

Chapter One

Introduction

1.1 Background to the Study

Feathers are a thin layer of skin covering the dermis that forms the characteristic outer coat or body covering of birds. Although feathers cover most of the bodies of birds, they only result from a certain well-defined region of the skin ¹. Also, feathers are an important derivative in the poultry industry as they make up 5-7% of a chicken's body mass ^{2,3}. When feather waste is in the natural state or unprocessed, it can cause a hazard to the well-being of individuals in the surroundings due to the presence of harmful microorganisms and the release of different pollutants such as nitrous oxide, ammonia, and hydrogen sulfide ⁴. As a result of this, investigations are being carried out by research workers on the cost-effective procedure of transforming feathers into a useful commodity ⁵.

Studies have revealed that the keratin component of feathers is useful in the manufacture of different products such as pillow stuffing, papers, upholstery padding, insulation, diapers, plastics, and feather meal. The fiber in feathers has qualities similar to polysaccharides, the starch that forms wood and paper, however, the area is far larger due to the diameter of the fibers which is smaller. As a result, the fiber will absorb additional wetness than wool or cellulose fibers. The crystal structure of feather fibers additionally makes them naturally stable and durable. Over sixteen million diapers, made of pulp, are discarded every year. A year's value of feathers might replace around 25% of the wood pulp used annually for diapers. Recently companies are working on the production of the absorbent feather-based product together with diapers, filters, insulation, upholstery padding, paper, and even outfits ⁶.

In the domestic animal industry, the production of animals that have various uses such as meat, fibers, and hides is increasing therefore it is essential to feed the livestock with

adequate healthy nutrition for good health and growth promotion in a cost-effective way ⁷. However, feeds available do not contain the adequate amino acids needed the search for adequate quality nutritious feed, has led to the discovery of the high protein content present in feathers ⁸. The protein found in feathers is known as Keratin which is commonly present in nails of mammals, wools hair, and feathers of birds ⁹. The total weight of a bird is made up of 7% of keratin protein which is about 90% of the total composition, however, only 5% can be utilized and absorbed into the body of a bird and this has led to findings on how feathers can be degraded to make it absorbable¹⁰. Recently, feathers are transformed to ingestible protein by the enzyme known as keratinase and this is a preferred conversion method as compared to the conventional use of a mechanical(physical) or synthesized (chemical) process which consequently leads to loss of important nutrients such as methionine, lysine, and tryptophan ¹¹. The capacity of degradation of feathers by keratinase producing microbes and keratinases is a depiction of the prospects that recycling using biological procedures will sustain the livestock sector of the agricultural production ¹². These microorganisms include bacteria, actinomycetes and fungi. Several microorganisms isolated from various environments which have rich deposits of keratin have been utilized for the degradation of keratin –contaminated wastes from different resources¹³. Bacillus strains are the most predominant keratinase producing bacteria. The species generally known to produce keratinase are *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, and *Bacillus cereus* ^{14, 15}. The production of keratinase enzyme can be done using solidified milk agar, peptone, yeast extract, and sterile non-fatty milk as substrate by several fungal species. Fungal species like *Chrysosporium indicum*, *Microsporum gypseum*, *Acremonium strictum*, *Acremonium sp.*, *Malbranchea sp.*, *Chrysosporium tropisms*, and *Aspergillus candidus* have been isolated using feather as substrate in skimmed milk agar at 28 ° c for 12 days. Clear

zones on the agar plates were used to indicate keratinase activity with the fungal species showing different keratinase producing activities ¹⁶.

1.2 Statement of the Problem

One of the most commonly produced sources of protein for human consumption is poultry birds. In recent times it is scarce and costly to provide enough protein supplements into these birds' feeds for proper growth and development. Feathers have been found to contain high protein content but there has been concern about its availability in form of utilizable protein for feed production because keratins proteins present in feathers are highly inaccessible if the structural orientation contributed by different chemical groups is not significantly dismembered and therefore indigestible by animals. To prepare feather meal using the normal conventional method of feather degradation (heat and chemical treatment) usually result in loss of certain heat labile amino acids.

1.3 Justification of the Study

The biotechnological process of degrading feathers poses some advantages over the physicochemical method because of their low energy consumption and also the ability of retaining the essential amino acids. The feathers from chicken have the potential of being used as raw materials for, highly economical protein rich feed due to its low cost (because it is a waste), large availability and chemical composition. There is need to put it into more use as a protein supplement or possibly using the feather meal as a sole protein source to be added to animal feed or ration.

1.4 Aim and Objectives of the Study

The aim of this work is to evaluate the potential of a microbially hydrolyzed feather as an effective alternative source of animal protein for growing broilers.

The Specific Objectives are to:

- i) isolate and select thermophilic keratinolytic bacteria from feather dumps located in Ibadan.
- ii) produce keratinase and determine the optimum production condition for this keratinase by the selected isolate.
- iii) apply partially purified keratinase on laboratory scale sized feather to achieve feather meal.
- iv) scale up objective (iii) and integrate the recovered feather meal into poultry broiler ration at graded concentration and give as feed to broilers.
- v) evaluation of the effect of '(iv)' above on the bird.

1.5 Research Questions

1. Can Thermophilic bacteria that are also keratinolytic be isolated from degrading feather dumps?
2. Can these bacteria be used to produce considerable keratinase and what are the best condition for the keratinase to be produced?
3. Will this keratinase be able to make the protein in feather more available for broilers when integrated into their ration?
4. What is the impact of the feather meal on the growth of broilers when fed with it at different concentration as compared to the usual commercial feeds?

1.6 Significance of the Study

This study will be of benefit to poultry farmers because using feather waste processed biotechnologically will be a cheaper source of protein and a source of dietary supplements in animal feedstuff. It will also serve as a method of recycling feather waste.

1.7 Scope of the Study

Isolate, screen and select keratinolytic and proteolytic bacteria that are also thermophilic in nature.

Identification of selected bacteria, produce keratinase at optimum production conditions using the bacteria.

Use the keratinase to hydrolyze ground feather and use it at graded portion as protein source in broilers' ration.

1.8 Limitation of study

The feather dump sites in the poultry industries were not accessible therefore samples were obtained from local poultry processing dump sites.

There was no time to carry out proximate analysis on the chicken to evaluate the protein content of chicken fed with the experimental and control rations.

1.9 Operational Definition of Terms

Hydrolysis: A chemical process of decomposition involving the splitting of a bond and addition of Hydrogen cations and the Hydroxide anion of water

Isolate: To separate something such as chemical from another substance

Keratinolytic: It is the ability of a microorganism to possess an enzyme that may be used to enhance the digestibility of feather keratin.

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Endnotes

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Chapter Two

Literature Review

2.1 Structure and Formation of Feathers

A feather is made up of single filaments or entails many fiber or projections, that can be connected by a rachis, forming branched structures. The fiber or projections is extracted from the cavity of a calamus, which get increased in size from ring shaped epidermal walls around the dermal papilla, both being inserted in a follicle¹. Modern feathers are the most complex outgrowth of vertebrate skin, and plumage has evolved significantly throughout geological time, with certain arrangements remaining apparent while others have disappeared. However, most mammals have a single filamentous hair that grows from the skin cone on top of a subcutaneous papilla, both of which are lodged in a follicle. Despite the geological time, the creation of the hair shaft, which is predominantly made of cysteine-rich keratins with amorphous keratin-associated proteins, has not changed significantly from its origin².

The dermis and epidermis work together to generate a conjunctive papilla, which is the first placode in the feather creation process. Later, this splits from the epidermal layer to produce sheaths that contain down radii. By separating the sheath that is already covering the developing down, the final stage of feather development is completed. The core is located in the lower strata, and entire placode is taken up by the follicle³.

The plumage has three distinct sections when it is fully matured, including the rachis, the feather's primary shaft is connected to the barbs as the secondary arrangement. Outer projections which are connected to the barbs, make up the third formation of feathers⁴.

Calamus and rachis are very light structured component that provides the necessary toughness to keep the axis rigid, form the axis which forms a (medulla) with a cross-section that is nearly rectangular in shape and taper toward the feather's tip fills the rachis. In contrast, the rachis is made up of layers of fiber that are oriented in opposition to one another. The medulla core is comprised entirely of keratin, has two levels of porosity, and has reduced feather weight since it takes up a larger portion of the rachis volume. Aside from the rachis, it is largely made up of dead material like protein, colour, and other elements that are produced during the formation of a feather. The calamus, also known as the quill, is the lower portion of the rachis that is wider and hollower, and it is often nude in color. This is where the feather enters the skin. The inferior umbilicus, located in the bottom portion of the calamus, is an opening through which the feather receives nutrition while it is developing ⁵.

A second opening known as the more advanced umbilicus by which the laminar body of the plumage emerged as it began to expand, is located immediately where the calamus finishes and on the surface level where the rachis starts to flatten out. The physical body and the biggest area of the feather, created by a complex network of interwoven hooks is called barbicels which connects barbules to one another, joining nearest barbs, and producing thickness that is able to carry a heavy load per area unit. For the birds to fly, the vane grows in the lateral edges of the rachis and gives rise to a lamina-like structure that is separated into two opposed halves ⁴.

The feather's rachis, which may measure 7 inches in length, runs the length of the entire feather. Depending on how far from the rachis they are, barbs can be anywhere between 1 and 4.5 cm long. The rachis base barbs are longer than its tip barbs. The barbules, which are

tertiary structures, feature hook characteristics in the tips and measure between 0.3 and 0.5 mm in the length ⁶.

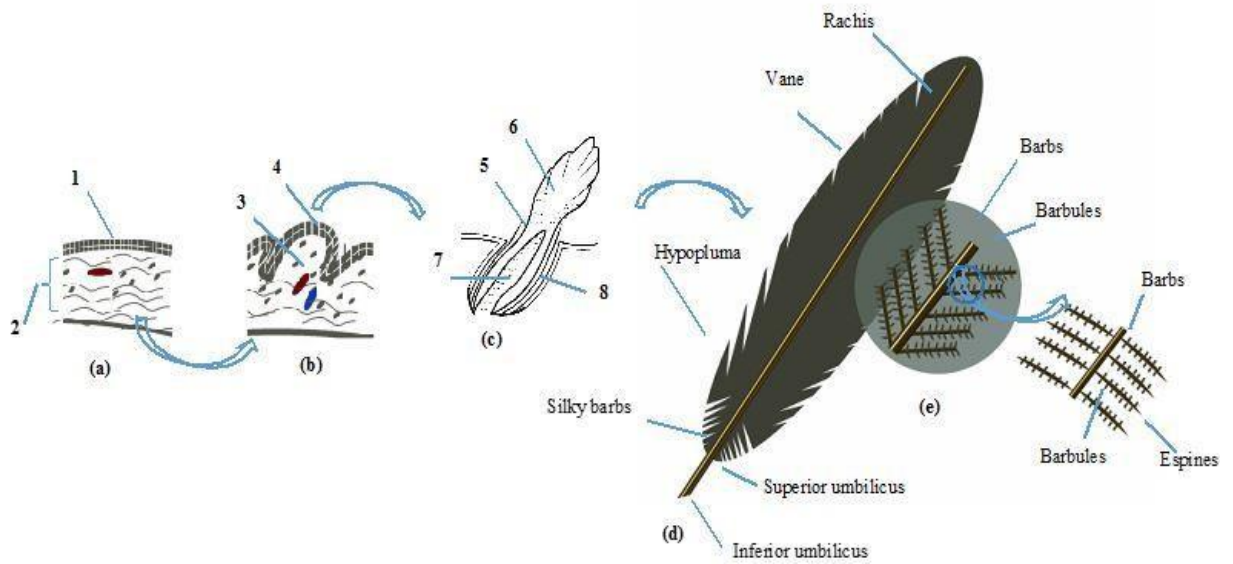


Figure 2.1: Parts of A Typical Feather

Source: Ref ⁴

2.1.2 Types of Feather and Their Function

The primary feather structural kinds and the location on the bird's body are divided into seven categories.



Figure:2.2: Different Types of Feathers

Source: Ref⁴

2.1.3 Types of Feather

Wing feather

The vanes, or consistent windproof surfaces, on either side of the central shaft of the wing feathers that are designed specifically for flight are produced by an interconnecting microstructure. These asymmetrical feathers, also known as remiges, have a shorter, less flexible leading edge that prevents twisting in flight ⁷.

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Figure 2.3: Parts of a Typical Winged Feather

Source: Ref⁴

Tail Feather

The majority of tail feathers, have an interlocking microstructure like wing feathers. These feathers support precise fly control when arranged in a fan form. Birds generally have six pairs of tail feathers, with the outer pairs showing progressively more asymmetry. Some birds' tail feathers have developed into ostentatious decorations that are ineffective for flight ⁷.

