# Chapter 1

## Introduction

Animal feed safety has become one of the priority areas in the animal production. The live-stock and poultry industry has historically been one of largest agricultural business. A cording to the National Rendering Association. N .R.A., (2003), the market for N .R. A. meat and meat based products requires slaughtering of roughly products of 139 million head of cattle, calves, sheep, hog, and other live-stock, as well as 36 million pound (Ib) of poultry ( broiler chickens , layer chickens ,and turkeys). Meat is the most valuable livestock products,. composed of protein, (amino acids), minerals, fat, fatty acid, vitamins and other bioactive component, and small quantities of carbohydrates All animal protein meal are good sources of vitamin A ,B, and D, as Well as fatty acid. Protein is essential key ingredient of animal feeds necessary for animal growth J. Webster and Award(1982). One - third to one-half of each animal produced for meat, milk, eggs, and fiber is not consumed by humans. These raw materials are subjected to rendering processes resulting in many useful products. Meat and bone meal, meat meal, poultry meal, hydrolyzed feather meal, feather meal, blood meal . fish meal. and animal fats are the primary products resulting from the rendering process. The most important and valuable use for these animal by-products are as feed ingredients for livestock, poultry, aquaculture, and companion animals David et al,( 2006). By-products obtained during poultry slaughtering are considered very valuable raw materials for production of highly valuable animal feeds and energetic feeds Okaanovic *et al*,( 2008) .People in the large American meat-packing industry were the first to realize that it was of great financial and sanitary advantage to make the fullest used

of each slaughtered ,dead or condemned animal Mann(1967).Although the a value of by-products constitutes only a small fraction of live animal value ,it is of considerable economic Importance to the entire live -stock and meat industry ,and influences the price of meat and the price paid to the producer for live- stock Wain *et al* (2014).

**Research problem:** The issue of this study is how to benefit from by-products of animal and poultry slaughter houses which contaminate the environment and cause serious diseases

**Important of the study** :

Incorporating these by-products and residues in animal and poultry nutrition due to their high nutritional value.

**Objectives:**

1- Determination of chemical composition and nutritive value of rendered animal and poultry by-products.
2- To investigate the contamination level and the contaminant organism of rendered animal and poultry by-products.

# Chapter 2

## Literature Review

Judge *et al,* (1989) detailed the edible organs and glands, such as tongues brains, hearts, livers and kidneys and considered them a variety meat for excellent sources of many essential nutrients required in human diet. The inedible by-products include inedible bones, horns,, inedible raw blood and fats .broiler carcass yield is approximately 65% of live weight which means that approximately 35%comprise feather, blood, viscera, feet and head which are considered inedible by-products Silverside and Jones,( 1992).

On the basis of usage as food, the by-products can be categorized into edible and non-edible, however, some of the by-products have medicinal values and classed under pharmaceutical category. Edible by products generally include all those organs which can be consumed viz. liver, lungs, heart, brain, intestine etc. They are also known as variety meats of 'fancy meat Malav *et al.,* (2018) All those by- products that cannot be consumed directly as food are non-edible by-products e.g. hides, horns, ears, hooves, nails, bristles etc. all the condemned parts of animal carcass also fall in this category. This demarcation of edible and non-edible by-products is not universally acceptable as it is largely dependent on customs, traditions, purchasing power, food choices etc. of consumers. In one region, consumption of a particular by-product may be a taboo and in other region it may be a delicacy. The yield of edible by-products from animals varies tremendously depending on species, sex, live weight, fatness and methods of collection Malav ,*et al*.,( 2018).

## 2.2 By-products Plant:

A by-products plant may be a complementary part of the slaughterhouse building, provided it is strictly separated from the slaughter house itself and not under the same roof and that only material from the slaughter house is processed. It's better to have the slaughterhouse combined with by-products plant in rural areas. The building should be provided with a separate entrance and a clean area for handling and storing the processed products. The floors should be impervious, cleanable and sloped to open channels To reduce contamination the raw material may be transported to the by-products plant by overhead pipes extended from the slaughter house toward the by-product plant. In large factory-abattoirs the by-products plant is situated in the ground floor under the killing or boning floor so that the raw materials may be fed directly through chutes. Franco, (2002).

## 2.3 Inedible slaughter by-products as sources of pathogens:

Inedible meat band condemned meat constitute sources of contamination to the environment. Contamination of these product is derived mainly from the animal and slaughter house environment.( Pseudomonas,) were recovered from viscera and surfaces of walls and floors with in the abattoir Newton .*et al* (1980).Eltoum (2000) isolated ,Staphylococcus *spp* from normal and abnormal lymph nodes collected from goats. Also Mackenzie,(1976 ) *et al* isolated streptococcus ,and Clostridium from condemned sheep livers, these organs are used as ruminants by-products for preparation of poultry additives by-products .and found Bacillus anthrax, Mycobacterium tuberculosis ,Salmonella *spp* .

Smyser ,*et al*,(1963) found that *salmonella, Escherichia* and *proteus* were frequently present in poultry by-products and bone scraps Sonnenshein, *et al* (1977) . rendering.

## 2.4 Treatment of inedible slaughter by-products:

A number of different methods are available for treatment of inedible by-products, all of them are concerned with three objectives:

(l) Elimination of water.

(2) Sterilization of the products.

(3) Separation of fats.

The best and most economical method of processing is by heat treatment in a jacketed vessel which gives complete sterilization and maximum return from the treated material.

The rendering process results in separation of fats and production of protein concentrates which are used almost exclusively in feed for livestock and poultry Webb and Price,( 1987). Kumar (1989) divided the rendering methods into simple cooking. Open pan rendering, wet rendering and dry rending and considered the simple cooking and open pan rendering methods as simple old procedures which have low capital investment.

### 2.4.1 System of rendering:

### 2.4.2Wet rendering:

Wet rendering is a process in which the material together with added water are subjected to direct high steam pressure (40 pound/ square inch(psi)) for 5 to 7 hrs. in vertical cylindrical vessels. This method became absolute and is not recommended because the products lose proteins in the water and need a long time to completely dry Kumar, (1989).

### 2.4.3 Dry rendering:

In this method all the unwanted moisture eliminated without loss of nutrients and allow an approximately 20% higher yield than the wet rendering, as the water containing water-soluble extracts and proteinases suspended matter is not discarded and at considerable amount of labor and steam are saved Mann, (1967).

#### 2.4.3.1 Dry rendering cooker:

Dry rendering cooker is a horizontal steam jacketed vessel equipped with a set of agitators, which keep the charge in continuous motion. The steam is applied to the jacket only and not to the material to be processed (Fig.1) at a pressure of 40 psi Kumar,( 1989).

