checked.

7. 8,37 ml HCl (37%) in 1000 ml flask

8. Acetanilid 99%, 10,36% N 64,75% Protein

**Procedure**

Analyze also the standard control sample present on the lab for daily quality control of the analysis. The results of the control sample must be imported into the Shewart Chart in MS Excel. Deviations in the control sample must be treated according to the procedure AM000.

1. Grind the sample through a 1 mm sieve. Thorough homogenization.

2. Weigh in 1.00 g of the sample with an accuracy of 1 mg into a mineralization tube.

3. Weigh 0.50 g of Acetanilid (4.8) and also use an empty tube for the blank.

4. Add 1 Kjeltab(4.2) and 12.00 ml of sulphuric acid (4.1)
5. Place tubes in the digestor equipment and boil for 1 hour at 420°C. (do not forget the scrubber)
6. Cool the tubes to the temperature of about 40°C, than start distillation, and titration.
7. Titration with the acid solution 0.1 M (4.7) until the color changes (from green to light pink).

- **Calculation and results**
  
  Nitrogen (%) = \[1,4 \times N \times (V_2 - V_1)\] / W

  - W  weight of sample (in g)
  - V1  volume of hydrochloric acid in blink titration (in ml)
  - V2  volume of hydrochloric acid in sample titration (in ml)
  - N  normality of hydrochloric acid

  Protein (%) = 6,25 x nitrogen

- **Source**

  Method:  
  EG L 179/8 - 10, 22 - 7 - 1993

  Repeatability: The difference between two analyses of the same sample must not be higher for the content of crude protein then:

  - to 20,0%  0,20%
  - 20,0 - 40,0%  1,00%
  - over 40,0%  0,40%
3. Crude Fat

• **Purpose and scope**
  Compound animal feeds and most solid raw materials except oil seeds, milk powders or feeds based on milk powders.

• **Principle**
  The fat is obtained by the direct extraction of the sample with warm light petroleum ether, boiling range of 40-60°C. The solvent is removed and the dry fat weighed. This treatment does not extract oxidized oils, phospholipids or fatty acids combined as soaps.

• **Equipment**
  • Desiccators
    1. Grinding machine Retsch ZM 200, 12 teeth rotor and 1mm sieve
    2. Analytical scale, Readability: 0.1 mg
    3. Extraction tubes or thimbles (26x60), fatless cotton-wool
    4. Extraction cups
    5. Extraction apparatus by Soxtec.
    6. Electric oven thermostatically controlled at 103±1°C.

• **Reagents**
  Petroleum ether (boiling range 40 - 60°C)
• **Procedure**

Analyze also the standard control sample present on the lab for daily quality control of the analysis. The results of the control sample must be imported into the Shewart Chart in MS Excel. Deviations in the control sample must be treated according to the procedure AM000.

Grind the sample and pass it through the 1 mm sieve. Mix the sample thoroughly and store in an airtight container.

1. Weigh in 2.5000 g – 3.5000 g of the sample with the accuracy of 1mg into a thimble.
2. Close the thimble with cotton wool.
3. Weigh the extraction cups
4. Place the thimble into the Soxtec, add Petroleum ether at the extraction cups 40 – 50 ml and place them also in the Soxtec.
5. Start Soxtec program (100º C, step 1=15 min, step 2=30 min, step 3=10 min, step 4=5 min)
6. Dry in the oven for 60 minutes at 103ºC.
7. Cool by the room temperature and weigh with the accuracy of 1mg.