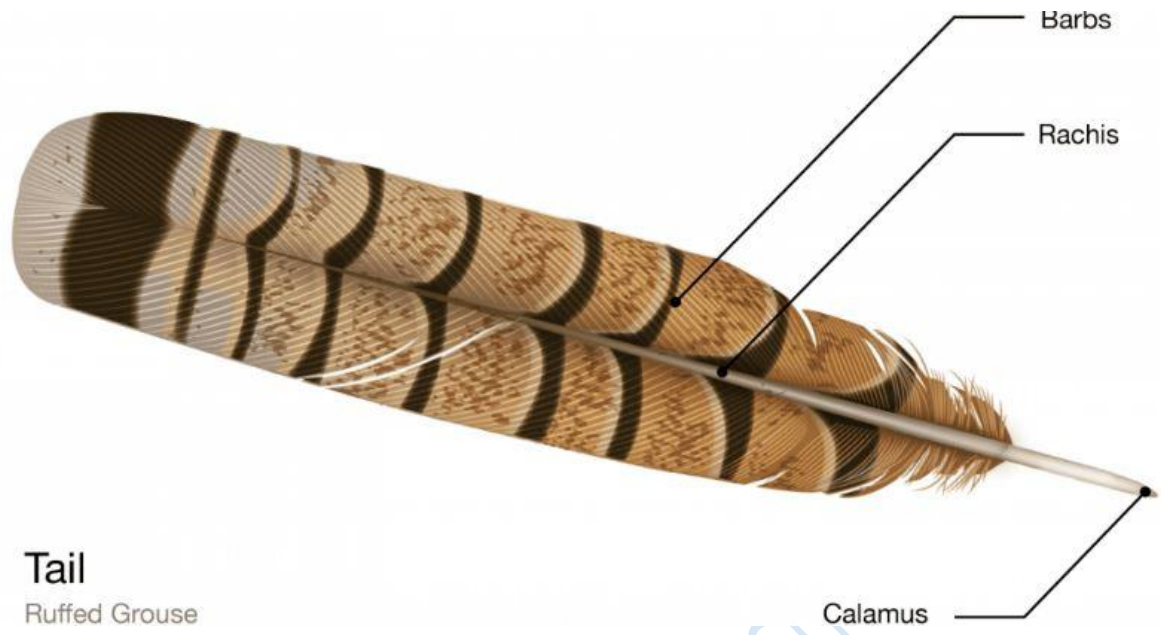


Figure 2.4: Parts of a Typical Tailed Feather

Source: Ref⁴

Contour Feathers

The bird's skin is covered in contour feathers, which give it a more streamlined appearance. The fluffy bases are nestled tight to the body and the waterproof points are arranged in an overlapping pattern like shingles. Contour feathers can also assist the bird in displaying or remaining concealed. Feathers can occasionally be vividly colorful or uniformly drab. The wing's covert feathers, which smooth over the area where the flight feathers attach to the bone, mold it into an effective wing⁷.



Contour

Helmeted Guineafowl

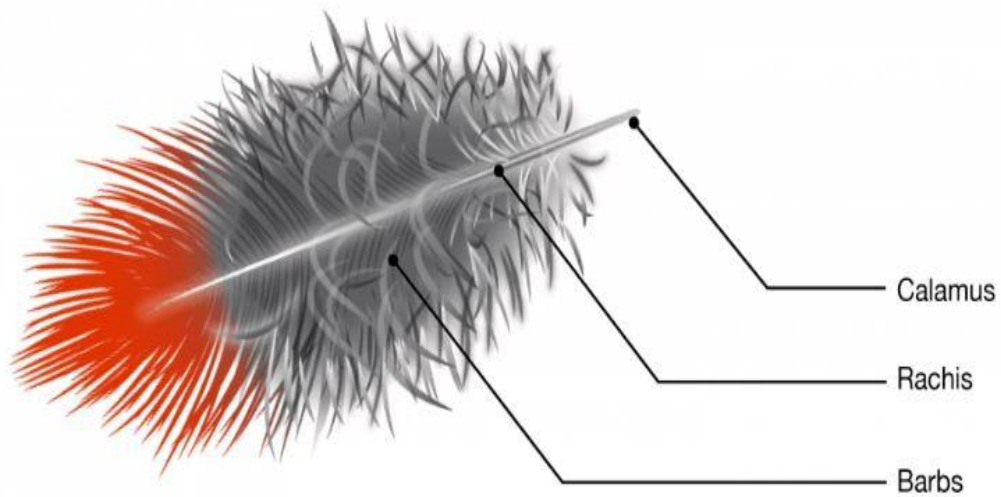
Figure 2.5: Parts of a Typical Contour Feather

Source: Ref⁴

Semi Plume Feather

Semi plumes have a developed central rachis but no barbule hooks, resulting in a fluffy insulating structure that is mostly concealed behind other body feathers ⁷.

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Semiplume

Northern Cardinal

Figure 2.6: Parts of a Semi Plume Feather

Source: Ref⁴

Down Feathers

Down feathers are comparably short and located closest to the body, where body heat is stored, like semi plumes, but with an even looser branching structure and little to no central rachis ⁷.

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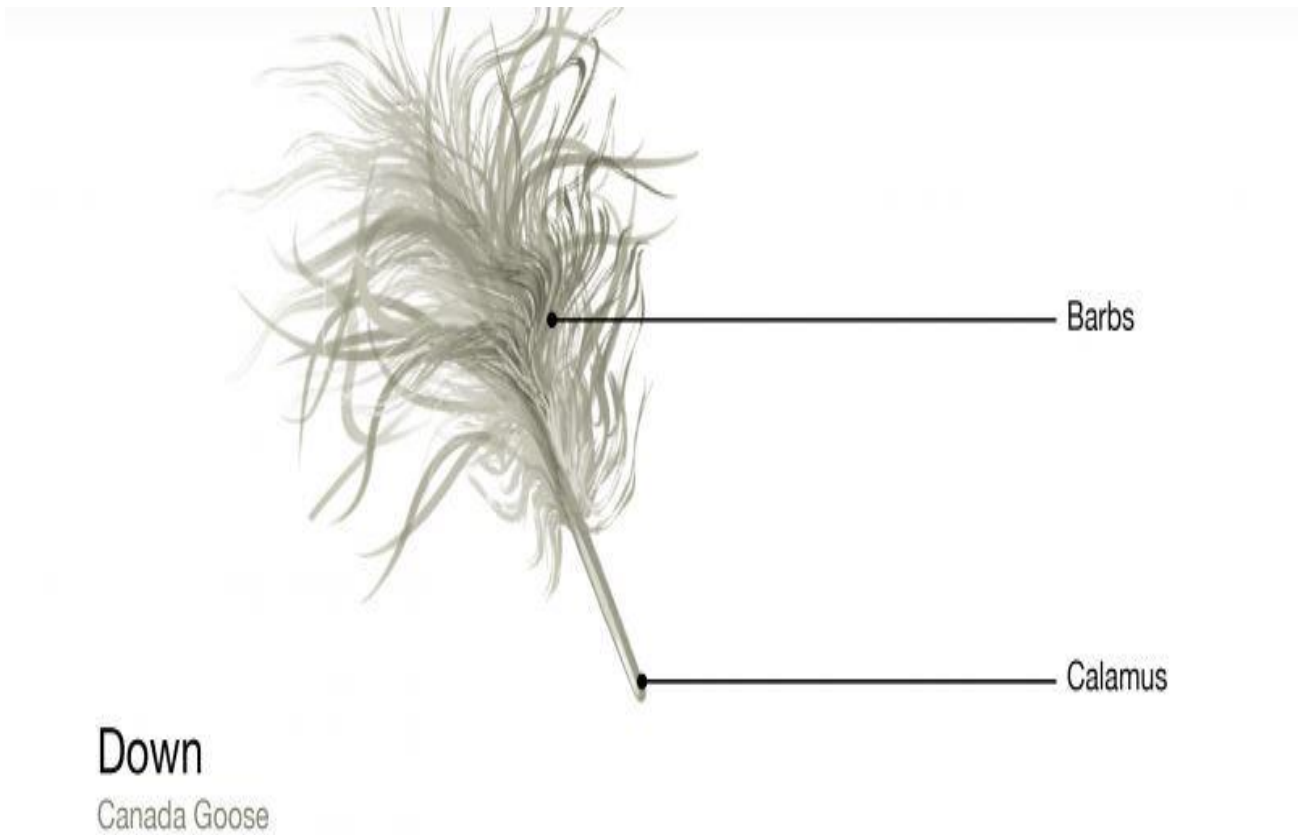


Figure 2.7: Parts of a Down Feather

Source: Ref⁴

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Filo plume Feather

Like human eyelashes, filo plumes are short, basic feathers with few barbs that perceive the location of the contour feathers ⁷.

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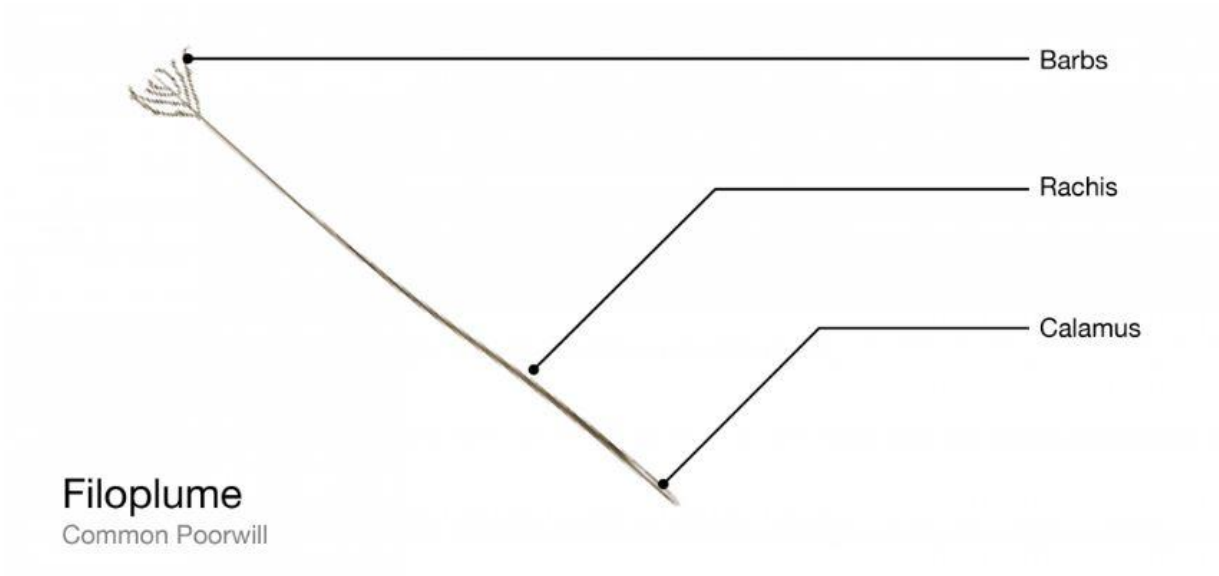


Figure 2.8: Parts of a Filoplume Feather

Source: Ref⁴

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Bristle Feather

The simplest feathers are bristles, which have stiff rachis and typically no barb branches.

Bristles, which are most frequently seen on the head, may shield the bird's eyes and face ⁷.

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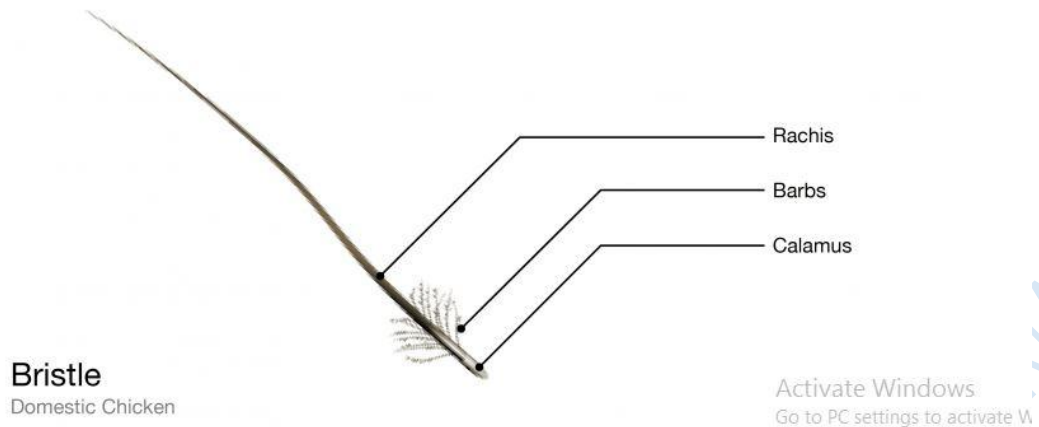


Figure 2.9: Parts of a Bristle Feather

Source: Ref⁴

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2.1.4 Functions of feathers

The exterior layer of every vertebrate animal, known as the outer layer or epidermal development, is made up of feathers. Flight, heat insulation, and water repellency are all benefits derived from feathers. Additionally, the color of the feather aids in communication and protection ⁸.

Feathers offer numerous possibilities to examine this occurrence since the development of function in physical features typically involves the addition of a unique function to an already-existing trait. For instance, flight feathers that produce lift can lengthen and stiffen to help with abrupt airborne maneuvers, brace over vertical surfaces, or even grow very extended for reproductive expression. Bright colors for mating signals or secretive hues to assist a bird fit in with its surroundings can be included in body feathers that serve as heat regulators or water resistance. It can be challenging to pinpoint which function may have developed earlier in some situations where numerous distinct functions are so crucial to a single feather. Body feathers that keep a bird warm and dry, for instance, might communicate a bird's attractiveness to prospective mates or assist a bird hide from predators and prey by increasing its concealment. Although some research implies visual signaling, it may never be known which purposes may have triggered the formation of body plumage in primitive flying reptiles⁹.

Protection from Solar Radiation

Birds typically have black skin in locations with strong ultraviolet radiation when feathers are absent or insufficient to protect from sun radiation ¹⁰.

Communication (Mate Choice)

Whenever a feather features a genuine indicator of an individual's quality, such as a colouring mechanism that must represent a high-quality diet or metabolic requirements, such as carotenoid pigment, this is one instance in which sexual selection can modify feathers ¹¹.