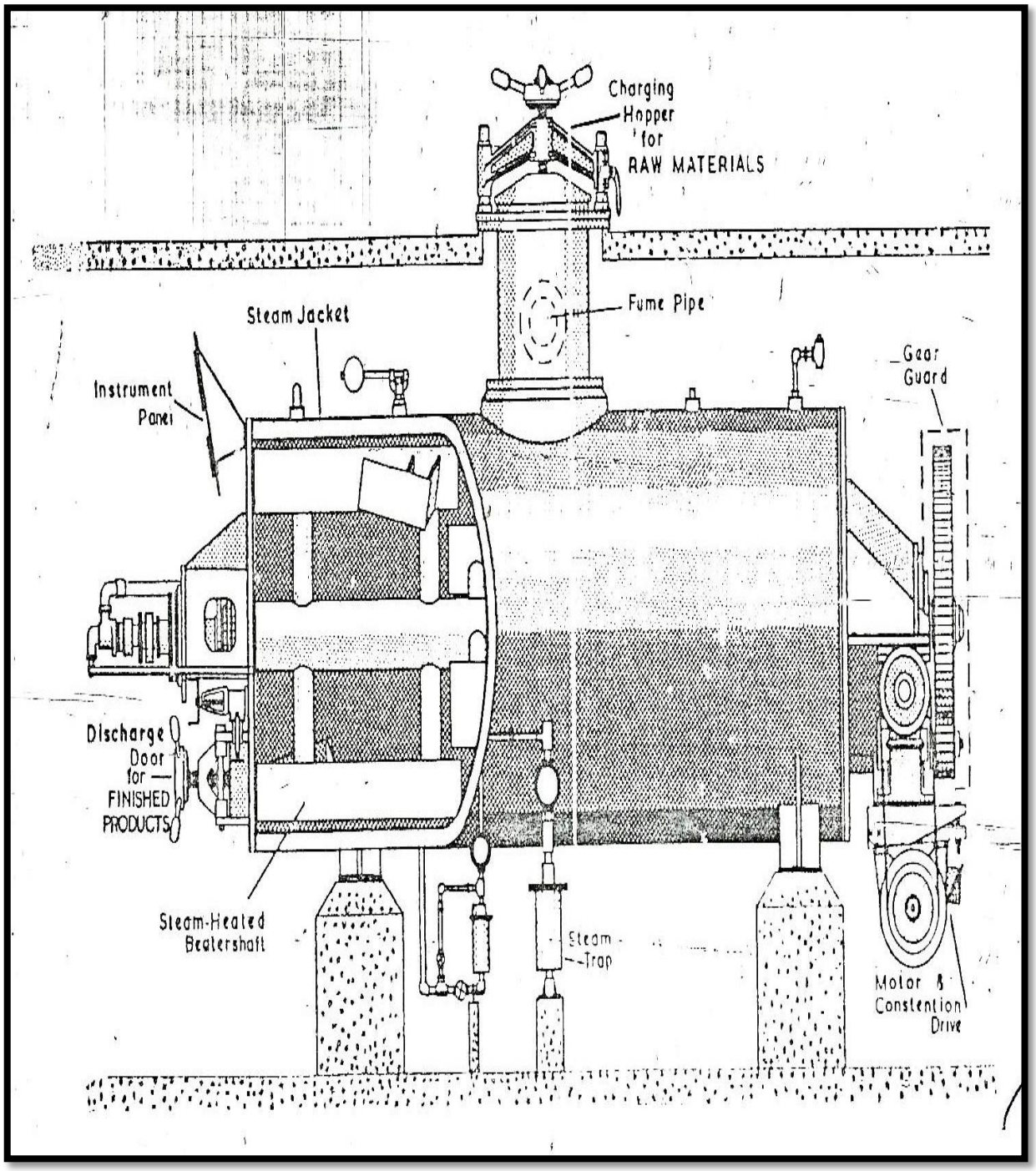


**Fig. 1: Typical pressure dry melter (cooker), showing steam trap and drive arrangement (Grace1986)**

The dry heat is then transmitted from the steam jacket to the raw material and converts its moisture into steam, which gradually builds up the internal,

pressure .This pressure combined with continuous agitation, disintegrates the material and breaks down the fat cells Mann, (1967);Grace, (1986) and Kumar,( 1989). The cooking time depends upon the quantity and quality of material.

After cooking the fat is extracted by chemical or mechanical ways. Then the product is milled fine flour (Mann, 1967).

## 2.5 Definition of Rendering Process:

Rendering is practical example of effective heat treatment to destroy microorganisms in raw animal and poultry by-products and its conversion into rendered safe material almost free from pathogens. The most important and valuable used for these rendered by-products is as feed ingredient as for livestock (Samah *et al*., (2018). Burial, incineration, composting and rendering are different method used for disposal of animal and poultry carcasses and their wastes. Salminen and Rintal,.( 2002). Rendering is a process of both physical and chemical transformation using a variety of equipment and processes and separation of fat. Cooking is generally accomplished with steam at temperatures of 240to290 of (approximately 115 to 145C) for 40to 90 minutes depending upon the type of system and material David and Hamilton,( 2006).

### 2.5.1 Concerns Associated with the use of inedible by-products:

Production of animal feed through recycling of animal waste to ease cost of feed has been in operation for over forty years Taylor ,*et al*., (1995).

Rendering is the main process used by the industry, and this involves using heat to stabilized, sterilized, and separate the dry-hydrated materials into dried products, namely animal protein meals and rendered animal fat Chen, (1992)..Rendering can be considered sustainable in three areas

economics, social and environmental. Environmental sustainability is especially clear as rendering requires a high level of energy, input to operate and renewable fuels Rendering is a classical example of effective heat treatment to destroy microorganisms and separate water, fat and protein contained in animal or poultry issues under controlled and specific processes David *et al*(2006). Rendering converts raw inedible animal tissue into stable value added materials resulting in many useful products like poultry by-products meal. Temperature and length of time of the cooking process can impact the quality of the finish product Hamilton, (2004).National rendering Association. N.R.A., (2006) found that ground raw parts of slaughtered poultry carcasses as head, feet etc. are highly contaminated with microorganisms including bacteria, virus-like particles, fungi, yeast and associated microbial toxins that constitute a potential risk to animal and human health Chen, (1992). During rendering process raw materials are cooked at predetermined, continuously monitored temperature and atmospheric pressure in batch steam cookers ($115^0$C-$145^0$Cat 40 PIS) , (N.R.A,( 2003).

### 2.5.2 Principles of carcass rendering:

The concept of rendering is heating or cooking of carcass materials (with complex or simple mixtures of proteins, minerals and fatty substance) to liquefy the fat and break down membranes or other structures that may hold the fat Romans *et al*,( 2001). Modern carcass rendering is a process of using high temperature and pressure to convert a variety of high perishable protein and fat materials including condemned, fallen, culled, and experimental animals with little or no value into safe nutritional and economically valuable products. Rendering plants employ inedible rendering processes convert the fat, protein, and keratin (hoof and horn) materials found in dead carcasses into inedible tallow, carcass meal and fertilizer,

respectively. In these plants the rendering process is accomplished by removing undesirable materials (such as ferrous metals) by passing through metal detectors or carcass part by cutting mixing and preheating and separating fat and protein materials. The hide is not usually removed from hogs and small animals .Under atmospheric pressure, ground carcass material entering the cookers with maximum particle size of 40mm is heated up to the maximum temperature range of 120 – $140^0$C for the average cooking time of about 3 hours(Mann 1967).

### 2.5.3Definition of By-products:

The by-products can be divided into primary and secondary by-products. Primary by-products also known as principle by-products are harvested directly from the animal whereas the secondary by-products are derived from principle by-products Wain, *et al*, ( 2014).A by-product is defined as a secondary products obtained during the manufactured of a principal commodity of product David ,*et al* (2006).Animal by-products including everything of economic value other than carcasses obtained from animal during slaughter and processing these products are classified as either edible or inedible for human judge *et al*, (1989).Analysis of animal by-product meals shows that they contain many essential nutrients. They have a wide use and substantial amounts that used in feed for poultry and other animals. When calculating ration formulas, nutritionists compare the costs of nutrients supplied by animal by-products with the cost of the same essential nutrients supplied by alternate products and select the ingredients that give the desired nutrient at least cost Webb and price, (1987). In poultry rations, meat meal fed at levels of 8% provides not only amino acids but a substantial amount of the required vitamins, calcium and phosphorus, as well as some trace minerals. A small percentage of blood meal increases the lysine and tryptophan content. The protein content in poultry meal should not be less

than 58% and the fat and moisture contents should not be more than 13% and 10% respectively. The protein content in meat meal should not be less than67 % whereas the fat and moisture should not be more than 12% and 10% respectively Sudanese Standards and Metrology organization SSMO,( 2002).

**2.6 –Types of by-products meal**

a) Meat and bone meal.

b) Meat Meal.

c) Hydrolyzed Feather Meal.

d) Poultry by-Product Meal.

e) Blood Meal.

f) Specialized Protein Blends.

**2.6.1 Major benefits of using animal protein meals are:**

- They contain moderate to high levels of amino acids like lysine, methionine and threonine.

- If processed properly, the amino acids are highly available.