**Calculation and results**

\[
\text{Fat [\%]} = \frac{100 (W_2 - W_1)}{W} 
\]
W = weigh of sample (in g)

W1 = weigh of flask (in g)

W2 = weigh of flask + fat (in g)

(W2 - W1) = weigh of fat (in g)

Source

Method: NEN 3148

Repeatability: The difference between two parallel analyses of the same sample must not exceed the content:

lower than 5% 0.20%

from 5 to 10%

4% from the highest result higher than 10% 0.40%

4. Crude Fiber

Purpose and scope

This method describes the way to determine crude fiber content. The content of fat free, unsoluble in acid and alkali, organic material, is determined to be crude fiber.
Principle

The sample is boiled for 35 minutes in diluted sulphuric acid. After filtration and washing the sample is boiled again for 35 minutes in diluted potassium hydroxide. After filtration, washing and drying the residue is ashed at 500°C. The difference in weight before and after ashing is called crude fiber.

Equipment

Desiccators

1. Grinding machine Retsch ZM 200, 12 teeth rotor and 1mm sieve
2. Analytical scale, Readability: 0,1 mg
3. Fibertec system, hot and cold extractor
4. Drier 130°C
5. Oven 500°C
6. Vacuum pomp (via water suction)
7. Glass filter crucibles; filter porosity of 40 - 90 μm.

Reagents

1. Sulphuric acid 0,13 M = 0,26 N H2SO4

   = 7,3 ml H2SO4 conc. / 1000ml water

2. Potassium hydroxide 0,23 M

   15,2 g KOH / 1000ml water
3. Acetone

4. Anti foaming agent, e.g. 1-Octanol

5. Sea sand

- **Procedure**

Analyze also the standard control sample present on the lab for daily quality control of the analysis. The results of the control sample must be imported into the Shewart Chart in MS Excel. Deviations in the control sample must be treated according to the procedure AM000.

1. Add 1 g sea sand in the filter crucible.

2. Weigh in 1.5 g of sample (=a) and bring this in the filter crucible.

3. Boil a sufficient amount of sulphuric acid (4.1), put the heating of the fibertec between the position 4.5 and 6. Set the levers to position "closed". Place the filter crucibles in the fibertec and carefully add the boiling sulphuric acid up to the first mark on the cooler. Close the front panel and add a few drops of 1-Octanol (4.4). Open the water tap for the coolers. As soon as the fluid is boiling put the switch "heater" to position 4½. Boil for 35 minutes (use a timer).

4. When 35 are passed remove the front panel and switch off the heater. Put on the water suction. Set the levers to position "vacuum" and the sulphuric acid is removed from the crucible. Wash the residue on the
filter with vacuum three times with ± 30 ml of hot tap water. After each washing step the residue must lose all water. Then put the levers back to “closed”.

5. Then add boiling potassium hydroxide and repeat all proceedings from the moment of adding sulphuric acid, until the washing with hot tap water.

6. Make sure to get rid of the water before connecting the filter crucibles to the cold extractor (3.4). Wash the residue under vacuum with ± 25 ml acetone (4.3)

7. Dry the filter crucibles for 90 minutes in a drier at 130 °C. Then cool the crucibles and weigh (=b).

8. Place the filter crucibles in an oven and ash for 90 minutes at 500 °C. Then cool and weigh (=c).

**Calculation and results**

\[
\text{Crude fiber} \, [\%] = \frac{(b - c) \times 100}{a}
\]

- **a** = mass of the sample, in g
- **b** = mass of the crucible including residue after drying, in g
- **c** = mass of the crucible including residue after ashing, in g
Notes

Products containing more than 10% fat must be defatted before analysis. Put the filter crucible to the cold extraction unit and wash 3 x with acetone. Remove the acetone by vacuum.

Source

Method: EG 26-11-1992; Nr L 344/35-37

Repeatability: 3 g/kg at < 100 g/kg, 3 % of the highest result at 100 g/kg or more

5. Crude Ash

Purpose and scope

This method is used for compound animal feeds and solid raw materials. Isn't acceptable for fat, oil, milk and milk products.

Principle

Organic substance is removable by self-ignition at a temperature not exceeding 550°C. The burning process takes place in two phases: in the first phase the water evaporates and in the second phase the organic substance is burned.