These feathers can be used in complicated activities, and in birds of paradise, the visual decoration of the feathers is positively connected with the complexity of the behavioral presentation ¹². Brief Bustards' pink coloring, which results from the fluorescence of porphyrin pigments, has been theorized to mark a male's first mating display in a given season in one extreme case where it can disappear in as little as 10-15 minutes ¹³.

Social Selection

A process known as social selection occurs when individuals within a species fight for supplies (such territories or access to mates)¹⁴. More than 25% of hummingbird species have females with colors similar to males, according to Diamant et al. (2021), and female-limited diversification is linked to ecology, migratory status, and social dominance ¹⁵. When examining relationships between feather ornaments and interpersonal relationships, future studies should take into account both sexes. However, the identification of young parasites does not seem to have a role. More research is required to understand why developing birds originally adopt ornate colors ¹⁶.

Sound Generation

The majority of birds' flight feathers appear to be able to make some kind of sound through aeroelastic flap, therefore the ability of multiple species to produce sound with feathers may be owing to a trait inherent to feathers that has concurrently developed in different genera ¹⁷.

Hummingbird feather bird chirps during mating presentation appear to be produced by aeroelastic flutter ¹⁸.

Cosmetic Coloration

Since pigmentation, nanostructure, and microstructure cannot be changed, feather colors are typically established after they have grown. Nevertheless, certain species have developed a number of ways to change the color of their feathers before molting. Pulviplumes, often known as powder down, are one method. Many different bird groups have unique down plumes that the bill may quickly crush and transfer over other feathers. The pigments in these powders may be transferred externally by the bird to other feathers, changing the apparent color of the plumage. Although pulviplume powder is often white, it can also include carotenoids, which give it colors like yellow, orange, or red ¹⁹.

Thermoregulation

As opposed to mammals, animals with feathers (birds) typically employ a specialized technique to regulate their body temperature ²⁰. This is because they rely on their plumage or enhanced breathing, which raises their metabolic rates. Feathers serve as insulators in addition to their crucial function in flight, which may be the reason why birds have the highest body temperatures of all vertebrate animals. They can attain thermoneutrality in cold weather thanks to this process, but in hot weather, they are more likely to experience heat stress, which necessitates the deployment of additional systems for preserving homeostasis. Bird species and differences in their plumage in various body parts are two additional characteristics that affect their ability to regulate their environment ²¹.

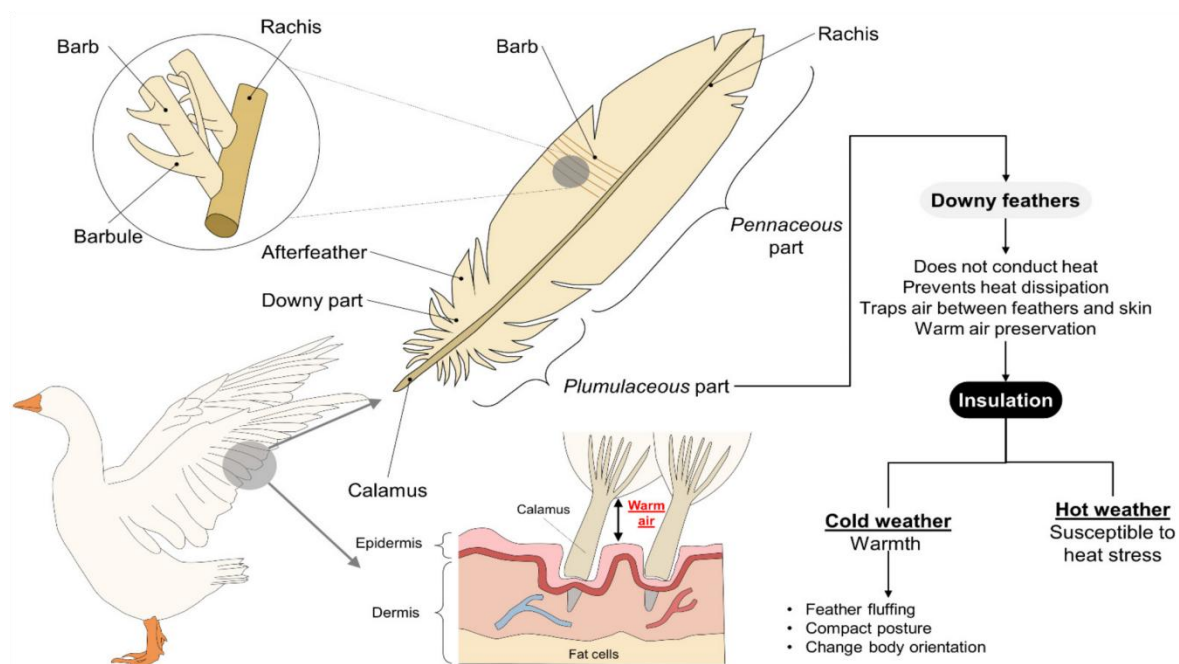


Figure 2.10: The structure of feathers and birds' use of thermoregulation

Source: Ref²⁰

The primary structures that regulate thermobalance are found in plumulaceous and downy feathers. Birds engage in activities like fluffing their feathers to trap air between them and their skin when exposed to chilly weather. Their body temperature is raised because heat dissipation is avoided and warm air is preserved. Nevertheless, the insulating effect of the feathers makes birds more heat vulnerable in hot weather since they hinder structured thermoregulation²⁰.

2.2 Feathers as an Environmental Pollutant

About 3.1 million tons of feather waste were produced by the chicken sector in Europe in 2014 and more than 28,000 tons of feather waste are produced annually in South Africa²². Australia produces between 86,000 and 111,000 tons of feather waste annually from chicken farms. According to reports, an average farm in eastern Europe produces 7 tons of chicken

feathers every day, and the country as a whole generates 77,000 tons of feather waste annually.

The improper discarding of feather waste in the environment has been linked to the unfavorable environmental impacts. Feather trash is typically disposed of by burning, burying in landfills, and dumping in major sewage lines²².

This bio-improper waste's disposal results in a variety of health issues, including foul odors, flies, rodents near poultry farms, landscape deterioration, environmental harm, and the spread of illnesses. The buildup of feathers and their gradual decomposition create sulphurous chemicals that smell extremely bad and serve as a breeding ground for germs that cause sickness. Since the majority of the microorganisms enter domestic homes, rats and birds spread illnesses that they bring to humans²³.

The implications of disposing of feathers in rivers and main sewer lines have also had a harmful impact on the ecosystem. The sewage system as well as the rivers become clogged as a result of dumping in main sewage lines and rivers. This causes the sewage waste pipes to rupture, and the stagnant river water encourages the growth of disease causing microorganisms and mosquito breeding grounds that spread illnesses like malaria. One technique used to get rid of feathers is burning, however this has disadvantages because it pollutes the air. When burned, disulphide bonds found in feathers release carbon monoxide and sulfur dioxide. The generated Sulphur dioxide is subsequently transformed into acid rain, which damages roofs and alters the PH of the soil and water²².

To control feather pollution in the environment, several initiatives have been undertaken. Incorporating feathers in various ways, both conventional and contemporary techniques have been utilized to reduce feather pollution. Feathers have historically been utilized as

embellishments for hats and costumes worn for traditional dances in many African cultures. For instance, in India, feathers have been utilized as a remedy to cure coughs, infertility, and snake bites ²⁴.

Feathers were used to measure the temperature of boiling oil or as droppers for ear drops. When the feather's tip burns, the oil is hot. Similar to how they were used as a writing tool in communication and education. In order to write distinct messages, educated members of the society would dip the tip of a feather in ink. African towns like Nairobi are faced with the enormous quantity of feathers that still wind up in the environment, despite the fact that the ancient methods only used extremely little amounts of them. Feathers are presently used to make paper, paper products, diapers, upholstery padding, and stuffing for pillows ²⁴.

Feathers may also be reprocessed manually into a feathery meal by being cut into little pieces and given to animals. The feathery meal is difficult to digest in this state, making attempts to use feather as animal feed unsuccessful. The digestive tract of an animal absorbs protein in the form of amino acids, not in the raw keratin found in feathers that has not been broken down ²⁵.

Animals lack the enzyme keratinase, which dissolves the keratin framework found in feathers. The recycling of feathers from slaughterhouses using enzyme hydrolysis is a great way to lessen environmental pollution and add value to feathers. Because of the existence of a highly cross-linked protein matrix that is protected by hydrogen bonds and hydrophobic interactions, Keratin, which is present in feathers, is difficult to degrade. Additionally, resistance to decay by an endopeptidase like papain and trypsin is provided by the cross-linking of the polypeptide chains brought on by the existence of disulfide bonds ²⁵.

2.3 Physicochemical Composition of the Feather

Keratin makes up the majority of feathers. It is a protein with a modest molecular weight (10 kDa) and a semi-crystalline shape ²⁶. Feathers also contain a lot of amino acids, including leucines and serines ²⁷.

The availability of keratin in the surroundings has also been discovered, and it is the primary ingredient in the hair, nails, wool, and horns of mammals, reptiles, and birds. After cellulose and chitin, it is the third most prevalent and biggest polymer type. The non-toxic, biocompatible, and non-degradable qualities of keratin are particularly exceptional. Keratin may be structurally altered and produced into a variety of forms, including gels, films, and nano- and microparticles. The many intra- and intermolecular disulfide crosslinks are what give rise to this stability. The high percentage of cysteine residues in the polypeptide, which is connected by disulfide bonds substantially affects the degree of strength and toughness ²⁸. In light of this, keratin may be exploited as a source of the most modern and sustainable raw materials to satisfy human requirements ²⁹.

2.3.1 Keratin

In several hard tissues, keratin is a crucial structural protein that forms barriers between the organs and their surroundings in important to secure them. Keratin has demonstrated resistance to destruction by certain proteases, such as trypsin and pepsin, due to its structure, which is maintained by disulfide bonds and hydrogen bonds. One of the most common proteins in nature, keratin may be located in a variety of organs including the feathers, hair, wool, and nails of animals ³⁰. It is also among the most prevalent renewable organic polymers in nature after celluloses, lignins, hemicelluloses, pectins, and chitins ³¹.

Feathers from the bird business because of presence of keratin are also valuable source of carbon, sulfur, and nitrogen that may be used to create other goods ³².

According to research, keratin has been discovered in avian and reptile tissues as well as mammals. Mammals are the major source of -keratin. Through disulfide connections created by cysteine residues, hydrogen bonds, and hydrophobic interactions, the polypeptide chains are tightly packed into the final structure ³³. Based on the amount of cysteine, keratin may also be divided into two categories: hard keratin and soft keratin ³⁴. Hence, using reasonably quick and affordable extraction-decomposition techniques is essential for the widespread usage of keratin. Currently available extraction techniques include physical, chemical, physical-chemical, and biological techniques ³⁵.

Although the manufacture of keratin hydrolysates by chemical or physical means frequently requires the use of high temperatures, this leads in the breakdown of heat-sensitive amino acids, such as methionine, lysine, and tryptophan, which decreases the nutrient benefit of the hydrolysates. Utilizing keratinolytic microorganisms or, probably better, specialized microbial keratinases that can accelerate the microbial degradation of keratin is a potential strategy ³⁶.

2.4 Microbial Keratinase

Through cleavage with the peptide bonds, keratinases are a subclass of proteases that can break down keratin ³⁷. Several keratinase enzymes have various molecular mass, ideal hydrogen ions levels, together with appropriate temperatures are created by microbes, according to studies. When keratin or substrates containing keratin are present, the majority of microbial keratinases are released into the matrix outside the cell ³⁸.

The keratinase-producing microbes may easily be secluded from the surroundings where they are commonly found. Bacteria including *Bacillus licheniformis*, *B. subtilis*, and *Stenotrophomonas maltophilia* produce keratinases. *Streptomyces albidoflavus* and

Streptomyces fradiae, two actinobacteria, also produce keratinases. *Trichophyton rubrum* and *Microsporum canis* are the two primary sources of fungal keratinases ³⁹.

2.4.1 Degradation of Keratin by Keratinases

One of the main causes of keratin's resistance to enzymatic breakdown is the existence of numerous disulfide bonds in the protein. The disulfide links must first be disrupted (reductively cleaved) for the majority of keratinases to catalyze keratin breakdown ⁴⁰. This indicates that sulfitolysis (S-S bond breakdown) and proteolysis may be two phases in the procedure for keratinolytic ⁴¹.

The first breakdown of disulfide bonds is required to weaken the keratin structure and make the amino acid chains accessible for keratinase assault in order to extract amino acids from keratin-rich materials utilizing keratinolytic enzymes. Enzyme hydrolysis also benefit from increased susceptible enzyme attack region ⁴².

The keratinolytic endoproteases major function is to carry out the keratinolytic substrate degeneration after the disulfide bonds in keratin have been disrupted. As oligopeptides break down oligo-peptides to liberate individual amino acids or shorter peptides, keratinolytic exoproteases cleave peptide chains at both ends. A mixture of enzymes, as well as the presence of a reducing agent or a reducing enzyme to aid cleave the S- S bonds, appear to be necessary for the effective and complete breakdown of keratin to free amino acids ³⁹. A further indication of synergy between keratinolytic and other enzymes is the rapid breakdown rates of resistant keratinous material attained by bacterial mixed culture ⁴³.