- They are rich sources of available phosphorus, calcium and trace minerals.

- They help sustain animal agriculture by transforming waste animal tissues into valuable products for further economic use.

- They are palatable when used in diets that are balanced for amino acids, especially lysine, methionine and cysteine, and tryptophan, threonine and (blood meal) N.R.A(.2006).

### 2.6.2 Meat and Bone Meal:

Meat and bone meal (M.B.M) is the protein residue after the moisture and fat has been extracted in the normal rendering process. It includes bone, but is exclusive of blood and extraneous material such as hair, hoof, horn or manure. It is golden to medium brown in color, with a fresh meaty odor and is available throughout the year. The quality and composition of the raw materials used will have some effect on the quality of finished product. Raw materials may vary in different geographic areas. Consequently, the composition of Meat and Bone Meal (MBM.) will vary from plant to plant. MBM customers can manage this variability by identifying individual MBM. manufacturing facilities having low variability, or by relying upon MBM blenders that with the capability of reducing the coefficient of variation in protein content to < 3%. Processing has the greatest effect on amino acid digestibility. Advances made in processing methods and equipment has resulted in marked improvements in the digestibility of meat and bone meal in the past 20 years. Meat-and-bone-meal may be used as an amino acid source in formulating feeds for all classes of poultry, swine, many exotic animals, some species of fish and pets feed N. R.A.(2003). MBM is primarily considered as a high protein raw material which also has added value in supplying energy-minerals and vitamins (Australian, 2013) Meat and bone meal is the product obtained by rendering drying and grinding of mammalian tissues and bones from animals produced for human consumption –exclusive of hair .wool hide except where it is naturally adhering to head sand hoofs Australian,( 2013).

### 2.6.3 Meat Meal:

Meat meal is the solid protein residue derived from the rendering of meat. It is exclusive of blood, bone and other extraneous material. The

product is golden brown in color with a fresh meaty odor. The quality and composition of the raw materials used will have some effect on the color and composition of the finished product, but has no effect on digestibility. Raw materials may vary in different geographic areas. Processing has the greatest effect on amino acid digestibility. Advances made in processing methods and equipment has resulted in marked improvements in the digestibility of meat meal in the past 20 years. Meat Meal is available all year, and may be used as a protein source in formulating feeds for all classes of poultry, swine, exotic animals, fish and pet foods. As with M.B.M, meat meals are not to be fed to ruminants N.R. A.(2006)

### 2.6.4 Hydrolyzed Feather Meal:

Hydrolyzed feather meal is derived by cooking under pressure ,for clean, encompassed feathers from slaughtered poultry. It must be processed for sufficient time to break the cysteine bonds and produce a meal with a minimum of 70-75% pepsin digestibility N.R.A (2003).The prime factor which will influence the quality of hydrolyzed poultry feathers is the degree of hydroxylation. Too high a hydroxylation (that is, a pepsin digestibility of 90 percent) will produce overcooked meal with reduced amino acid digestibility. Likewise, too little hydroxylation (i.e., a pepsin digestibility below 65 percent) will result in an undercooked meal, also with low amino acid digestibility. Raw feathers have high cysteine content and during processing the cysteine linkage is broken, which increases the value of the feather meal. If too many cysteine bonds are broken, however, excess sulfur amino acids are destroyed and unnatural compounds are produced. These compounds are digestible in pepsin under laboratory conditions, but are unavailable to the animal. The physical properties of feather meal vary according to the feathers used; feathers of a light color result in a light golden brown meal; feathers of a dark color result in a dark brown-black meal.

Feather meal has a fresh odor. If blood is added to the meal after processing, the color will be darker, but the meal will benefit accordingly from its inclusion. Feather meal is resistant to rumen degradation and is a valuable bypass protein source for ruminant reaction .The protein content of feather meal is about 80 percent. The fat content in feather meal varies significantly depending on mixture of the feathers with skin tissue. High quality feather meal should contain less than 5 percent fat. Moisture should not exceed 10 percent. Very low moisture content may indicate overheating, which would destroy amino acids. Its digestibility will vary with type of equipment used to process feather meal. If properly hydrolyzed (under pressure), the digestibility will be around 80 percent. N.R.A.(2003).

### 2.6.5 Poultry By-Product Meal:

Poultry By-Product Meal**:** consists of the ground rendered parts of the carcasses of slaughtered poultry, such as heads, feet, undeveloped eggs and intestines, exclusive of feathers, except in such trace amounts as might occur unavoidably in good manufacturing practices. With consumers purchasing poultry meat cuts rather than whole carcasses, the protein content of the poultry by-product meal approximates 58 percent, reflecting the higher bone content of the raw material. Adulteration with raw feathers will alter the amino acid content and decrease digestibility. This product should be treated with an anti-oxidant immediately after processing to ensure fat stability. Poultry by-product meal will be golden to medium brown in color, with a fresh poultry odor. The product may be used as an amino acid source in formulating feeds for all classes of poultry, livestock, many exotic animals and pet foods N.R.A,( 2003).

**Low ash poultry meal:**

The principal sources of raw material come from unused chicken and turkey materials created at each level of the meat processing chain. Chicken and turkey skin, carcasses, offal, and fat are collected and processed daily. Various processing methods are employed to remove mineral containing components resulting in a product with reduced mineral content, increased digestibility and increased levels of essential amino acids. Benefits include renal health in pets and improved water quality of intensive aquaculture systems. The product is golden brown, having a typical poultry meal N.R.A,( 2006).

**2.6.6 Blood Meal:**

Blood meal is a finely ground protein residue derived from clean, fresh blood, excluding all extraneous material such as hair, stomach belching's and urine except in such traces as might occur unavoidably in good manufacturing process Ahmed *et al* (1995)

Moisture is removed from the crude blood by dewatering, followed by ring, flash or spray drying N.R.A,( 2003).The method of drying blood is probably the greatest single factor that will influence the quality of the finished product. Sustained high drying temperatures can bind or inactivate a large percentage of the lysine, as well as other amino acids, making them unavailable to mono-gastric animals. Spray drying is one method that produces blood meal that is highly digestible. Lysine digestibility improves as the method of drying moves from ring drying -flash drying- spray drying. In many regions, the plasma is harvested from whole blood, resulting in plasma proteins and blood cells, which are usually spray dried. Both products are high in quality and are primarily used in diets for young pigs or in milk replacers. The protein content of whole blood meal is at least 80 percent and

the protein digestibility is a minimum of 95 percent. Blood products are a rich source of essential amino acids for swine and poultry. Whole blood is a prime example of a highly ungraded protein for ruminants with more than 75 percent of the protein by- passing to the small intestine. This bypassed protein has a good quality of amino acids that are highly digestible in the lower gut. The product may be used as a protein source in formulating feeds for all classes of poultry, livestock, many exotic animals and some species of fish N.R.A (.2006).

### 2.6.7 Specialized Protein Blends:

These are blends that can contain blood meal, feather meal, meat and bone meal, meat meal, and poultry by-product meal designed to contribute specific nutrient advantages to the diets of various species. Specific advantages might include cost effective contributions of metabolizes protein, available phosphorus, and digestible amino acids N.R.A.(2003).

## 2.7 Chemical composition of animal and poultry by- product:

### 2.7.1 Chemical composition of animal by- product:

Chemical composition of animal by- products was 59% for protein content, fat content as 10% ash18%, moisture content as 10 %, as stated by N.R.A,( 2003). Chemical composition of animal by- products moisture content was 2 - 7% Protein content as 60 - 67% fat content as 8-15%ash content as 20-25%.which concluded by (Naga, 2014).

The protein content in animal by- product should not be less than 67% whereas the fat and moisture content should not be less than 12%and 10% respectively. Sudanese standards and Metrology SSMO ( 2002)

### 2.7.2 Chemical composition of poultry by- products:

Chemical composition of poultry by-products meal by was 68% for protein content, fat was15%, ash was 9%, moisture was 4% stated by N.R.A (2006).