Equipment

1. Desiccators
2. Grinding machine Retsch ZM 200, 12 teeth rotor and 1 mm sieve
3. Analytical scale, Readability: 0.1 mg
4. Porcelain dishes 4 - 5 cm diameter, 3 - 4 cm deep. Before the first use, burn dishes for 3 hours in a furnace.
5. Muffle furnace, thermostatically controlled at 550°C.

Procedure

1. Analyze also the standard control sample present on the lab for daily quality control of the analysis. The results of the control sample must be imported into the Shewart Chart in MS Excel. Deviations in the control sample must be treated according to the procedure AM000.

2. Grind the sample through a 1 mm sieve.

3. Weigh a porcelain burning dish. Weigh in 2.5 g of the sample into the dish.

4. Burn in a muffle furnace until carbon is totally removed. Common burning time: 4 hours at 550°C.

5. Cool in a desiccators until constant weight.

6. Weigh with an accuracy of 0.1 mg.

Calculation and results

Crude ash (%) = \[
\frac{100 \ (W_2 - W_1)}{W} \]
W weight of the sample [g]

W1 weight of the dish [g]

W2 weight of the dish and the sample after ashing [g]

Source
Method: EG L 155/71 1-7-72

Repeatability: 0.1 % abs.

6. Moisture

Purpose and scope

Compound animal feeds and solid and liquid raw materials

Principle

The sample is dried out at 103 °C ( ± 1 °C ) for 4 hours to a constant weight.

The weight difference represents the water content.

Equipment

1. Electric oven, thermostatically controlled at 103°C ( ±1°C ).

2. Dishes, diameter 5 cm, depth 2 cm, with well sealed lid ( for solid materials).

3. Dishes, diameter 6 cm, depth 2,5 cm (for liquid materials).
4. Sea sand, purified and dried.

5. Glass stirring rod, 8 – 10 cm.

6. Desiccators

7. Grinding machine Retsch ZM 200, 12 teeth rotor and 1mm sieve

8. Analytical scale, Readability: 0.1 mg

**Procedure**

1. Procedure for animal compound feeds

2. Grind the sample to the 1 mm grain fineness. Mix well and store in a airproof container. Avoid warming up of the mill during grinding

3. Weigh the dish including the lid (3.2) (=W), after that weigh in 5 g (± 0.1mg) of the sample into the dish (=W1)

4. Analyze also the standard control sample present on the lab for daily quality control of the analysis. The results of the control sample must be imported into the Shewart Chart in MS Excel. Deviations in the control sample must be treated according to the procedure AM000.

5. Remove the lid, place the open dish and the lid in the oven and dry 4 hours at 103°C. The time starts when the oven is back at 103°C.

6. Remove the dish and lid from the oven. Put the lid on the dish.

7. Cool in a desiccators to room temperature and weigh (=W2)
Procedure for liquid materials:

1. Weigh the dish including sand (=W).

2. Weigh in ± 5.0000 g of the sample into the dish with sea sand (=W1).

3. Dry the sample in the drier at 103°C for 4 hours. The time of 4 hours starts when the oven is back at 103°C.

4. Remove the dish from the oven, cool in a desiccators to room temperature and weigh (=W2).

Calculation and results

$$\text{Moisture\%} = \frac{100 \times (W1 - W2)}{(W1 - W)}$$

when: $$W = \text{weight of dish (in g)}$$

$$W1 = \text{weight of dish + sample ( in g )}$$

$$W2 = \text{weight of dish + sample after drying (in g )}$$

All weights are at 0,1 mg precise.