2.5 Feather Meal

The feathers acquired after slaughtering a bird are processed to create feather meal which can be used as fertilizer or protein supplement for livestock. Broiler, turkey, in addition with other poultry processing plants produce feathers as a byproduct. The weight of the living

bird's feathers, which ranges from 3 to 7 percent, which results in meaningful amount of protein ^{44,45}.

Protein is the main component of feather meal. Typically, it has a protein content of around 85%, along with some fat (9%), and minerals (6 percent). Its protein composition is significantly lacking in lysine compared to many other animal-based products. It has a high cysteine content (4.6 percent protein) but low levels of methionine, histidine, and, to a lesser extent, tryptophan (0.6 percent protein). To be sure that it has been processed appropriately, feather meal has to undergo a pepsin digestibility test. More protein sources should be utilized in diet formulation to compensate for the inadequate amino acid profile of feather meal ⁴⁶.

2.5.1 Methods of Processing Feather Meal

The feather keratin can be hydrolyzed by three different methods which are: Mechanical, Chemical and Biological treatment.

Physical Method

The two main categories of physical treatment are mechanical treatment and thermal treatment. The chicken feathers are milled and ground during mechanical treatment to reduce the size of the particle substrate. Feather hydrolysis is made easier during the subsequent treatment by milling and grinding the feathers. Nevertheless, this technology is not frequently used for carrying out comprehensive processes because of its reputation for consuming massive amounts of energy and being costly ⁴⁷.

To degrade the biomass of feathers, ultrasounds are used as another mechanical approach. The frequency, duration, and characteristics of the substrate all have an impact on how

effective this approach is. Conversely, there are techniques that can be employed to disrupt the arrangement of the amino acid component found in the feathers and this include, boiling and pressure cooking ⁴⁸.

These techniques nevertheless need a lot of energy. For the disruption, other therapies are also used, such as microwave irradiation. The breakdown of cell structure is made possible through this method because the heat generated permeates into the feathers ⁴⁸.

Chemical Treatment

Acids or bases are utilized in chemical treatment to break the disulfide bonds and release the disintegrated keratins from the birds feathers ⁴⁹. In a research, it was shown that employing KOH (0.6 percent) for 24 hours at 70°C, chicken feather hydrolysis could be increased to more than 85%, producing about 326 mg/mL of free amino acids. The hydrolysis nevertheless with acid under comparable conditions is ineffective ⁵⁰.

Investigation from another research, employing chemical hydrolysis over 24 hours at room temperature results in feather degeneration of around 78% accomplished. For hydrolysis to break the disulfide bonds, oxidizing chemicals such as permanganate, bromine, hydrogen oxide, and reducing agents like sodium sulfide, thioglycolate, and potassium cyanide have also been found utilized ⁵¹.

At pH 10–13 for six hours at 30°C, feather hydrolysis with reducing agents was used to produce proteins that is capable of being dissolved. To generate the liquid product, feathers were treated with calcium hydroxide (abundant with amino acids and polypeptides). When

this solution was carbonated, 54 percent of calcium was extracted that comprises of a comparable nutrient such as found in soybean meal and may be used as animal feed. Common techniques for getting rid of feathers include chemical hydrolysis and incineration. These processes consume a lot of energy, which lowers the protein and crucial amino acid quality (lysine, methionine, and tryptophan). Lysinoalanine and lanthionine are two unhealthy amino acids that are produced during the process⁴⁹.

Biological Treatment

Alternative therapies are being investigated as a result of several drawbacks related to mechanical and use of chemicals, and these limitations include harsh chemicals, higher energy consumption, and extra costs. For the degeneration of feathers, biological therapies are used as a solution to these issues. Microbial and enzymatic treatment are the two categories under which biological treatment is categorized. As keratins are the main protein found in feathers, microorganisms that produce enzymes are being researched for the degradation of feathers. Plumage is transformed into disintegrated feather by microbes that makes the absorbable protein utilized as feed. After the release of enzymes by the microorganisms, feather's keratin, collagen, and elastin are hydrolyzed by the proteinase enzyme. Through this technique, feather waste is transformed into nutritiously sound and digestible feather lysate. The widespread conception that some bacteria can breakdown keratin has been backed by the frequent natural processes of microorganisms that readily and, in some circumstances, has the advantage to thrive on keratinaceous surfaces ⁵². Additionally, several *Bacillus* species are recognized as major keratinase enzyme producers.

Numerous *Bacillus* strains have been found as keratinolytic bacteria, including *B. licheniformis*, *B. amiloliquefaciens*, *B. subtilis*, *B. pumillus*, and *B. cereus* ⁵³.

As opposed to alternative therapies such as mechanical and chemical treatments, this approach has the benefits of being effective, affordable, and environmentally friendly ⁵⁴.

2.5.2 Amino Acid Profile of Feather Meal

Several significant amino acids, including histidine (His) and lysine (Lys), are present in high concentrations in the accumulated fermented feather, according to investigations ⁵⁵.

There are correlations between the amino acid profiles of fish meal and feather meal ⁵⁶. This is the reason why feather meal has a tremendous deal of prospects for improvement as an animal feed component ⁵⁷.

The body is modified by amino acid metabolism and the process of separation of protein to various smaller constituents is known as protein hydrolysis. Protein degradation, which can be defined by transformation in the form of a reduction in solubility, is brought on by the hydrolysis of peptide bonds and also globular structures are affected by the hydrolysis process. Chemical substances like acids or bases can have an impact on the denaturing process. Broiler feather waste (BFW) that has been degraded using both acids and bases is known as broiler feather concentration (BFC). With the aid of water molecules, molecules are broken down into simpler compounds during the hydrolysis process. The process of converting a protein's peptide bond into a smallest molecule is known as protein hydrolysis. The peptide link will undergo hydrolysis, which will alter the protein. The protein constituents of the feather's protein linkages can be eased by using alkaline solutions (NaOH) ⁵⁸.

Since the amount of ammonia is increased and the molecular weight of proteins or polypeptides is decreased as a result of these modifications, the solubility is also increased.

Moreover, this is due to the fact that proteins' globular structure has been impaired. Heat, enzyme activity, and chemical substances (acids and bases) can all impact the hydrolysis process. Groups of amino acids make up proteins. Like other proteins, keratin is made up of many kinds of amino acids, although disulfide compounds predominate among the usual amino acids. Amino acid composition and structure can change during hydrolysis with acids and bases ⁵⁹.

Table 2.1: The Nutritional Composition of Feather Meal

Nutrient Content	Quality (%DM)
Dry matter	92.1
Crude protein	85.7
Metabolizable energy	23.0
Crude fiber	0.9
Calcium	12.7
Potassium	1.3
Phosphorus	8.2
Sodium	1.3
Magnesium	0.9
Zinc	142
Copper	10
Iron	625
Manganese	16

Source: Ref ⁶⁰

2.5.3 Protein Availability in Poultry Feed

Due to the ability to feed on both animal and vegetable substances, the nature of the majority of poultry species, their digestive systems are often uncomplicated, and their carcasses are not operative. Ostriches and geese, which have functioning caeca that are well-developed, are exceptions to this general norm. In comparison to other domesticated animals, the digestive system of birds contains more organs but is also shorter. The crop, a storing organ, and the gizzard, a grinding organ, are two of this digestive tract's distinctive characteristics and in less than three hours feed moves from the mouth where cloaca and nutrients are broken down and absorbed in fast-growing meat chickens. High-efficiency birds require absorbable, nourishing foods to make up for the relatively small digestive tract with quick digestion and absorption transport time ⁶⁰.

Daily nutritional protein serves as a source of amino acids for bodily maintenance, muscular development, and egg protein synthesis. Twenty amino acids, all of which are metabolic necessities, are needed for the production of muscle and egg proteins. Ten of these are classified as necessary food components because they are either not produced at all or are formed too slowly to fulfill metabolic needs. They must be included in the diet. Other amino

acids can be used to make the balance and these are referred to as non-essential dietary ingredients which are not needed to be taken into account when preparing feed ⁶⁰.

The synthesis of different proteins in the body requires the synthesis of all 20 amino acids, according to physiological theory. It is generally of the opinion that glycine is crucial for young birds. Because they may be produced from methionine and phenylalanine, respectively, cysteine and tyrosine are regarded as semi-essential amino acids. The three limiting amino acids in the majority of realistic poultry diets are lysine, methionine, and threonine ⁶⁰.

Protein requirement is generally not specifically needed for poultry. To synthesis non-essential amino acids, therefore, an adequate dietary supply of nitrogen from protein is required. This prevents the synthesis of non-essential amino acids from using the necessary amino acids as a source of nitrogen. Thus, ensuring that the supply of all necessary amino acids is met, the required requirements for both protein and essential amino acids are satisfied ⁶⁰.

Numerous variables, including as production level, genotype, sex, physiological condition, environment, and health status, have an impact on the amino acid needs of chicken. As an illustration, a lot of lean meat accumulation calls for a lot of lysine. A Significant amount of methionine is necessary for high rates of egg production or feather development. The relative quantities of the various amino acids generally do not alter, despite variations in the amino acid needs. Therefore, there is an equal ratio of dietary amino acids that is optimum for chickens, and variations in amino acid needs are often described in respect to an ideal or balanced protein⁶⁰.

2.5.4 Protein Source for Broilers

Soya Bean Meal

The majority of nutritional protein (or nitrogen) needs are met by sources of protein which is from plants. Soybean meal is the primary source of chicken feed and is a typical plant protein source utilized in the production of animal food. Depending on how many hulls are removed and the method used to extract the oil, soybean meal contains 40 to 48 percent crude protein. Soybean protein provides an excellent balance of necessary amino acids compared to other oilseed meals, making it a good addition to the majority of cereal-based diets. In comparison to other oilseed meals, soybean meal has a greater amino acid availability. Additionally, compared to other oilseed meals, the energy that is capable of being utilized in metabolism content is significantly larger. Protease inhibitors, one of the naturally occurring substance found in plant derived food that interferes with absorption of nutrient included in raw soybeans, can impair avian performance and protein digestion. However, during the preparation of soybean meal, heat renders these inhibitors inefficient. There is no restriction on the usage of properly processed soybean meal as a great source of protein for all types of poultry. In general, plant protein sources lack critical amino acids, especially lysine, which is the first limiting amino acid in cereals.

Plant-based diets could not provide enough of the essential amino acids needed for the production of eggs and meat when they're not added with the animal protein sources and crystalline amino acids⁶⁰.

Animal Protein

Animal protein components are typically utilized to stabilize the amino acid levels of diets rather than as primary sources of protein due to their high cost. In many nations, feed producers make sure that animal protein content in chicken diets does not fall below required levels, particularly for young birds with high-demand amino acid requirements. It is achievable to fulfill the demands of matured birds with diets that contain lower quantities of

animal protein a substantially larger amounts of plant protein since the requirements for critical amino acids gradually decrease ⁶⁰.

Fish Meal

The animal protein sources that are most frequently utilized in domesticated birds' diets are fishmeal and meat meal since they are excellent sources of high grade protein, and their cost typically reflects this. Additionally, it offers an abundance of B vitamins, vital fatty acids, and minerals (including calcium, phosphorus, and trace minerals). Another characteristic of fishmeal is the presence of unknown growth agents. Therefore, feed production work to ensure that diets include a minimum amount of fishmeal ⁶⁰.

Dried and pulverized fish carcasses make up the majority of fishmeal. Fish type and manufacturing procedures exert influence on the color of fishmeal, which is typically brown and of excellent. The extremely dark color indicates warming, which can denature amino acids, diminish the availability of amino acids, and significantly impair the quality of the protein. In the majority of underdeveloped nations, fishmeal is a significant source of animal protein ingredients—and occasionally the primary source ⁶⁰.

Local fishmeal is either imported or manufactured locally, and it generally contains 40–50% crude protein compared to more than 60% protein in imported fish meals. Due to lack of control over the raw fish quality, processing methods, and storage conditions, local fish meals are often of inferior quality. They are frequently contaminated with inexpensive dissolution medium, such as urea, non-nutritive diluents like sand, and protein sources of low quality (dry poultry manure, oilseed meals)⁶⁰.

Putrefaction, contaminants, or an excessive amount of salt may make some fishmeal unpalatable. It is not unusual for samples to include up to 15% salt. The current predicament highlights how few developing nations have quality control mechanisms. The

salt content of fishmeal should be closely managed since it should be less than 3% for optimal outcomes but legally may be up to 7% because salt has gastrointestinal and growth-depressing effects ⁶⁰.

The variety of cereal and oilseed meals used in the feed formulation will determine how much fishmeal should be used. Another significant factor is the price of fishmeal. In general, typical inclusion levels may range from less than 4% for older meat birds and layers to up to 8% for young birds. Higher concentrations must be prevented in finishing and laying diets since they might provide meat and eggs a fishy aroma. Where agriculture conditions are lower than perfect, the use of fishmeal can partially substitute ⁶⁰.

Future opportunities for fishmeal production development are limited; due to the constraints on global fisheries, the output does not appear to have expanded over the previous 20 years and is unlikely to do so in the future. The entire animal protein banned in Europe includes fishmeal, and there is also a persistent concern about potential impurity levels (like dioxin) in fishmeal⁶⁰.