Chemical analysis of poultry by- products meal was that fat was 15%, protein as 68%, moisture as 4%, ash content was 9% as reported by Ahmed (2008).

The chemical analysis of poultry by- products meal revealed-that crude protein was 60%, fat content was 8-15%, ash contentw as 10-15%, moisture content was 6-10 reported by David and Hamilton (2006). protein content in poultry meal should not be less than 58% and the fat and moisture contents should not be more than 13% and 10% respectively. Sudanese Standards and Metrology organization SSMO (2002) specified That

### 2.8 Bacterial Load in Animal Feed Concentrates:

Wadi (2002) . Investigated variation in colony forming units per gram (C.F.U/ gm.) for animal feed He found that bacterial load in rendered poultry by- products ranged from- $1.6\times10^4$ to $8\times10^{5.}$ He considered feeds with 500 C.F.U/gm. were suitable and could he used as animal feed. Bacteria count in poultry feed ranged from $1.0 \times 10^5$ to $8.8 \times 10^5$ C.F.U/ gm.

Banerjee and Shetty, (1992) .coliform count in fish meal , meat and bone meal ranged from 0.0 to.$5.6\times10^5$respectively. Veldman *et al.*,(1995).

### 2.9 Bacterial contamination of animal feed concentrates

Animal feed get contaminated with bacteria in several ways. Air and dust are the most important environment sources of bacterial contamination especially bacillus ,clostridium and micrococcus which contaminate the feed during processing Jay( 2000). Samaha and ezzat, (1986) isolated, E .coli,,Loken *et*

*al,*. (1968) isolated Salmonella from environmental swabs. Animal feed may contain several bacteria that are harmful to animal; these bacteria can pass through the food production chain and cause human food borne diseases. Crump *et al*., (2002) reported an outbreak of human salmonellosis in the United States and linked that with eating of contaminated chicken and livers. The infection of these chickens was traced back to a chickenfeed made with bone meal that had been contaminated with salmonella Strains of *Clostridium* were also isolated from fish and meat meal Pupavac and Lalic,( 1990). Siebrits, (2003) reported that the greatest risk in animal feed manufacturing was cross-contamination which might happen during milling.

## 2.10- Effect of Heat on Bacteria and Bacterial Load:

The effect of dry heat or heat with moisture on bacteria was studied by Lui, *et al*, (1969).They found very resistant spores after heat treatment at 100 C of 30 min which belong to bacillus. Bacillus spore were present in all treated feed concentrate (Moran Row and Hagan (1990).

Sale *et al.* (1970, Mills, *et al*.,(1998). demonstrated that bacillus spore had the highest microwave tolerance.

## 2.11- Bacteria Associated with Slaughter By-products and Feed concentrates

### 2.11.1Enterobacteriaceae:

Enter bacteria uses are used for the assessment of microbiological quality of feed components, and those isolated from feed stuffs were predominantly thyrotrophic Veldmam *et al*.,(1995). *Psychrotrophic Enterobacteriuases* are widely distributed in meal industry and occur commonly on meat and meat by-products Newton *et al (*1980).

### 2.11.2 Salmonella:

Animal feed constitute one of the major sources of various *Salmonella* sera – rovers. Theses rovers have a path of infection from animals and ultimately to human beings Molye, (1966) Mackenzie and Banis, (1976).

Several investigators isolated *Salmonella* from products of animal origin which are used in preparation of animals and poultry by-products feed Watkins *et al,* (1959). Bensink and Boland, (1979) isolated 35 Salmonella species from samples Taken at various points of processing of slaughter by-products. They did not isolate Salmonella from any of the samples of freshly cooked material, however the material became contaminated immediately after leaving the cooker, and the rate increased as the products moved along the processing line. Wedman, (1961) isolated some *Salmonella* front a variety of animal by-products and condiment that animal by-products in rations were responsible for specific field occurrences of salmonellosis .Tanios, (1997) investigated *Salmonella* species in animal feed composed of meat and bone meal and poultry meal by-product , and found that poultry meal samples had the highest contamination rate of 20%.*Salmonella* isolated represented nine namely S. muenster, S. cerro, S. tyhimurium, S. anatum , S. Kingsto S. reubeuss, S. Stockholm, S. binza and S. boecker. Larainore and Moritz, (1969) isolated Salmonella from fish, feather and meat meal, and the highest contamination was found in meat meal, but Veldman *et al.,* (1995) found that fish meal had the highest contamination of salmonellae. Wadi, (2002) recovered salmonella from bone meal and feed concentrates. Mackenzie and Bains, (1976) found the same types of salmonella isolated from poultry farms and feed, in broiler carcasses from a processing plant. Jay, (2000) indicated that *Salmonella* had not been found in rendered products or finished feeds. This agreed with the results obtained by Larainore, and Moritz (1969), Veldman *et al*., (1995) and Tanios (1997) ,. However *S.Enteritidis* and *S.*

*typhimurium* were detected in environmental samples taken from poultry houses Soun *et al*., (1999). The survival of *S. enteritis* was highest in deep litter and lowest in fish meal and feed Manual once used the designation *S. gallinarum* for both non-motile S. pulllorum and s gallinnrum which resulted in confusion, however they are now listed as *S. gallinarum–pullorum.* Calnek *et al*.(, 2000).Wedman (1961) detected non-motile *Salmonella* in animal by-products. El toum,( 2000) isolated *S. sandiego* from lymph nodes collected from slaughtered goats.

Salmonellosis is a very important disease not only from the economic point of View, but also from the public health aspect as it is zoonotic disease and the control of infection from animal feed depends mainly on the selection of salmonella- free raw materials from reputable sources and an effective heat treatment of the ingredient Tanios (1997) ,. The protein content in poultry meal should not be less than 58% and the fat and moisture contents should not be more than 13% and 10% respectively. The protein content in meat meal should not be less than67 % whereas the fat and moisture should not be more than 12% and 10% respectively Sudanese Standards and Metrology organization SSMO,( 2002).

### 2.11.3 Escherichia:

E. coli is an indicator bacteria of faucal contamination and the major source of the bacteria in the environment is the faucal of infected humans but there may also be animal reservoirs Wagner and Jr,( 2000). E. coli is least affected by the conditions on meat surfaces and is likely to be the main hazard On meat of normal pH held at room temperature Newton and Gill,( 1980). *E. coli* population on the slaughterhouse equipment is heterogonous ,therefore the simple washing of equipment after Several hours of processing with a jet of water is not sufficient to remove them Banerjee, (1992).

## 2.12 Animal feed hygiene:

Public concern about food safety of animal derived foods was highlighted due to food borne bacterial infections outbreaks, outbreak of bovine spongiform encephalopathy (BSE) and the discovery of the control role played by controlling infected meat and bone meals. This is also concern about microbial resistance to antibiotics caused by veterinary drug residues Siebrits, (2003).Bacteria do not multiply in feed under normal circumstance because of their low moisture content, but they do so readily if water is added to feed Carlson and Snoeyenbos, (1970). In general good feed manufacturing practice, heat treatment and correct handling and storage of raw materials and finished feeds, which includes keeping moisture level very low are considered the main measures that must he used to minimize the risk of infection from animal feed Ahmed et al, (1995).

# Chapter 3

# Material and Method

This study was conducted in the laboratory of meat Department, college of Animal production Science and Technology, Sudan University of Science and Technology (SUST) from January 2017-to December, 2017.

## 3.1 Collection of samples and rendering:

Fifteen samples of rendered meat meal of animal and poultry by-products were used in this study. Nine samples of raw animal by-product were collected from different slaughter houses .The samples were brought to the meat laboratory and subjected to dry rendering using presto cooking suit. The rendering animal by-product were subjected to dry in the sun for 7 days for each sample .The dried rendered samples were ground to powder, then the powdered samples were packed in plastic bags and stored for chemical and biological assessment.