Notes

1. To check the temperature in the oven use a calibrated thermometer.

Source

Method: EG 83 / 73 - 30.3.73
Repeatability: The difference between two parallel analyses of the same sample must not exceed for the content: 0,2% at 5,0 - 20,0%

7. Aflatoxin

1. Add 100 μL conjugate to red marked mixing wells.

2. Add 100 μL controls and samples to the red marked mixing wells.

3. Mix. Transfer 100 μL to antibody wells. Incubate for 2 minutes.*

4. Dump liquid from antibody wells.

5. Wash wells thoroughly 5 times with deionized water.

6. Tap out water on absorbent paper towel.

7. Transfer 100 μL substrate from reagent boat to antibody wells using 12-channel pipettor. Incubate for 3 minutes.*

8. Transfer 100 μL Red Stop from reagent boat to antibody wells.

9. Read results using a microwell reader with a 650 nm filter.

*AST incubates for 5 minutes

VeratoxVeratox-AST

Lower limit of detection:  2 ppb                        3 ppb

Range of quantitation:  5 ppb - 50 ppb                 5 ppb - 150 ppb

Controls provided:   0, 5, 15 and 50 ppb            0 ppb
Testing time: 5 minutes - 10 minutes

Antibody cross-reactivity: Total aflatoxins

Total aflatoxin (B1, B2, G1, G2) (B1, B2, G1, G2)

8. Minerals

ICP Spectrometer

Routine Performance of the 72X is determined by two tests:

Analytical Tests

Net Intensity

Signal to Background Ratio

Short Term Precision

Calibration Accuracy

Detection Limit Test

Enables to isolate problem areas

Stable ambient Environmental conditions

Torch, nebulizer, spraychamber, pump tubes etc. are clean and in good working condition

Polychromator must be stable at 35 oC for several hours

Boost purge must be turned on for at least 2 hours
Plasma should be ON for at least 30 minutes

Peltier cooling should be on for at least 5 minutes

A Dark Current Scan should be completed

A valid Wavelength Calibration must be run before testing

Vertical and Horizontal pre-optics positions optimized with the Mn 257.610 line

Adjust nebulizer flow by aspirating 1000 ppm Y. Adjust flow until red bullet just protrudes above the top of the torch

Wavelength Calibration Solution

5 ug/ml each of Al, As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Se, Sr, Zn and 50 ug/ml K

Prepared in 1% AR grade HNO3 made with pure distilled, de-ionized water

Blank

1% AR grade HNO3 made with pure distilled, de-ionized water

Select new method from VISTA_QC in the “supplied worksheets” folder

Run blank, Standard and run Standard as a sample
The Precision for 10 replicates of sample readings on the multielement standard should be:

Specification

Axial < 1.5% RSD

Radial

- Al 167, As, Pb& Se < 2.5% RSD
- All other lines < 1.5% RSD
- The individual concentration read back value of the multi-element standard measured as a sample immediately after a calibration should be within
- Specification 3%
  - Select a new method based on VISTA_DL in the supplied worksheet folder.
- Ensure optimum viewing height and nebulizer flow are set
- Aspirate Blank and then Standard for calibration
- Ensure blank is not contaminated, and allow sufficient time after the standard solution.
- For samples 1-5, aspirate BLANK
Appendix (2)

Plate (1) Balance

Plate (2) Desicator

Plate (3) oven 550 for ash

Plate (4) Titration unit
Plate (5) fiber (hot extraction unit)
Plate (6) samples store
Plate (7) fiber cold extraction unit
Plate (8) oven moisture 103
Plate (9) Digester (protein)

Plate (10) ash extraction

Plate (11) fat unit

Plate (12) grinding machine
Plate (13) kjeltec for protein

Plate (14) Records

Plate (15) weighing samples

Plate (16) soxtec for fat
Appendix (3)

Chemical Composition of the Main Poultry Feed Ingredients (Dry matter basis)