Meat Meal

The amounts of protein, calcium, and phosphorus that are readily available in meat meals are all rather high. Meat meal is a product made from dry-rendered animal tissues, which excludes stomach contents, blood, hooves, horns, and trimmings from hides unless they are present at levels acceptable with appropriate slaughtering practices. Meat meals are mostly made from bones and tissues close to them, including tendons, ligaments, some skeletal muscle, the gastrointestinal tract, lungs, and livers that have been condemned. The considerable differences in beef meal quality are a result of changes in the amounts of various basic components. The final product can be referred to as either meat meal (having more than

55 percent crude protein and less than 4.4 percent phosphorus) or meat and bone meal, depending on the ratio of bone to soft tissue utilized in the manufacturing process (containing less than 55 percent crude protein and more than 4.4 percent phosphorus). In this product, there is no tryptophan in collagen, the main protein found in bone, connective tissue, cartilage, and tendon. Collagen may make about 50 to 65 percent of the total protein in meals with low-quality meat ⁶⁰.

The quality of the protein found in bone, including its amino acid content and digestibility, can vary substantially. The temperature at which the beef meal is prepared also has an impact on the protein quality. Meat meal is a lower-quality dietary supplement for cereal-based diets than fishmeal or soybean meal. For birds given maize-based diets, tryptophan is the first limiting amino acid in meat meals; lysine and methionine are also limiting. Generally, it is advised to use no more than 10% meat and bone meal in chicken diets since phosphorus requirements are covered at that percentage⁶⁰.

The bovine spongiform encephalopathy (BSE) issue, which was linked to the feeding of beef meal to ruminant animals, serves as an example of the growing safety concerns feed makers must address in currently. In several regions of the world, the use of meat meal in the production of animal feed is now banned, and the persistence viability of this raw material is unknown ⁶⁰.

2.5.5 Feather Meal as Protein Source in Broilers Feed

Chicken crossbreed designed for quick development and completion are known as broilers. They are generally produced between 7 and 9 weeks of age after they have reached a weight of approximately 5 pounds. Roasters are birds that can be processed between 9 and 12 weeks of age and normally measure between 7 and 10 lbs. By planning ahead, an unseasonable supply of chicken is achievable⁶¹.

The processing of poultry meat faces a number of difficulties, and one of these is feed. This is because there is competitive pressure among humans, animals, and businesses for the use of traditional feed ingredients, which has led to high prices for these ingredients and, as a result, increased variability in the cost of manufacturing of feed ⁶².

The use of nontraditional feed sources that may be able to deliver the same results as conventional feeds while doing so at a cheaper cost must thus be explored in order to reduce feed costs in order to reduce the overall costs associated with the production of poultry. For poultry production to maximize profit, it is crucial to be economical by adopting unorthodox and inexpensive feed components to minimize feed costs ^{63,64}.

Table 2.2: Effect of Feeding Broilers with Protein Diets

Protein Source	Recommended Level in the Diet	Effects of Feeding Excessively
Soya bean meal	Broilers: Up to 25% in the starter feed and 20% in the finisher feed. Layers: 12-15%	Large amounts of soybean meal may cause troubles from pasting up in which excreta sticks to the vent. This can lead to renal dysfunction due to retention of the urates which interferes with passing of the droppings. Soybean meal may also produce goiter
Alfalfa meal	2-3% for broilers and up to 10% for the layers.	The high content of carotenoids will impart a deep yellow pigmentation of skin which is not desired by consumers
cotton seed meal	May replace up to 50% of the soybean meal in the diets of broilers, but should not be fed in excess of 5% of the layer's	When large amount of cottonseed meal is fed to the layers, the gossypol found in greenish cast to egg yolks. Cyclopropenoic fatty acids, also

	diet.	present in cottonseed meal, will impart a pink color to egg whites on storage.
sunflower meal	3-5% for broilers and layers.	Sunflower meal contains anti-nutritional components such as pectin's and arabinoxylansz which affect digestibility and bioavailability of protein. This product is also remarkably low in essential amino acids, and is high in chlorogenic acid content which
Fish meal	2-5% for broilers and layers.	Feeding of fishmeal in excess of the recommended level will lead to fishy flavor in meat and eggs. Also, the high cost and the low palatability of the fishmeal are important factors
Source: Ref ⁶⁴		

The main benefit of alternative feed supplies is that they may be utilized to feed animals without facing opposition from the food processing industry or humans⁶⁵. The rising use of these nontraditional feed components, depending on the standard and amount of the substance, will be crucial for future ambitions of protecting animal feeding and enhancing their food security. Application of these unusual feed components, which may be considered trash, can thereby reduce the price of producing poultry⁶⁶.

The disposal of these wastes from the poultry processing house is a significant task; other disposal options include burning or burying, both of which are unfavorable to the environment. Chicken processing factories that are profit minded handle enormous numbers of hens on a daily or weekly basis⁶⁷. The protein keratin, which makes up 90% of unprocessed feathers' protein composition, accounts for 7% of the live bird's weight. There has been a lot of study concern in the prospect of processing feather to increase its edibility

because raw feathers are mostly hydrophobic and have a very low absorption of approximately 5% ^{68,69}.

2.5.6 Advantages of Using Feather Meal as Broilers Feed

Pulverized Feather meal is a high-protein feed that is frequently added to the diet for animals, poultry, and aquatic life. With the exception of lysine and methionine, which are lower than fish meal, feather meal has an amino acid composition that is generally complete and can contain up to 80% protein. All other trace elements are also greater than fish meal. Additionally, it has the highest cysteine concentration of any natural diet, making it an abundant source of protein.

Extruded feather powder is a protein feed ingredient undergoing research and development. This will aid certain nations, like China, which are experiencing a protein-feed resource scarcity. The world's demand for fish meal is on the rise, and nations that produce a significant amount of it, like Peru and Chile, are protecting their marine fish resources and promoting sustainable development, which has led to a lack of commodities and driven up the price of fish meal ⁷⁰.

The cost of imported fish meal has currently grown by more than 50% since the commencement of the year, which has put tremendous strain on the animal husbandry and small profit feed industries. The lack of animal protein supplies may be effectively solved by developing and using animal waste products in reasonable ways. Feather protein content can range from 75% to 85%. (imported fish meal is around 65 percent). However, the keratin protein that makes up feather protein has a high concentration of hydrophobic amino acids. Since the protein chains are joined together by two sulfur bonds to create a solid and compact spatial structure, they are often unable to be broken down by proteases into peptides and amino acids, limiting their direct usage as animal protein feed ⁷⁰.

Ecological contamination can be minimized by using squeezed feather meal. Feathers that have not been treated cannot be utilized as feed. Nevertheless, in certain nations each year, the feather industry or chicken slaughterhouses create thousands of tons of discarded feathers. If they are left lying around without being disposed of, they will adversely pollute the environment ⁷⁰.

2.5.7 Feather Meal as Fish Feeds

In many types of fish, inexpensive feather meals can be used in place of expensive fish meal as a beneficial source of protein. The manner in which the feathers are degraded determines their nutritional content, but there are several other elements that might affect the quality of the meal. Restrictions typically permit the use of feather meal in fish food ⁶⁰.

Enzymatic feather meals had a higher protein nutritive value and led to growth rates that were 10.5-11.5 percent higher than those of steam-processed feather meals in juvenile rainbow trout when compared to the other two types of feather meals, which had increasing levels of arginine (10 g/kg, 13.5 g/kg, and 15 g/kg). To keep arginine levels below 13.5 g/kg, which was determined to be harmful to fish growth, it was proposed to provide enzymatic feather meal at a maximum rate of 100 g/kg of food ⁷¹.

A diet comprising 29 percent digestible protein may be successfully replaced with feather meal for 16 weeks while feeding juvenile red hybrid tilapia (37 g BW) as a substitute for fish meal. Compared to fish fed the control diet, which comprised fish meal, soybean meal, and corn gluten meal as protein sources, fish fed on feather meal exhibited greater weight increase, the speed at which the number of organism increases, and feed conversion efficiency ⁷².

Further study on younger red tilapia juveniles (24 g BW) over 84 days came to the conclusion that 9 percent dietary inclusion of feather meal was the ideal level, as it produced the

maximum life expectancy, unaffected growth rate, feed intake, and unaltered carcass composition ⁷³.

Fermented feather meal and steam-processed feather meal were utilized to replace 50% of the fishmeal in young (37.5 g) turbot produced superior growth results than steam-processed feather meal at a 24 percent dietary intake. However, performance was poor when enzymatic feather meal was included in diets at a rate of exceeding 8%, whether it was supplemented with lysine and methionine or not. It was proposed to use 8% feather meal in place of fish meal, without adding any additional amino acids ⁷⁴.

In seabass juveniles (*Dicentrarchus labrax L.*), substituting hydrolyzed feather meal for 76 percent of the fish meal led to higher nitrogen losses due to better protein digestibility, but it also enhanced phosphorus digestibility and decreased Phosphorus losses. Feed intake, growth performance, feed conversion, body composition, and health indices were not impacted. So it was proposed that feather meal might substitute fish meal in seabass diets up to 76 percent ⁷⁵.

2.6 Other Uses of Feather Meal

In order to decrease the amount of pollution and disease spreading, broiler feathers can be used in a number of ways and methods for products with added value or as a raw resource for practical industrial products ⁷⁶.

Manufacture of Clothing

The researchers of a study that looked into the usage of chicken feathers as filler in cold weather apparel revealed that the fiber derived from the feathers had good thermal properties than fillers made from artificial sources. The scientists also mentioned that as opposed to synthetic fiber, feathers from broiler chickens might be a more affordable source of raw

material for the industrial production of winter clothing. When cutting broiler feathers into smaller pieces, which are utilized as industrial raw materials in the clothing manufacturing industry, improved insulating property is acquired. This is because doing so significantly diminishes the absorbing capacity of broiler feathers⁷⁷.

Manufacture of Organic Fertilizer

Users can also process and use broiler feathers as organic fertilizer. According to reports, *Vigna unguiculata* grew more when organic fertilizer made from chicken feathers was used and administered at a rate of 3kg per 10kg of soil. In a similar vein it was observed that Treated Poultry Feather Waste (TPFW) accelerated plant development, produced healthier leaves, enhanced plant growth, and improved plant weight. The authors also demonstrated how TPFW increased the amount of leaves per plant. The quantity of leaves on a plant is typically interpreted as an indicator of higher plant yields. Their research indicated that as TPFW spray rates were increased, there were more plant stands⁷⁸.

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Chapter Three

Methodology

3.1 Materials

Decomposed feather samples (5g each collected at different dumpsites), 100kg of freshly plucked feathers, Ziploc, plastic bags, Auger, 24 broilers chicks, Test tubes (5ml)(Pyrex), Cello tapes, Foil paper, Petri dishes(Pyrex), Spatula Plain tubes, Inoculating loop, Ethanol, Absorbent Cotton wool, Marker, Bijou bottles, Distilled water, Paper tapes, Hand gloves, Face mask, Measuring cylinders (100ml, 500ml) (Pyrex), Conical flask (100ml,500ml) (Pyrex), Beakers (Pyrex), Micro pipette, Wash bottle, Matches, Burner, Injecting needles, Skimmed milk, Micro cuvette, DNA extraction kits ,Gel extraction kits, Rearing cage, wood

shavings, Drinkers, Feeders, Feeds, Vaccines and Medicines, Weighing scale, Good source of water, lamps.

3.2 Equipment

Oven (CHF 097 Gallenkamp England), Refrigerator (HR 195 Thermocouple), Water bath (CB.2 Sq2, Gallenkamp England), Incubator (IH.- 100 Gallenkamp England), Autoclave (PM 0552 Boxer lab. Equ. Ltd), Weighing balance Electronic pH Meter (Mettler Toledo), inoculating chamber, UV/Visible Spectrophotometer (Shimadzu, U V 1800), Centrifuge PAC, (1236MG Pacific), Cold centrifuge, Industrial milling machine(Longer machinery), poultry cage(height 12feet per 1.350cm²to contain 3birds).

3.3 Chemicals/Reagent

Some of the chemical used include: NaCl .KH₂PO₄ K₂HPO₄, NH₄Cl, MgCl₂, Yeast extract, Nutrient Agar, Nutrient Broth, Keratin azure, NaOH, HCl, TCA, Na₂CO₃ CuSO₄ .5(H₂O), Na₂ Tartrate .2(H₂O), Bovine Serum Albumin (BSA). Folin Ciocalteu's Reagent, Mineral Salt Media (MSM) Cupric Sulphate, Cadmium sulphate, Manganese chloride, Zinc sulphate, Mercury chloride.

3.4 Methods

3.4.1 Sample Collection

Freshly plucked feathers (100kg) were collected from a poultry factory along Eleyele road, Ibadan, Oyo state. The feathers were collected into a clean plastic bag and labeled to be taken to the laboratory. The feathers were washed properly with distilled water and dried in the oven at 60°C for many hours until it was satisfactorily dry which is confirmed by its crispy texture. This feather was used as part of media for isolation of keratinase producing microorganisms ¹.

Decomposed feather samples were collected from dumpsites into well labeled clean Ziploc bags around the area where feathers have degraded overtime using Auger to pick the feather samples from within the dump. This was done so that thermophilic isolates can be obtained since temperature within a dump is usually higher. The sampling points are poultry slaughter sides located at the following areas;

- Eleyele market
- Bodija market
- Ido market
- Sango market

3.4.2 Feather Meal Powder Preparation

Dried feather samples were milled mechanically in a milling machine (Model SC-002F) and sieved through a sieve with mesh size of 0.5mm to achieve particle size of 0.5mm and used as feather meal to be added in preparation of culture medium.

3.5 Isolation and Screening of Protease and Keratinase-Producing Bacteria

3.5.1 Isolation of Bacteria

Serial dilution was carried out to 10^6 for degraded feather samples. This was done by mixing 1 gramme of each sample in each test tube containing 9 ml of sterile distilled water each. Each test tube was diluted continuously until dilution factor 6 was attained for each tube, blank tubes were made and each sample was plated.