Six samples from rendered poultry by-product were taken from two different poultry rendering units. the samples were taken every 15days interval from different rendering batches. The rendered poultry by-product were subjected to chemical analysis and cultured for total bacteria count and to identify the contaminant organism.

## 3.2 Chemical analysis of rendered animal and poultry by-product:

### 3.2.1 Moisture analysis :

-The moisture removed from the samples by heating at 105C$^{o}$ in a force – draught oven for 3 hour.

#### 3.2.1.1 Apparatus:

- Metal dish.
- Drying oven.
- Sensitive balance.

#### 3.2.1.2 Procedure:

- Place dry crucible or dish in a forced – drat oven for a minimum of 1 hour.
- Transfer to a desiccators and allow cooling to room temperature, and weighing.
- Weigh 5 g of sample in to the dish and heat in the dry oven for at least 3 hours

#### 3.2.1.3Calculation:

**Moisture%= 100 - % dry matter]**

$$\text{dry matter \%} = \frac{(\text{Wt of dried sample} + \text{dish}) - (\text{wt of dish})}{(\text{Wt of original sample “5 gm”})} \times 100$$

### 3.2.2 Determination of Ash and organic matter Principle:

The sample is ignited at 500-550 C$^{o}$ to burn off all organic material. The inorganic material which does not volatilize at that temperature is called ash. The difference between sample and ash gives the organic matter.

### 3.2.2.1 Equipment:

- Muffle furnace set at 550C$^{o}$.
- Crucibles or metal dish.
- Sensitive balance.

### 3.2.2.2 Procedure:

- Heat a clean basin or metal dish for 1 hour in oven, cool and weigh.
- Weigh 5 g of sample in to the dish.
- Place it in the cooled furnace and ash the sample at 550C$^{o}$ for 4 hours.
- Turn off the muffle and leave to cool (100C$^{o}$).
- Remove the sample from muffle and transfer to desiccators, cool and weigh.

### 3.2.2.3 Calculation:

$$\text{Ash\%} = \frac{(\text{wt of Ash} + \text{dish}) - (\text{wt of dish})}{(\text{Wt of original sample})} \times 100$$

**Organic matter% = 100 - Ash%**

### 3.2.3 The determination of Crude Fat (sox let):

### 3.2.3.1 Principle:

The sample is extracted with petroleum spirit, the solvent is distilled off and the extract dried and weighed.

Reagent:

Petroleum spirit, boiling point (60-80 C$^{o}$)

### 3.2.3.2Procedure:

1- Weigh accurately 2.5g of sample.
2- Press small piece of cotton wool in to the top to stop loss of sample occurring.
3- Insert the thimble in extractor tube.
4- Weigh a 250 ml round, flat –bottomed quick fit- flask.
5- Add 100 ml of petroleum spirit in the 250 ml flask.
6- Connect the flask to quick fit-extractor and quick fit-condenser.
7- Turn on condenser water and heater.
8- Adjust heating to produce slow, regular boil.
9- The extraction of crude fat lasted for 5 hours.
10- Take the thimble from the extractor tube and assemble the apparatus to collect the evaporated solvent for re-distilling and re-use.
11- Put the flask +oil in oven (105C$^{o}$ 3hours).
12- Cool to room temperature in desiccate) and weigh accurately.

**Calculation:**

$$\text{Fat } \% = \frac{(\text{WT. of flask } + \text{oil } - \text{ WT. of flask})}{\text{WT. of original sample (2.5)}} \times 100$$

### 3.2.4 Determination of total nitrogen (crude protein):

### 3.2.4.1 Principle:

Total nitrogen is determined using the method described by (Johan KjeldahL,1883). Organic nitrogen is converted in to ammonium ions by digestion with concentrated sulphuric acid in the presence of a catalyst such as a mixture of copper sulphate with selenium**.**

As the digestion proceeds, some of sulphuric acid is reduced to sulphuric dioxide which in turn reduces the nitrogenous material to ammonia. The ammonia combines with sulphuric acid to form ammonium sulphate. Amonia is liberated by boiling with sodium hydroxide, steam distilled in to boric acid plus indicator and determined by titration.

### 3.2.4.2 Equipment:

- Digestion system.
- Distilling unit.
- Digestion tubes or flask.
- Flask 50 ml.
- Burette.

### 3.2.4.3 Reagent:

- Conc. Sulphuric Acid.
- Catalyst (Copper sulphate+selenium).
- Sodium hydroxide solution 50%.
- Standard solutionof ammonium sulphate.
- Standard acid 0.01 N -HCL.
- Boric acid+ bromocresol green/methyl red indicator solution.

### 3.2.4.4 Procedure:

- Weight accurately, 0.5 gm. of sample on crucible and transfer to kjeldahl tube or flask.
- Add catalyst, and then add 10 ml of cone sulphuric acid.
- Place the tube in the digestion and bring the temperature to 350 C.
- The reaction mixture will turn black due to the dehydrating action of sulphuric acid and the formation of free carbon.
- Digest for 1.30-2 hour until color change to light blue or colorless.

- Remove the tube from the block digester and allow to cool.
- Add carefully 20 ml of distilled water to digestion flask and then transfer to 75 ml kjeldahl tube and dilute to volume with distilled water.

### 3.2.4.5 Distillation and titration:

- Pipette 3 ml from digested sample in to the distillation tube.
- Wash with 3 ml distil- water.
- Add 3 ml of Na OH.
- Wash with 3 ml distil - water.
- Add 10 ml of boric acid + indicator in conical flask 50 ml.
- Place the flask so the head of the condenser is below the surface of the solution, turn the condenser water on.
- Start heating for 3 minute and collect steam distillation of ammonia (NH3) which involves trapping in boric acid + indicator.
- Titrate the distillate against a standard acid (0.01 N- HCL). The color change to light pink with the end point occurring.

### 3.2.4.6 Calculation:

$$\text{CP\%} = \frac{\text{Titrate} - \text{Blank}}{\text{Standard} - \text{Blank}} \times \frac{75}{3\ \text{ml}} \times \frac{1}{0.5\text{g}} \times 6.25 \times \frac{1}{1000} \times 100$$

**or** $\text{CP\%} = N_2 \times 6.25$

### 3.3 Biological assessment of rendered animal and poultry by-product:

#### 3.3.1.Vlable count:

Available count is a technique used in microbiology to determine the number of colony forming units in a bacterial suspension or homogenate. The technique was first described by Miles and Misra, (1938).

#### 3.3.1.1 Material:

- A calibrated dropping pipette delivering drops of 20 ml
- Petri dishes containing blood agar
- Phosphate buffered saline
- Bacterial suspension or homogenate

#### 3.3.1.2 Method:z

The inoculum /suspension is serially diluted by adding one ml of suspension to 9 ml of diluent .When the quantity of bacteria is unknown dilutions should be made to at least$10^8$-Three plates are needed for each dilution series. For statistical analysis an average of at least 3 count are needed.

- Plates are divided into equal sectors(it is possible to use up to 8 per plate). are labeled with the dilutions.
- In each sector1 ml of the appropriate dilution is dropped on to the surface of the agar and the drop allowed to spread naturally.
- The plates are left upright on the bench at 37C for18-24 hours in incubation conditions considering the organism.
- Each sector is observed for growth, high concentration will give a confluent growth over the area of the drop or a large number of small merged colonies.

- Colonies are counted in the sector where the highest number of full-size discrete colonies can be seen (Usually sectors containing between 2-20colonies are counted.)
- The following equation is used to calculate the number of colony forming units.
- C.F.U per ml =Average number of colonies for dilution ×50 ×dilution factor.

### 3.4 Preparation of sample:

One gram of samples dissolved in the ten ml of nutrient broth .After that samples must be incubated at room temperature for two hours.

### 3.4.1 Culturing of the samples:

Full loop from sample taken and streaked in plate medium E coli streaked in Ethel. Methylene Plue (E.M.P). medium and salmonella streaked in Xylose. Lysine Deoxy cholate agar. (.X.L.D.). medium .E coli give green metric chain And salmonella give black centrally colony .And subculture two organism in nutrient agar then primary and secondary test done

### 3.4.2 Primary tests:

### 3.4.2.1Gram's test:

Gram's stain was done as described by (Cruickshank *et al*., 1975).