<table>
<thead>
<tr>
<th>Composition (%)</th>
<th>Sorghum</th>
<th>Groundnut cake</th>
<th>Sesame cake</th>
<th>Wheat bran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude fat</td>
<td>2.65</td>
<td>8.34</td>
<td>13.42</td>
<td>3.45</td>
</tr>
<tr>
<td>Crude protein</td>
<td>14</td>
<td>45.68</td>
<td>43.94</td>
<td>18</td>
</tr>
<tr>
<td>Fiber</td>
<td>2.93</td>
<td>10.19</td>
<td>8.65</td>
<td>13.56</td>
</tr>
<tr>
<td>Ash</td>
<td>2.28</td>
<td>9.7</td>
<td>14.65</td>
<td>5.82</td>
</tr>
<tr>
<td>NFE</td>
<td>78.44</td>
<td>26.09</td>
<td>19.33</td>
<td>51.97</td>
</tr>
<tr>
<td>Metabolism energy (MJ/Kg)</td>
<td>15.22</td>
<td>12.01</td>
<td>12.29</td>
<td>8.46</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.05</td>
<td>0.65</td>
<td>2.12</td>
<td>0.18</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.33</td>
<td>0.59</td>
<td>0.98</td>
<td>0.78</td>
</tr>
<tr>
<td>Available phosphorus</td>
<td>0.099</td>
<td>0.177</td>
<td>0.294</td>
<td>0.234</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.23</td>
<td>1.32</td>
<td>0.96</td>
<td>0.65</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.16</td>
<td>0.47</td>
<td>1.289</td>
<td>0.25</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.34</td>
<td>1.11</td>
<td>1.1</td>
<td>1.16</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.16</td>
<td>0.34</td>
<td>0.65</td>
<td>0.47</td>
</tr>
<tr>
<td>Manganese (mg/kg)</td>
<td>19.8</td>
<td>52.17</td>
<td>71.96</td>
<td>147.61</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>41.01</td>
<td>68.54</td>
<td>136.45</td>
<td>104.269</td>
</tr>
<tr>
<td></td>
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<td>----------</td>
<td></td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td>118.22</td>
<td>215.7</td>
<td>304.92</td>
<td>154.17</td>
</tr>
<tr>
<td>Boron(mg/kg)</td>
<td>2.07</td>
<td>27.50</td>
<td>26.55</td>
<td>4.69</td>
</tr>
<tr>
<td>Cooper (mg/kg)</td>
<td>7.5</td>
<td>15.85</td>
<td>45.51</td>
<td>15.61</td>
</tr>
</tbody>
</table>

(Sulieman et al., 1999)
### Appendix (4)

**Poultry Feed Ingredients and feed Form**

<table>
<thead>
<tr>
<th>Factory</th>
<th>Feed Ingredients</th>
<th>Feed Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sorghum- Groundnut cake- Wheat bran- limestone-concentrates-enzymes-toxin binder-Dicalcium phosphate-Premix-Oil-Lysine-Methionine-salt</td>
<td>Mash/Pellets</td>
</tr>
<tr>
<td>B</td>
<td>Sorghum- peanut meal-limestone-concentrates-toxin binder-Dicalcium phosphate-Premix-soya Oil-Lysine-Methionine-choline chloride-salt</td>
<td>Mash/Pellets</td>
</tr>
<tr>
<td>D</td>
<td>Sorghum- Wheat bran- peanut meal- limestone-concentrates- toxin binder-Dicalcium phosphate-Premix-Oil-Lysine-Methionine-salt-choline chloride</td>
<td>Mash/Pellets/crumble</td>
</tr>
<tr>
<td>E</td>
<td>Sorghum- Groundnut cake- peanut meal -Sesame cake -limestone-concentrates-enzymes-toxin binder-Dicalcium phosphate-Premix-vegetable Oil-Lysine-Methionine</td>
<td>Mash/Pellets/crumble</td>
</tr>
<tr>
<td>F</td>
<td>Sorghum- Groundnut cake- Wheat bran- Sesame cake -limestone-concentrates-enzymes-toxin binder-Dicalcium phosphate-Premix-Oil- Lysine-Methionine-salt</td>
<td>Mash/Pellets/crumble</td>
</tr>
</tbody>
</table>