For each sample, mixture from 10^{-1} , 10^{-3} to 10^{-6} dilutions were each taken and spread into two different Petri plates containing Mineral salt medium. The mineral medium contained: 0.5% feather, 0.03% K_2HPO_4 , 0.04% KH_2PO_4 , 0.05% NaCl, 0.01% $MgCl_2$ and 2% agar ². Plates were incubated at 37°C for mesophilic isolates and 45°C for thermophilic isolates for 48 hours each. Pure culture of isolate was obtained from each of these plates by streaking an inoculum obtained from touching a colony carefully and aseptically with a sterile plastic inoculating loop of 0.5 cm loop diameter on Nutrient Agar plate.

3.5.2 Screening for Protease Production

Colonies that had hydrolysis around them were picked separately from mineral salt agar medium and transferred to skim milk agar. The skim milk agar contained: peptone 5g/L-1, beef extract 3g/L, skim milk: 5g L and agar 15g L A single colony of each isolate was streaked on skim milk agar and incubated at 45°C for 24 hours. Isolates with clearance around the colonies were selected as positive to hydrolysis of casein at 45°C ³.

3.5.3 Qualitative Screening of Keratinolytic and Proteolytic Isolates for Keratinase Production Using Keratin Azure as Standard Substrate

Obtained isolates were screened for keratinase production on a minimal medium containing 0.03% K_2HPO_4 , 0.04% KH_2PO_4 , 0.05% NaCl, 0.01% $MgCl_2$, 0.5 % of keratin azure (Manufactured by Sigma) instead of feather meal, and 2 % of agar. Plates were incubated at 45°C for 48 hours. Isolates whose growth turned the medium bluish were observed as positive for hydrolysis of keratin contained in keratin azure in the medium³.

3.5.4 Purification of Isolates

Four selected microorganisms were purified through a repeated subculture method. Streak plate methods were used for this purpose. Skimmed milk agar was used as a medium. When a plate yielded only one type of colony, the culture was considered pure.

3.5.5 Production of Keratinase by Selected Isolates in Liquid Mineral Medium

Selected isolates were each induced to produce keratinase using liquid medium made up of: (0.03% K_2HPO_4 , 0.04% KH_2PO_4 , 0.05% NaCl, 0.01% $MgCl_2$) with 0.5% (w/v) ground feather as the sole source of nitrogen and keratinase inducer. Each Erlenmeyer flask was made to contain 20 ml of the liquid medium and 1 ml of selected isolate containing 10^6 inoculum size was introduced into each flask aseptically. Flasks were incubated at $45^\circ C$ in a shaker incubator (Stuart S 150) at 120rpm for 24 hours. Replicates of these experiments were made along with a control flask which only contains the minimum medium without any inoculum added.

Another set of flasks were each filled with 20ml of liquid medium containing 0.5% (w/v) keratin azure as the sole source of nitrogen and keratinase inducer. Each flask was inoculated with 1ml of 24 hour culture of selected isolate containing 10^6 inoculum size. Replicates of these experiments were made along with control flask which only contains the minimum medium without any inoculum added. These flasks served as a standard for comparison ⁴.

3.6 Characterization of Bacterial Isolates

The characterization of selected bacterial cultures was initially based on colonial and morphological characteristics of each, which included shape, Gram reaction and microscopy using the light microscope (CH Olympus) at 100X objective. In addition to morphological

characters, the isolated bacterial cultures were cultivated in selective media and certain biochemical tests were also performed on them.

3.6.1 Gram Staining

A smear of the pure culture of selected isolate was prepared by placing a drop of water on a clean microscope slide and then homogenizing the microbial inoculum in the drop of water with a sterile cooled loop, it was then spread by means of a circular motion of the inoculating loop. The smear was air dried and heat fixed and was gently flooded with crystal violet solution (1%) for 1 minute. The smear was carefully washed under running distilled water and gently treated with three drops of Lugol's iodine for 1 minute, after which the iodine was washed off under running distilled water. The smear was decolorized with 95% ethyl alcohol reagent which was added to the slide drop by drop until no further violet color was coming out. It was then gently washed under running distilled water and counterstained with Safranin solution (0.5% w/v) for about 60 seconds. This was washed under running water and blot dried with sterile filter paper and examined under oil immersion objective of the light microscope ⁵.

3.7 Biochemical Test

3.7.1 Citrate Utilization Test

This test was used to study the ability of the organism to utilize citrate present in Simmon's media ($MgSO_4$ -0.2g/l, $NH_4H_2PO_4$ -1g/l, K_2HPO_4 -1g/l, sodium citrate-2g/l, Nacl-5g/l, Bromothymolblue-0.08ml, agar-15g/l, distilled Water-I liter) as a sole source of carbon for growth. The Simmon's media was prepared sterilized and poured into the tubes. The media

was allowed to cool and solidify in the form of slants. The slant was then stabbed with the isolated bacterial sample using a needle and incubated at 37°C for 36 hours ⁶.

3.7.2. Motility Test

The medium mainly used was SIM medium (Sulphide Indole Motility medium) which is a combination differential medium that tests three different parameters which are Sulfur Reduction, Indole Production and Motility. This media has a very soft consistency that allows motile bacteria to migrate readily through them causing cloudiness. The inoculum was stabbed into the center of a semisolid agar deep using a sterile inoculating needle. Bacterial motility was evident by a diffuse zone of growth extending out from the line of inoculation ⁶.

3.7.3 Catalase Test

This test was carried out in a lamina flow hood. Three to four drops of 30% H₂O₂ was added to an overnight (18-24 hrs.) pure culture of the test bacterial on agar plate and formation of vigorous bubbling signified positive result ⁶.

3.7.4 Sugar Fermentation

Glucose, Sucrose, Mannitol and Arabinose were used for this fermentation test. One gram of each sugar was weighed into different conical flask and labelled accordingly. Into each conical flask, 1g of peptone water was added and made up to 100ml with distilled water. Phenol red of 0.01g was added as indicator and 5ml each of the sugar solution was dispensed into different tubes with Durham's tube inserted into each test tube. The tubes were plugged with cotton wool and labelled appropriately; it was then sterilized in an autoclave for 15 minutes at 121°C. The tubes were then allowed to cool. The bacteria isolates were each inoculated aseptically into sugar solution in the tubes and incubated at 37°C for 2 days. The

change of color from red to yellow indicates acid production which implies the utilization of sugar by the organism and the appearance of bubble in Durham's tubes indicates gas production ⁷.

3.8 Molecular Identification of Selected Isolate

3.8.1 DNA Extraction

Pure culture from solid medium was aseptically transferred to 1.5ml of sterile liquid medium and cultures were grown in shaker incubator at 37⁰c for 48 hours. The method is according to procedure followed by Tridale et al ⁸. At the end of 48hours, each culture was centrifuged at 4600 for 5 minutes. The resulting pellets from each culture were re-suspended in 520ul of TE buffer (10mMTris-HCL, 1Mm Ethylenediaminetetraacetic Acid (EDTA) pH (8.0). Fifteen microliters of 20% sodium Dodecyl Sulfate (SDS) and 3 ul of proteinase K (20mg/ml) were then added. The mixture was incubated for 1 hour at 37⁰c, then 100ul of 5 M NaCl and 80 ul of a 10% Cetyl Trimethylammonium Bromide(CTAB) solution in 0.7 M NaCl added and vortexed. The suspension was incubated for 10 minutes at 65⁰c and Later kept on ice for 15 minutes. An equal volume of chloroform; isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 minutes and centrifugation at 7200 for 20 minutes. The aqueous phase was then transferred to a new tube and isopropanol (1:0.6) was added and DNA precipitated at 20 ⁰c for 16 hours. DNA was collected by centrifugation at 13000g for 10 minutes, washed with 500ul of 70% ethanol, then air dried at room temperature for approximately three hours and finally dissolved in 50ul of TE buffer.

3.8.2 Polymerase Chain Reaction (PCR)

PCR sequencing preparation cocktail consisted of 10 µl of 5x Go Taq colorless reaction, 3 µl of 25mM MgCl₂, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5' – AGA

GTT TGA TCM TGG CTC AG-3' and – 1525R, 5'-AAGGAGGTGATCCAGCC-3' forward and reverse primers respectively and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 μ l with sterile distilled water 8 μ l DNA template. PCR was carried out in a Gene Amp 9700 PCR System Thermal cycler (Applied Bio system Inc., USA) with a PCR cocktail profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C FOR 30 S, 50°C for 60s and 72°C for 1 minute 30 seconds; and a final termination at 72°C for 10mins. And chill at 4°C^{9,10}.

3.8.3 Integrity Check for Extracted DNA and PCR Product (Electrophoresis for DNA and PCR Product)

The integrity of the amplified about 1.5Mb (Myoglobin) gene fragment was checked on a 1% Agarose gel run to confirm amplification. The buffer (1X TAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 3 minutes. The molten agarose was allowed to cool to 60°C and stained with 3 μ l of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to allow formation of wells where comb was inserted. The 1X TAE buffer was poured into the gel tank to barely submerge the gel. Two microliter of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4 μ l of each PCR product and loaded into the wells after the 100 base pairs(100bp) DNA ladder was loaded into well number 1. The gel was electrophoresed at 120V for 45 minutes visualized under ultraviolet trans-illumination(MICROSIL) and photographed. The sizes of the PCR products were estimated by comparison with the

mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel ¹⁰.

3.8.4 Purification of Amplified Product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Approximately 7.6 μ l of Sodium acetate 3M and 240 μ l of 95% ethanol were added to each about 40 μ l PCR amplified product in a new sterile 1.5 μ l Eppendorf tube mixed thoroughly by vortexing and kept at -20°C for 30 minutes. Centrifugation was done for 10 minutes at 13000 g and at 4°C followed by removal of supernatant by inverting tube on trash once. The pellets were afterwards washed by adding 150 μ l of 70% ethanol, mixed and then centrifuged (Beckman Coulter) for 15 minutes at 7500 g at 4°C. Supernatant was removed by inverting tube on trash once and then the tube was inverted on paper tissue and allowed to dry in the fume hood(Kalstein) at room temperature for 10-15 minutes. The fragment obtained was resuspended with 20 μ l of sterile distilled water and kept in – 20°C temperature prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1 hour as previously stated, to confirm the presence of the purified product and quantified using a nanodrop (Model 2000 Thermo scientific)¹¹.

3.8.5 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer (Model 3130x1 Sequencer-Applied Bio systems) using manufacturers' manual while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis¹².

3.8.6 Phylogenetic

Phylogenetic analysis was performed on the sequences of isolated microorganisms using MEGA 6 software¹³. Sequences were prepared in FASTA format and aligned using ClustalW option of the program. A phylogenetic tree was constructed based on maximum likelihood with 1000 bootstrap and the evolutionary history was inferred using the Neighbor-Joining method. The resulting tree was analyzed and interpreted based on its clustering pattern and topology^{13,14}. The evolutionary history was inferred using the Neighbor-Joining method¹⁵.

3.9 Determination of Keratinase Activity of Isolate SN7 (*Bacillus subtilis*) On Feathers with Respect to Time.

The growth rate of *Bacillus sp* in minimal medium containing 1% feather was monitored every 6 hours over a period of 48 hours at 36°C by checking the absorbance at 600nm. This was done following the procedure of Labal et.al¹⁶. The Basal medium (100ml) contained 0.9g/L K₂HPO₄; 0.2 g/l KCl; 0.2g/L MgSO₄·7H₂O; 1.0g/L NH₄N₃; 0.002 g/L ZnSO₄; 0.002 g/L MnSO₄; 0.002g/L FeSO₄·7H₂O and 1g of feather powder. The medium was autoclaved at 121°C at 15atm for 20 min. The medium was allowed to cool. The seed culture (5ml) was aseptically transferred into the mixture of basal medium containing the feather powder. The culture was incubated in a rotary shaker incubator at 30°C for 48 hours at 150rpm. MSM medium that does not contain any organism was used as control. The Keratinase activity of *Bacillus Subtilis* on feather was evaluated and described below.

3.9.1 Crude Enzyme Activity Determination for Isolate 7(*Bacillus sp*)

At the end of hydrolysis, the mixture was reacted with TCA (10%) to stop the reaction, cooled for 30 minutes and centrifuged at 3,000 rpm for 10 min. The clear supernatant was assumed to contain the extracellular enzyme; thus the enzyme solution was used in the

enzyme assay. One unit of keratinase activity was taken to be 0.01 unit of absorbance per minute. Absorbance reading was taken at 280nm, with TCA used as the blank^{16,17}

3.10. Optimization of Keratinase enzyme by checking the effect of Nitrogen Source, Carbon Source, Temperature, PH, and Incubation time on Enzyme Production

3.10.1 Effect of Nitrogen Source on Keratinase Production

Effect of each of Peptone, KNO₃, Yeast extract and Casein which are nitrogen sources on the production of keratinase by selected isolate was done by introducing 10ml of basal medium into each Erlenmeyer flask. Each of the nitrogen sources (g/l) was added to each flask containing the basal medium. The medium was autoclaved at 121°C at 15 atmospheric pressure for 20 minutes, the medium was allowed to cool to ambient temperature(25⁰c) inoculated with culture (10³cfu/ml) and incubated in a rotary shaker incubator at 45°C and 150 rpm for 48 hours. The mixture was centrifuged at 5,000 rpm at 4 °C for 20 minutes after incubation¹⁸. The supernatant was collected after centrifugation and keratinase assay was conducted on the supernatants as previously described.