### 3.4.2.2 Oxidase test:

The organism was grown on nutrient agar. Oxidase reagents were added to a piece of filter paper. The test organisms was picked with a sterile bent glass rod and rubbed on the filter paper.

***Recorded result*** dark purple color that developed in 5 to 10 seconds was considered as a positive result.

### 3.4.2.3 Catalase test:

A drop of 3% aqueous solution of hydrogen peroxide was placed on a clean slide and a small amount of the bacterium colony was placed on the drop by glass rod.

***Recorded result*:** Production of gas bubbles indicated a positive result.

### 3.4.2.4 Motility test:

Motility medium was stabbed with straight loop and incubated at $37^0$C.

***Recorded result*:** Motile bacteria migrated outside craigie tube through the medium which become turbid.

Non-motile bacteria were confined to the stab line.

### 3.4.2.5 Sugar fermentation test:

The sugar media were incubated at $37^0$C and examined daily for up 7 days.

***Recorded result*:** A red color in the medium indicated acid production. Gas formed in the inverted Durham tube.

### 3.4.2.6 Oxidation-Fermentation (O-F) test:

Two tubes containing Hugh and Leifson's medium were inoculated, one being covered with a layer of sterile paraffin oil and both were incubated at $37^0$C for up to 14 days.

### 3.4.3 Secondary tests

#### 3.4.3.1Citrate utilization:

The test organism grown in nutrient agar was heavy inoculated into a slop of Simmons citrate agar. The inoculated medium was examined after 24 hours of incubation at 37° C and daily for up to 7days Blue color and growth of the organism indicated positive result green color and no growth indicated negative result.

#### 3.4.3.2 Urease activity:

A slop of urea agar medium was heavily inoculated by the test organism and incubated at 37◦ C and examined daily.

Record Result -Appearance of a red color indicated positive result

#### 3.4.3.3 Production of indole:

The test culture was inoculated into Peptone water was incubated with the test organism and incubated at 37◦ C for 24-48 hrs., Kcvac's reagent was poured down on the wall of the culture tube to make a top layer.

***Recorded result***: Development of a red color between the layers indicated a positive reaction.

#### 3.4.3.4 Methyl red (MR) test:

Glucose phosphate was inoculated with test organism and incubated at 37◦C for 2 days. Then 2 drops of MR reagent added and the tube was shaken.

Recorded result: A positive MR reaction was inoculated into glucose by color changing of culture to the red .Whereas yellow or orange color was negative result**.**

### 3.4.3.5 Voges-Proskaure (VP) test:

The test culture was inoculated into glucose phosphate medium and incubated at37◦C for 2 days added 1 ml reagent.

Record result –Appositive reaction was incubated by a strong red culture.

### 3.4.3.6 Hydrogensupplied$H_2S$ production test:

- Peptone water was inoculated with test culture and lead acetate paper incubated at 37◦ C.
- Record result –Blacking of paper indicted production of $H_2S$.

### 3.4.3.7Sorbitol Ma Cconkey agar test:

The test culture was inoculated into sorbitol Ma **Cconkey** agar and incubated at 37◦C for 24 hrs.

Record result produced colorless colonies were considered E. coli.

### Statistical analysis

The data presented as± standard deviation was subjected to statistical analysis of variance (one way ANOVA ) all calculation were per-formed using SPSS Version 17( Gomez and Gomes (1948).

# Chapter 4

# Results

The results of this study are presented in the tables (1- 9)

**4.1 Table (1 ) Averages values ± SD of Chemical Composition of Rendered Animal by- products from different sources:**

| Sources | Moisture % | Fat % | Protein% | Ash% |
|---|---|---|---|---|
| Sources1 | 11.11±1.05 | 11.11±1.05 | 60.78±1. 30 | 17.44±0.73 |
| Sources2 | 12.00±1.32 | 12.00±1.12 | 58.67±2.06 | 17.33±1.12 |
| **Sig** | **N.S** | **N.S** | **N.S** | **N.S** |

****Means there were significant different between treatments at (P<0.05).

N.S Means no significant different between treatments at (P<0.05)

Table (1) shows the average values ±SD of rendered animal by-products composition in source (1) moisture content was 11.11±1.05.Fat content was 11.11± 1.05 Protein content was 60.78±1.30 and the ash content was 17.44±0.73.

In sources (2) moisture content was 12.00± 1.23 Fat content was 12.00±1.12, protein content was 58.67 ± 2.06 and the ash content was 17.33±1.12There was no significant deference, at(P<0.05) in sources 1 and 2 in the chemical composition

**4.2-Table (2) Averages values ± S D of Chemical composition of Rendered Poultry by –products from different sources:**

| Sources | Moisture% | Fat % | Protein% | Ash% |
|---|---|---|---|---|
| Sources1 | 10.67±1.00 | 10.67±1.00 | 65.44±0.88 | 13.44±0.88 |
| Sources2 | 10.44±0.88 | 10.44±0.88 | 64.89±0.78 | 14.22±0.67 |
| **Sig** | **N.S** | **N.S** | **N.S** | **N.S** |

Table (2) shows the averages values ± SD of rendered poultry by-products composition in source (1) moisture content was 10.67 ±1.00.Fat content was10.67± 1.00 Protein content was 65.44±0.88, and the ash content was 13.44±0.88. In source (2) Moisture content was 10.44 ± 0.88 Fat content was 10.44±0.88.Proteincontent was 64.89±0.78 and the ash was 14.22±0.67.

Table (2) shows there is no significant difference at ($P<0.05$) in the chemical composition of the two sources.

**4-3-Table-( 3)- Averages values ± SD of Chemical Composition of Rendered Animal and poultry by-products**

| Sources | Moisture % | Fat % | Protein% | Ash% |
|---|---|---|---|---|
| Animal by-product | 11.56±1.25 | 11.56±1.15 | 59.72±1.99 | 17.39±0.92 |
| Poultry by-product | 10.56±0.92 | 10.56±0.92 | 65.17±0.86 | 13.83±0.88 |
| **Sig** | ** | ** | ** | ** |

Table(3) shows the comparison of averages values ±SD of rendered animal and poultry by-products composition. In animal by-product moisture content as.56±1.25whereas moisture content as 10.56±0.92 in poultry by-product. Fat content as 11.56±1.15 in animal by- product whereas fat content 10.56±0.92 in poultry by-product. Protein content in animal by-product was 59.72±1.99 whereas protein contentas65.17±0.86 in poultry by-product. The ash content as 17.36±0.92 in animal by- product whereas ash content in poultry by-product as 13.83±0.88.

There was significant difference at ($P<0.05$) in the average value± SD of rendered animal and poultry by product composition.

**4.4 Table (4) Nutritional values of rendered animal by-products:**

| Sources | Fat % | Protein% | Ash% |
|---|---|---|---|
| Animal by-products | 11.56 | 59.72 | 17.39 |

Table (4) shows the nutritive values of rendered animal by- products fat content as 11.56, protein content as59.72 and ash content as 17.3

**4.5 Table (5) Nutritional values of rendered poultry by-products:**

| Sources | Fat % | Protein% | Ash% |
|---|---|---|---|
| Poultry by-products | 10.56 | 65.17 | 13.83 |

Table (5) shows that nutritive values of rendered poultry by -products fat content as 10.56. Protein content was 65.17 and ash content was13.83.

**4.6 Table (6) Comparison of nutritive value of rendered animal and poultry by-products:**

| Sources | Fat % | Protein% | Ash% |
|---|---|---|---|
| Animal by- products | 11.56 | 59.72 | 17.39 |
| Poultry by- products | 10.56 | 65.17 | 13.83 |
| **Sig** | ** | ** | ** |

Table (6) shows comparison of nutritive value of rendered animal and poultry by –products .In animal by- products fat content was 11.56whereas fat content in poultry by- product as 10.56.Protein content in animal was 59.72 whereas protein content in poultry by –products was 65.17.ash content in animal by- product was 17.39 whereas ash content in poultry by- product as 13.83.