3.10.2 Effect of Carbon Source on Enzyme Production

Effect of carbon sources such as Glucose, Fructose, Mannitol and Starch on the production of keratinase by selected isolate was done by introducing 10ml of basal medium into each of Erlenmeyer flask. Each of the carbon source (1 g/L) of the different carbon sources to the production medium while keeping other component of the basal medium constant. The medium was autoclaved at 121°C at 15 atmospheric pressure for 20 minutes. The culture (10³cfu/ml) was incubated in a rotary shaker incubator at 30°C and 150 rpm for 48 h after the

incubation. The mixtures were centrifuged at 5,000 rpm at 4°C for 20 minutes. The supernatant was collected after centrifugation and enzyme assays were conducted on the supernatants^{2,19}.

3.10.3 Effect of Temperature on Enzyme Production

The effect of temperature on the production of the enzyme was carried out by the method of Barman et al.² Inoculum from 18 hours seed culture was inoculated into the basal medium containing 1.00g feather at different temperatures varying from 25 to 70°C. The culture was incubated in a rotary shaker incubator at 30 °C for 48 hours at 150 rpm. The mixture was centrifuged at 5,000 rpm at 4 °C for 20 minutes. Keratinase activity was assayed and subsequent analysis of residual activities under standard assay conditions was determined¹⁹.

3.10.4 Effect of pH on Enzyme Production

Effect of pH on the production of the keratinase was carried out by the method of Barman,² Inoculum from eighteen-hour old seed culture was inoculated into the basal medium containing 10% feather and the pH of the medium was adjusted to different pH varying from 2 to 10 using the following buffer systems of 50mM Acetate buffer for pH 2.0 - 5.0, 50mM Phosphate buffer for pH 6.0 - 7.0, 50mM Tris-HCl buffer for pH 8.0 - 10.0. The culture was incubated in a rotary shaker incubator at 30 °C for 48 hours at 150 rpm. The mixtures were centrifuged at 5,000 rpm at 4 °C for 20 minutes. Enzyme activity was assayed and subsequent analysis of residual activities under standard assay conditions was determined¹⁹.

3.10.5 Effect of Incubation Time on Enzyme Production

10ml of seed culture broth was added to 1 Liter of basal medium. The incubation time on the production of the enzyme was carried out by the method of Baman et al.,² Inoculum from 18

hours seed culture was inoculated into the basal medium containing 10% feather at varied incubation time. The culture was incubated in a rotary shaker incubator at 30 °C for 60 hours at 150 rpm. Fractions (5ml) of the mixture was collected and centrifuged at 5,000 rpm at 4 °C for 20 mm at intervals of 6 h for a total of 72 hours ². Keratinase activity was assayed and the subsequent analysis of residual activities under standard assay conditions was determined ¹⁹.

3.10.6 Degradation of Feathers by Isolates

Distilled water (100ml) is added to shredded unpretreated feathers of about 0.5g and 0.5g of the same quantity of shredded pretreated feather in two different Erlenmeyer flasks and properly labeled as stock mixture. From the stock mixture 5ml was added into each of 100ml beakers and properly labeled. Two other beakers with each containing the same volume of the stock mixture but without the isolate inoculated into them was used as control. Replicates of each of the categories of these sets up were also made. All the beakers were incubated for 24hrs at 45°C ².

After 24hr, 2ml of TCA was added to 2ml of each of the inoculated beakers content to stop the reaction. It was then allowed to cool by placing in the refrigerator for 30minutes. After 30minutes, the mixture was centrifuged at 3000rpm for 10minutes. 500µL of supernatant from mixture was taken and 0.7µL of Lowry solution was added. Sample was then vortexed and kept in the dark for 20minutes. 10 µL of Folin reagent was then added to each sample and vortexed. All plain tubes containing the samples were then kept in the dark for 30min. Absorbance of each sample was then checked by taking spec reading at 750nm using water as blank ¹⁹.

3.11 Degradation of Feathers by Isolates at Optimum Conditions

Shredded unpretreated feather of 0.5g was placed into two different 100 ml conical flasks with each containing 30ml of Tris HCl buffer. Each flask was properly labelled as stock mixture. Each mixture of about 20ml was taken into two different 50 ml beakers and 3ml of isolate 1, 0.02g of Yeast extract, and 0.02g of glucose was added into each beaker and properly labeled. The four (4) beakers and conical flasks containing the mixtures were then kept in the incubator shaker for 24hrs at 45°C. After 24hrs, 2ml of TCA was added to 2ml of each sample to stop the reaction in sterile plain tubes. It was then allowed to cool by placing in the refrigerator for 30min. After 30min, mixture was centrifuge at 3000rpm for 10min. 500µL of supernatant from mixture was taken and 700µL of Lowry solution was added. Sample was then vortex and kept in the dark for 20min. 100µL of Folin reagent was then added to each sample and vortex. All plain tubes containing the samples were then kept in the dark for 30min. Absorbance of each sample was then checked by taking spec reading at 750nm using water (H₂O) as blank ¹⁹.

3.12 Degradation of Feather Used as Feed (Feather Meal) for Broilers

Raw feathers were collected from poultry slaughter house. The raw feathers were washed with mild commercial laundry detergent, rinsed severally with good clean water. In the process, all unwanted materials other than the feather was removed. The cleaned feathers were dried in a laboratory oven at for four hours and later shredded. The Experiment was conducted in sterile 500 mL Erlenmeyer flasks containing 20 g of sterile feathers and 100 mL of basal medium at PH 8 and addition of Yeast extract and Glucose as source of Nitrogen and Carbon respectively. Flasks containing medium and feathers were autoclaved and then inoculated with 10ml of selected isolate. Flasks were incubated in a shaker incubator at 40°C and pH of 8. The control flasks were kept uninoculated. Degradation was carried out for 9 days. Every 3 days, feathers were filtered

through a sterile muslin cloth and washed severally with distilled water. Afterwards, it was dried at 60^oc for 4 h to remove moisture. The dried degraded feather was weighed to determine the estimated degree of degradation of feathers by the isolated bacteria ²⁰.

3.13 Feeding on Experimental Broilers with Feather Meal and Commercial Feed

The research for experimental feeding of broilers was conducted at the animal house of the university. The experiment was conducted using 18 broiler of chicken for a study period of 3 weeks (21 days). At the arrival of the broilers, the initial body weight of each broiler was recorded and measured again on days 7, 14, 21. The body weights of the birds were weighed in grams by using an electronic digital balance.

The broiler chickens were given recommended treatment and the chicken house was fumigated and left for a week. The chicken house was partitioned into six compartment. Each compartment housed 3 two weeks old broiler chicken. Each compartment was approximately labelled according to the feed given to the occupant. The feed was as follows: fish meal 100%, fish meal 50% + hydrolyzed feather 50%, Hydrolyzed feather 25% + fish meal%, hydrolyzed feather meal 100%, Non hydrolyzed feather 100% (control). The experimental birds were given feed in the morning at 8am and evening at 4pm on daily basis. Feed given were weighed using a top loader sensitive weighing scale to achieve the requisite amount of feed to be given to each treatment and the quantity per replicate. Water was supplied to the birds as often as necessary. All vaccination and medication programme were carried out as at the time recommended. On weekly basis, the weight of birds in each replicate was measured and record.

Endnotes

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Chapter Four

Results and Discussion of Findings

4.1 Results

4.1.1 Sample Analysis

Growth of organisms were seen to appear on the plates. Some of the organisms occurred at only one site, some at two sites while others at three different sites. A total number of 7 bacteria strains were isolated from the feather samples obtained at the dump sites.

4.1.2 Identification of Bacteria Isolated Based on Morphological Characteristics

The result of the colonial morphology characteristics of the isolated bacteria on Mineral salt medium is shown in table 4.1

Table 4.1: Colonial Morphology of Isolates on Mineral salt Medium(MSM)

Isolate Code	Size	Shapes	Elevation	Surface	Color
SN1	Large	Round	Flat, Dry	Smooth	White
SN2	Small	Round	Slightly Raised Muroid	Smooth	Yellow
SN3	Small	Round	Slightly Raised Muroid	Smooth	White
SN4	Large	Round	Dry, Flat	Smooth	White
SN5	Large	Round	Flat, Dry	Smooth	White
SN6	Small	Round	Slightly Raised Muroid	Smooth	Yellow
SN7	Large	Round	Flat, slightly Muroid	Smooth	White

Source: Author's Laboratory Result ,2022

Isolate SN1 is large in size, roundish, flat, smooth in surface and whitish in colour.

Isolate SN2 is small in size, roundish, slightly raised, smooth in surface and yellowish in colour.

Isolate SN3 is small in size, roundish, slightly raised, smooth in surface and whitish in colour.

Isolate SN4 is large in size, roundish, flat, smooth in surface and whitish in colour.

Isolate SN5 is large in size, roundish, flat, smooth in surface and whitish in colour.

Isolate SN6 is small in size, roundish, slightly raised, smooth in surface and yellowish in colour.

Isolate SN7 is large in size, roundish, flat, smooth in surface and whitish in colour.

4.1.3 Screening for Proteolytic and Keratinolytic Bacteria

Results of primary screening of the bacteria for proteolytic activities on the medium containing skimmed milk agar are shown in Table 4.2 and plate 2 while results for secondary screening for keratinolytic activity on mineral medium containing keratin azure as the inducing substrate are shown in Table 4.3. Bluish coloration as seen in plate 1 was observed in some of the secondary medium due to the degradation of keratin and these were recorded as positive for keratinolytic activity. The isolates that tested positive to keratinolytic activity test were thus selected for further work.



Plate 4.1: Isolation of bacteria using keratin supplemented agar (keratin Azure).

Source: Author's Laboratory Result ,2022

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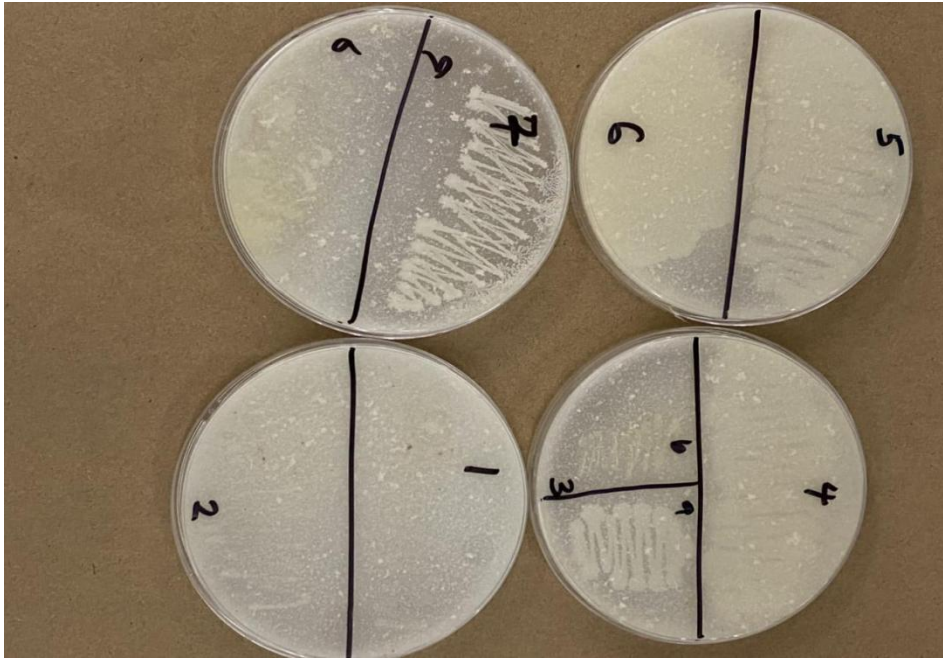


Plate 4.2: Proteolytic activity of isolates on skimmed milk agar

Source: Author's Laboratory Result ,2022

Table 4.2: Diameter of Clearance of Isolates in Casein Medium

Isolate Codes	Diameter of Zone of clearance (mm)	Proteolytic activity on casein
SN1	24	+
SN2	18	+
SN3	23	+
SN4	10	-
SN5	0	-
SN6	5	-
SN7	26	+

Source: Author's Laboratory Result ,2022

The result of the Diameter of Clearance of Isolates in the Casein Medium presented in table 4.2 shows that the highest zone of clearance (26mm) is shown by isolate SN7, followed by SN1(24) while the least clearance is shown by isolate SN5

Table 4.3 Diameter of Clearance and keratinolytic activities of Isolates on Keratin Azure Medium

Isolates Codes	Diameter of Zone of clearance	Keratinolytic activity
SN1	22	+
SN2	14	-
SN3	24	+
SN4	9	-
SN5	3	-
SN6	10	-
SN7	24	+

Source: Author's Laboratory Result ,2022

The result of the Diameter of Clearance of isolates on keratin Azure presented in Table 4.3 shows that isolate SN7 and SN3 has the highest diameter of Zone of clearance(24mm) followed by isolate SN1(22) while isolate SN5 has the least diameter of zone of clearance.

The result of the keratinolytic activities of Isolates on Keratin Azure Medium presented in Table 4.3 shows that isolate SN1, SN3 and SN7 are positive to keratinolytic activity while SN2, SN4, SN5 and SN6 are negative that is they show no keratinolytic activity.

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4.1.4 Biochemical Identification of selected keratinolytic Isolates

Tables 4.4 shows the biochemical characteristics of bacteria and their identities.