There was high significant difference at ($P<0.05$) between nutritive value of rendered animal and poultry by –products.

**4.7 Table (7) Total bacterial count in rendered animal by –products from different sources:**

| Sources | Colony count |
|---|---|
| Sources (1) | $5.66\times10^5$C f U/ml |
| Sources (2) | $5.86\times10^5$C F U/ml |

The total bacterial count in animal by-products source (1) was $5.6610^5\times$and sources (2) was $5.86\times10^5$.

**4.8 Table( 8)**

**(8) Total bacterial count in rendered poultry by-products from different sources:**

| Sources | Colony count |
|---|---|
| Sources (1) | $4\times10^5$ CFU/ml |
| Sources(2) | $5\times10^5$ CFU/ml |

The total bacterial count of rendered poultry by –product in source(1) was$4\times10^5$ and sources (2) was $5\times10^5$.

**4.9 Table (9) The Contaminant bacteria in the rendered animal and poultry by –products:**

| Sources | Isolated |
|---|---|
| Animal by-products | Salmonella + *E. coli* |
| poultry by-products | Salmonella + *E. coli* |

Table (9) shows that bacteria contaminants in the rendered animal and poultry by –products were Salmonella SP and E. *Coli sp*

# Chapter 5

## Discussions

In this study the chemical composition of rendered animal by-products revealed that the moisture content was11.56%, fat content was 11.56 %,protein content was59.72%, and ash content was 17.39 %. This result was agreed with N R. A ( 2003) in moisture content( 10%), fat content was( 10%), and ash content was (18%), and protein content (59 %).

. Chemical composition of rendered animal by – products reported by Naga,( 2014) moisture content as 2-7%, protein content as 60-67%, fat content as 8-15%, and ash content as 20-25% which agreed with the result of this study in protein and fat, but dis-agreed in ash and moisture content. Sudanese standard and Metrology organization(SSMO, 2002) specified that the protein content in animal meat meal should not be more than 67%whereas fat and moisture should not be more than 12% and 10%respectively thus the specification of SSMO,( 2002) was agreed with the result of this study.

Chemical composition of rendered poultry by-product in this result revealed that the moisture content was10.56%, fat content was 10.56.%, protein content was 65.17.%, ash content was13.38%,. N.R.A,( 2006) reported that, in rendered poultry by-products protein content was68%, fat content was15%, ash content was 9%, and moisture content was 4%which dis-agreed with this result- Chemical composition of low ash poultry by- products meal reported by Ahmed, (2008) showed crude fat as 15%, protein was 68%, moisture was 4%, ash content was 9%.The result which reported by Ahmed dis- agreed with this result. Chemical composition reported by David and Hamilton (2006) revealed that the crude protein was 60%, fat content was 13%, moisture content was 10% ,ash content was 12%.which agreed with result in moisture and ash content.

the protein content in rendered poultry by-product should not be less than 58%, and fat and moisture should not be less than13%, and 10% respectively, which dis-agreed with the present study in protein content but was in- line with moisture and fat content. Sudanese standard and Metrology organization SSMO,( 2002) specified that

The sampling cultured from the rendering animal and poultry by-products showed that aerobic total count in rendered poultry by-products ranged from $4\times10^5$ to $5\times10^5$ CFU/gm. And the aerobic total count in rendered animal by-products ranged from $5.66\times10^5$to 5.86 $\times10^{5.}$The bacterial load in rendered poultry of the present study ($4\times10^5$ - $5\times10^5$) was in average similar to that reported by Wadi, (2002) who found ($1.6\times10^4$ to $8\times10^5$). Banerijee and Shetty, (1992) found that bacteria count in poultry by-products ranged from ($1.0\times10^5$ - $8.8\times10^5$) which agreed with this study. Veldman *et al* , (1995) reported coliform count in meat meal, fish meal bone meal ranged from 0.0to $5.6\times10^5$. the result obtained by Veldman *et al* (1995) agreed with this result.

The two species of bacteria isolated in rendered animal and poultry by products were *Salmonella* SP and *E. coli SP* .this result was agreed with Newton *et al*., (1978) who reported that *Salmonella* and *E.Coli* were the most common bacterial isolated in rendered animal and poultry by-products.

*Salmonella SP*, also were detected from animal by-products by Veldman *et al*, (*1995*) and Watkins *et al*., (1959).similar result was obtained by Laramore and Moritz, (1969) they were found *Salmonella and Escherichia* coli were the most commonly isolated organisms from meat meal in rendered animal and poultry by- product which agreed with this result., S0um *et al*( 1999) Banerijee and Shetty,( 1992) isolated *E. Coli* in packed samples in poultry by- products. Which agreed with this study. They suggested that Samples

might be contacted with contaminant slaughter house equipment or chicks infected with .*E.Coli* or due to contamination during processing.

## Conclusion and Recommendation

**Conclusion**:

This study concluded that the rendered animal and poultry by-products were high nutritive value and safe for using in animal and poultry feed.

**Recommendation:**

1-Modernization of slaughter house and construction of by-products processing units in each slaughterhouse

2Development of appropriate technology for collection of slaughters by-products.

- Proper heat treatment of cooking by –products more than 4 hrs. Attention should be given to separation of raw material from processed products and there should be separate areas for storages of the former and areas where the latter are milled packed and stored.
- Proper hygiene during handling and storage of end products at low moisture to minimize the risk of contamination.
- Standard of bacterial load must be specified for slaughter by-products meal.
- More work is needed to find efficient method for recovery of contamination potential pathogens from slaughters by-products and to determine the pathogen city of each one and is economic important

## Reference


Ahmed, M. M., Sotohy, A. S., Ismail, A. A. and Ismail, M. A. (1995). Bacteriological evaluation of poultry feeds and feed ingredients in Assiut government. *Assiut Vet. Med.* 34 67-43.56

Ahmed, S. M. E (2008). Processing of protein concentrate from some poultry waste. Vol (5) No.121-127.

Australian Renders Association (2013). Section 7, Animal proteins standard.

Bains, B. and Mackenzie, M. (1974). Transmission of *Salmonella* through an integrated poultry organization. *Poultry Sci.* 53, 1114-1118.

Banerjee , A. and Shetty H.S (1992) microbial load in poultry feed and detection of afla toxin Letters APPL Microbial 14-89-91.

Bensink, J. C. and Boland, P. H. (979). Possible pathways of contamination of meat and bone meal with *Salmonella*. *Aust. Vet. J.*, 55, 521-524.

Calnek, B. W., Barnes, H. J., Beard, C. W., Mc Dougaid, E/ R and Sait, Y. M. (2000). *Diseases of Poultry.* 10th Ed. American Association of Avian Pathologists Ames lawa U.S.A. P. 82-83.

Carlson, V. L. and Snoeyenbos, G. S. (1970). Effect of moisture on *Salmonella* populations in animal feeds. *Poultry Sci.*, 49,717-725

Chen,T.C.(1992) poultry meat microbiology in Encyclopedia of food science and technology pp-2140-2145.

Cruickshank,(1975). Medical Microbiology. The practice of Medical Microbiology12 ED.churchill Livings stone Edinburgh vol.

Crump, J. A., Griffin, P. M. and Angulo, F. J. (2002). Bacterial contamination of animal feed and its relationship to human. foodborne illness. *Clinical infectious Diseases*. 35, 859-865.

David .L Meeker, and, C.R., Hamilton (2006). An overview of rendering industry in essential rendering: All about the animal by-products. National Render Association.

El toum, G. A. E. (2000). Aerobic bacteria in lymph nodes of goats slaughtered at Omdurman Abattoir. M.Sc. Thesis. University of Khartoum.

Franco, M. A. (2002). Animal by-products order (Northern Ireland 2002). Statutory Rules of Northern Ireland 2002 No. 209.

Gomez .K.A .and Gomes .A.(1948)-statistical procedures for Agriculture research 2nd education John Wiley and Sons New York.

Grace, J. F. (1986). *Meat Hygiene*. 8th Ed., Bailliere, Tindall, England. P.97-108.

Hamilton, C. R.(2004). Real and Perceived Issues Involving proteins. In Protein Sources for the Animal Feed Industry. Expert Consultation and Workshop. Bangk

J.webster and Award meat science. (1982). Hand book of Rural Technology for the Processing of animal by-products food and agriculture organization. FAO United Nation, Rome.

Jay, J. M. (2000). Modern Food Microbiology. 4th Ed. Van Nostrand, Reinhold New York.

JinnChyi, W.ShuHui.H.Chin Yang, L.JC, W.SH. H. and CY.L(2003). Lethal effect of microwaves on spores of Bacillus spp J of food protection .4-496 609.

Judge, M. D., Aberle, E. D., Forrest, J. C., Hedrick, H. B. and Merkel R. A. (1989). Principles of Meat Science. 2nd Ed. Kendall Hunt, U.S.A. P. 355-343.

Kumar, M. (1989). Handbook of Rural Technology For The Processing of animal By-products. Food and Agriculture Organization F.A.O. United Nations, Rome.

Larainore, C. R., and Moritz, C. W. (1969). Fluorescent-Antibody technique in detection of *Salmonella* in animal feed and feed ingredients. *Appl. M7icrobiol.*, 3, 352-354.

Loken, K. I., Kathleen, H. C., Solee, R. E. and Pomeroy, B. S. (1968). Microbiological quality of protein feed supplements produced by rendering plants. *Appl. Microbiol*. 7, 1002-1005.

Mackenzie, M. A. and Bains, B. S. (1981). Dissemination of *Salmonella* serotype from raw feed ingredients to chicken carcasses. *Poultry Sci.*, 55, 957-960.

Malav ,op. Birla, Virk, K. S.Sandhu. H. S.MehatN. Kumar and Pand Wagh R. V. (2018). Safe Disposables of slaughter house waste.143-152

Mann, J. (1967). Processing and Utilization of Animal By-products. Food and Agriculture Organization. United Nations, Rom

Mills, G., Earnshaw, R. and Patterson, M. F. (1998). Effects of highly hydrostatic pressure on Clostridium..  26, 227-230.

Mils, A. A. and Misra, S. S. (1938). The estimation of the bacterial power of the blood. *J. of Hyg., camb*. 38, 732.

Molye, A. I. (1966). Salmonella in rendering plant by-products. *J. of Am. Vet. Med. Asso*. 9, 1172-1176.

Moran, L., Row, M. T. and Hagan, J. A. (1990). The effect various heat activation treatment on fast, intermediate and slow germinating spores of *Bacillus spp.Letters in Appl. Microbiol.*, 10,43, 46.

Muzquiz, M. J., Alonso, M. J., Sanchez, A. C., Saachez, F. A., Moracho, J., Martinez, J., Acedo. C. and Franco, A. (1981). Isolation of bacteria from *Fasciola hepatica. RevistaIberica de Parasitologia*, 41, 111-117. Abstract.

Naga V. R.(2014). Faculty of food processing technology. banats University of Agricultural Science and Veterinary Medicine .Egypt

National Renders Association (NRA). (2006). The source of essential high quality products. 2nd edition.

National Renders Association (NRA). (2003). Pocket Information Manual; A buyer's guide to rendered products. Published by the National Renders Association.

Newton K. G and Gill C.O(1980) the effect of ph on growth Gram –negative bacteria from meat and meat waste.49-315-323.

Newton, K. G,Harrison, J.C.I and wauters .aGill, A.M. (1978). Sources of psychotropic bacteria on meat at the abattor.J of APPI Bacteriol 45-75-82.

Okaanovic, D. M. Ristic, Kormanjos. Sfilipovic, B. Zivovic (2008). Biotechnology CRA Characteristics of poultry.

Pupavac, V and Lalic, M. (1990). Effects of bacterial contamination of animal feed on the health of animals. *Krmiva,* 32, 11-12.

Romans J. R.costello W .J ..Carlson .C W. GreaserM. L and Jones K .W .(2001). The meat we eat 14th edn Interstate publishers.Danville.

Sale, A. J. H., Gould, G. W. and Hamilton. W. A. (1970). Inactivation of bacterial spores by hydrostatic pressure. *J. of General Microbiology,* 60, 323-334.

Salminen E. and Rintala J. A. (2002). Anaerobic digestion of organic solid poultry slaughter house waste. A review bio-resource technology, 83: 13-26.

Samah, H., and Ezzat, M. (1986). Some pathogenic microorganism of zoonotic importance isolated from rodents .17-34-159-165.

Samaha, E. G. Z., Moustafa, W., Anwar and Badsway, E. M.(2018). Global veterinarian microbial load of poultry by-products following rendering process. P. 756-759.

Siebrits, F. (2003). Safety and hazards in animal feed to food chain. *International Meat Symposium*. South Africa. January 2003, 103-107.

Silverside, D. and Jones, M. (1992). Small-scale poultry processing. FAO Animal Production and Health paper 98. Rome: Food and Agriculture Organization. United Nations.

Smyser, C. F., Bacharz, A. and Van Roekel. H. (1963). Detection of *Salmonella* from artificially contaminated poultry feed and animal by-products. *Avian Dis.,* 7, 423-434.

Sonnenshein, B., Hahn, G., Bringewatt, W., Bisping, W. and Moch, R. (1977). Microbiological supervision of sterilization procedure in carcass disposal plants. *DeustcheTieraztlicgeWochenschrift.*, 84, 168-170. Abstr.

Soun,, O., Ermel, G., Rose, V. Rose, N., Drouin, P. Salvat, G. and Colin, P. (1999). Identification by a multiplex PCR – based assay of *Salmonella typhimurium*, and *Salmonella enteritidis* strains from environmental swabs of poultry house, *letters in Appl. Microbiol.*, 29, 1-6.

Sudanese Standards and Metrology Organization (2002). Standardization of animal feed stuffs.( May 2002).

Tanios, A. I. (1997). *Salmonella* in animal feedstuffs. *Assiut Vet. Med. J.*, 37 (74), 117-142.

Taylor ,D.M.S.L. wood gate and M.J Atkinson. (1995).Inactivation of the Bovine sponge from Encephalopathy path Agent by Rendering procedures Veterinary Record 137-605-610.

Veldman, A., Vahl, H. A., Borggreve, G. J. and Fuller, D. C. (1995). A survey of the incidence of Salmonella species and enter bacteria in poultry feed and components. *The Vet. Record.*, 136, 169-172.

Wadi, A. A. (2002). *Bacterial load of animal feed concentrate.* Thesis for M.Sc. Degree. University of Khartoum.

Wagner, A. I. B. and Jr. (2000). Cited by El Hussein (2003). Bacterial food poisoning. Texas Agricultural Extension Service. www.@yahoo.com

Wain, S. A., Gazalli, L. L., Sohi Ali. (2014). Animal By-products. Efficient utilization vis-à-vis benefits in: Compendium conference of India. Meat Science Association and National Symposium on Sustainable meat

production for nutritional security and consumer wellbeing: Challenges and strategies, 28-30. November, Duvasu, Mathura, India.

Watkins, J. R., Flowers, A. I. and Grumbles, L. C. (1959). *Salmonella* organisms in animal products used in poultry feeds. *Avian Dis.*, 3, 290-301.

Webb, N. B. and Price, J. F. (1987). *The Science of Meat and Meat Products*. 3rd Ed. Food and Nutrition Press. Inc. Westport. Conn. U.S.A

Wedman, E.E(1961) salmonella and other disease-producing organisms in animal b y-product Survey J of Am med Asso 1172-1176