The biochemical result shows that Isolate SN1 is Gram negative catalase positive, oxidase negative and degraded glucose, Sucrose and mannitol but did not utilize lactose and arabinose.

Isolate SN2 is Gram negative catalase positive, oxidase negative degraded lactose but was unable to utilize glucose, arabinose, sucrose and mannitol.

Isolate SN5 is Gram negative catalase positive, oxidase negative but was unable to utilize lactose, glucose, arabinose, sucrose and mannitol.

Isolate SN 7 is gram positive catalase positive, oxidase negative was able to utilize lactose, arabinose, sucrose and mannitol but could not degrade glucose.

Table 4.4: Biochemical Characteristics of Isolates

Isolate Code	Catalase	Oxidase	Gram Stain	Lactose	Glucose	Arabinose	Sucrose	Mannitol
SN1	+	-	-	-	+	-	+	+
SN2	+	NA	-	+	-	NA	-	-
SN5	+	NA	-	-	-	-	-	-
SN7	+	NA	+	+	-	+	+	+

Key: + = Positive reaction, - = Negative reaction, NA-Not Available

Source: Authors Laboratory Result,2022

4.1.5 Molecular Identification of Isolate with Positive Keratinolytic Activity

Isolate coded SN7 was confirmed to be *Bacillus subtilis* after subjecting the isolated DNA to 16s ribosomal RNA gene amplification by polymerase chain reaction (PCR) +allowing it to gel by electrophoresis and analyzing the base sequences of the amplified strand by Sanger sequencing following the manufacturers instruction. The gel image obtained for the isolated DNA from isolate 7 is shown in Plate 4.3.

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Plate 4.3: Gel Image of Extracted DNA Strand of SN7 After PCR

Source: Author's Laboratory Result 2022

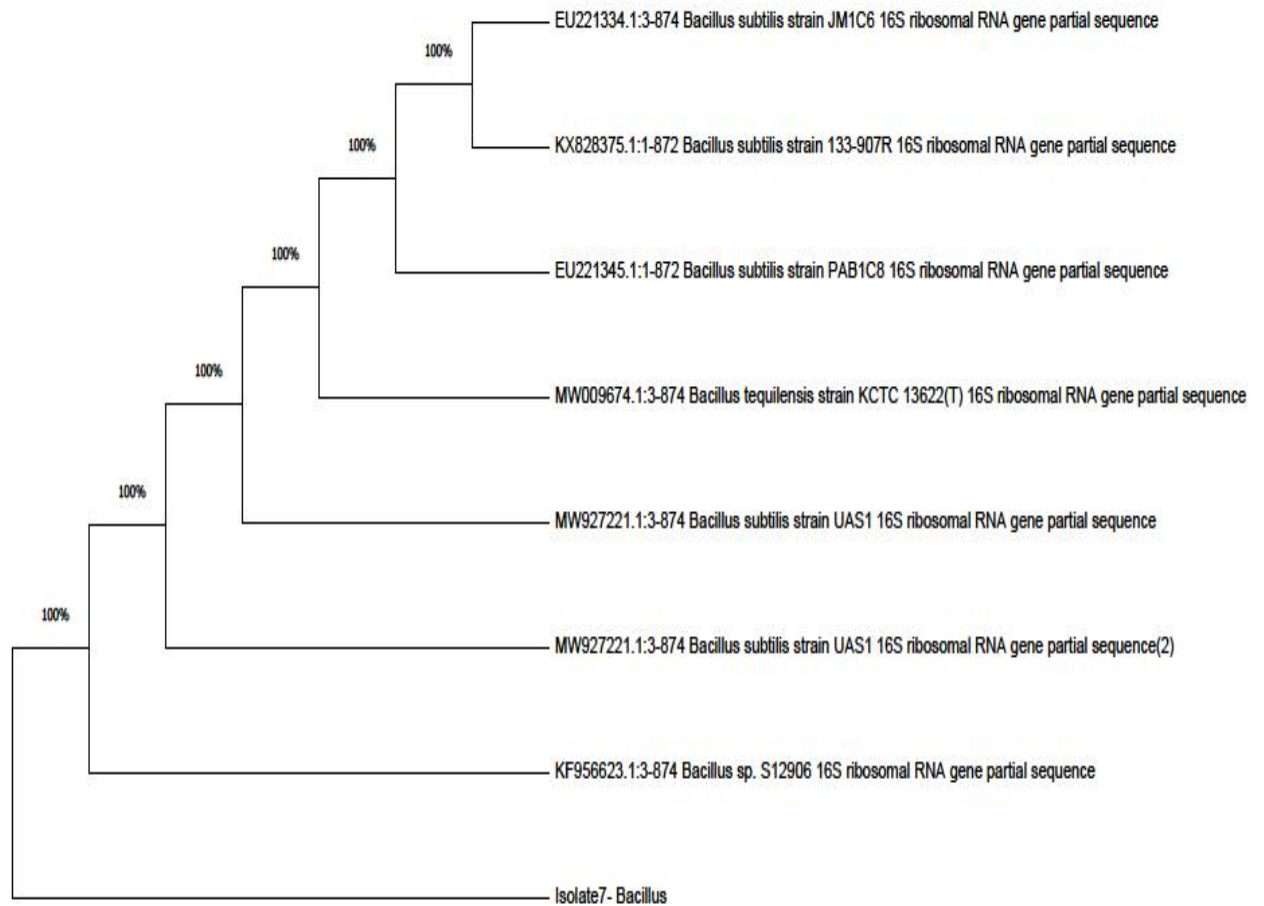


Figure 4.1: Phylogenetic tree built with the neighbor-joining method based on 16SrRNA gene sequence of *Bacillus subtilis*(SN7)

Source: Author's Laboratory Result 2022

4.1.6 Changes in The Keratinase Activity (U/ μ l) Of *Bacillus subtilis* with Time

Keratinase activity of *Bacillus subtilis* on feathers was observed between 24 to 72 hours. The highest keratinase activity (70 μ /L) was observed at 48 hours while the lowest keratinase activity 21.3 μ /L was recorded at 24 hours.

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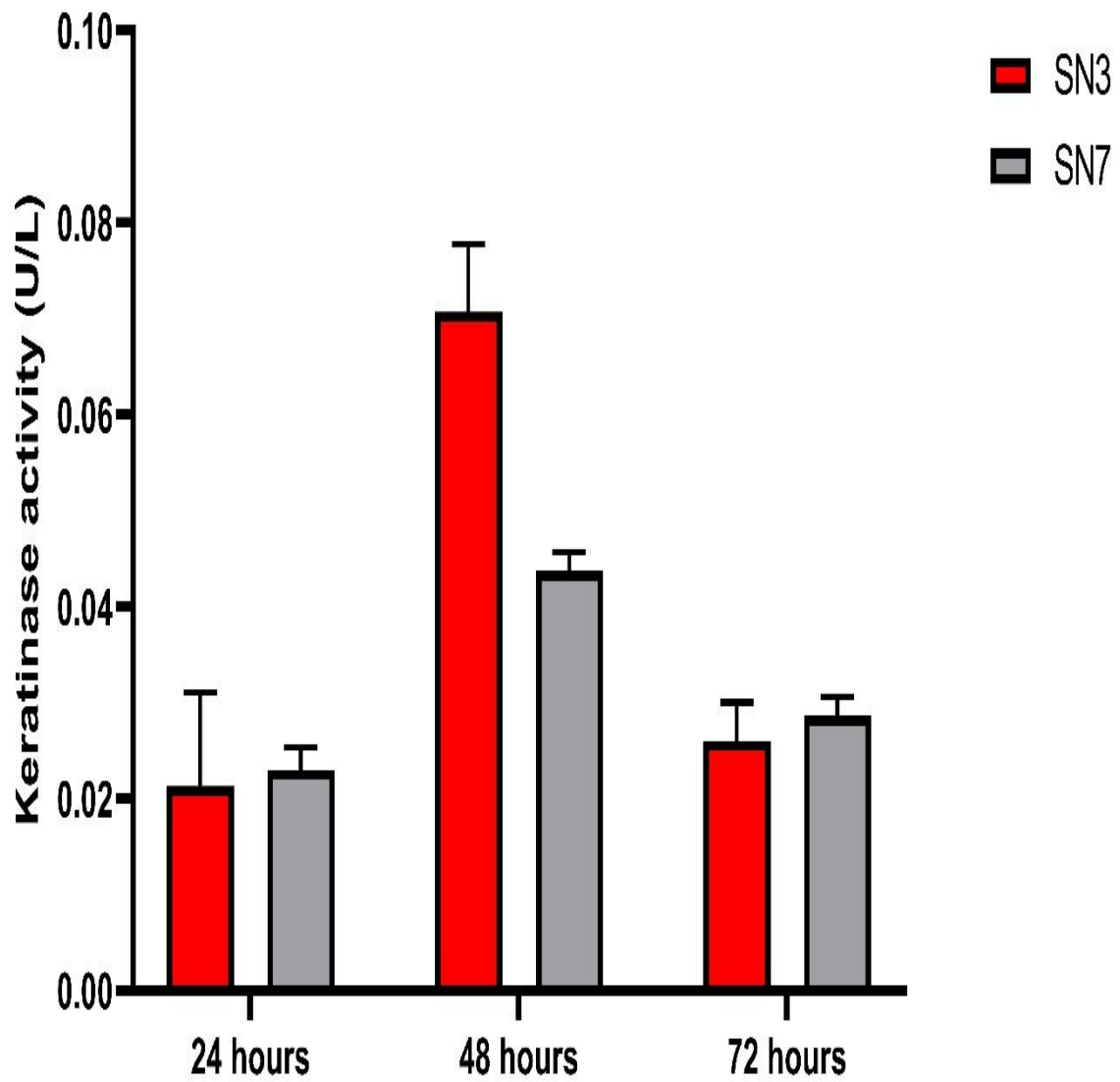


Fig 4.2: Changes in Keratinase Activity of Bacteria Isolates with Time

Source: Author's Laboratory Result ,2022

4.1.7 The Effect of Different Nitrogen Sources on Keratinase Activity of *Bacillus subtilis*

Bacillus subtilis was subjected to test for production of the keratinase enzyme using different nitrogen sources using Casein, Peptone, KNO₃, NaNO₃, and yeast extract. The highest activity of 86.33 µl/L was observed when casein was supplemented into the production medium while the least activity of 0.33 µl/L was seen when NaNO₃ was substituted into the medium.

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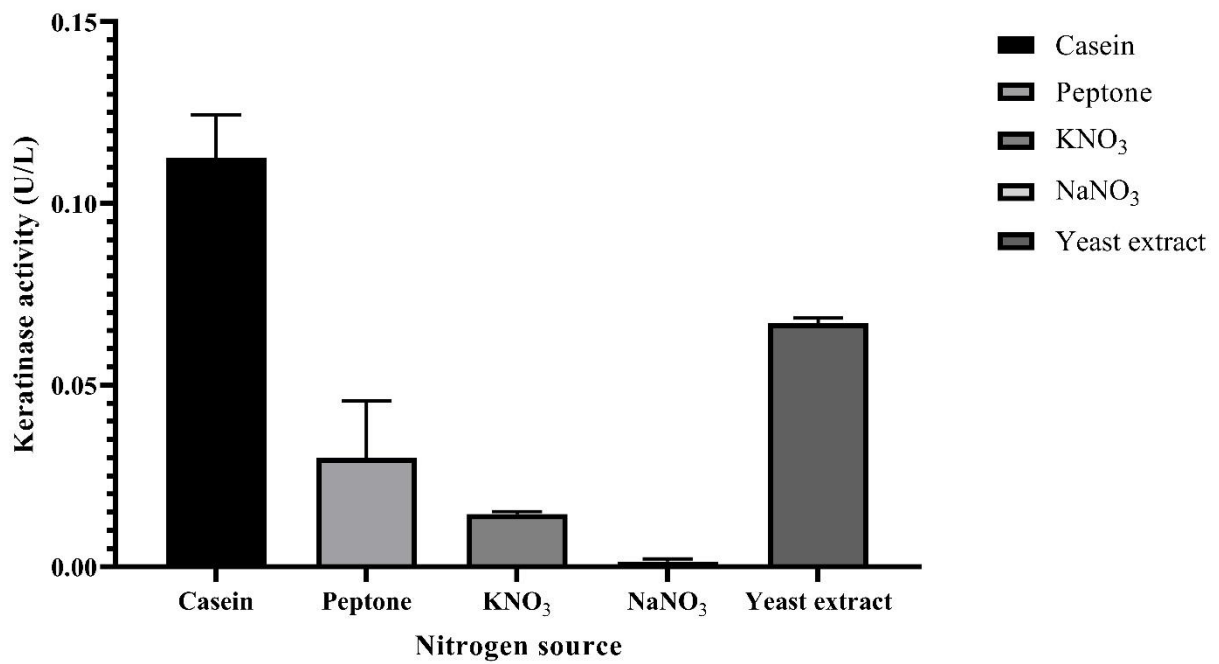


Figure 4.3: Keratinase Activity (U/L) of *B subtilis* with Different Nitrogen Sources

Source: Author's Laboratory Result, 2022

4.1.8 The Effect of Different Carbon Sources on Keratinase Activity of *Bacillus subtilis*

Bacillus subtilis was subjected to test for the production of keratinase using different carbon sources namely glucose, sucrose, mannitol and starch. The highest activity of 581.33 μL was recorded when starch was supplemented into the production medium when the least keratinase activity of 24.6 μL was seen when glucose was supplemented into the medium.

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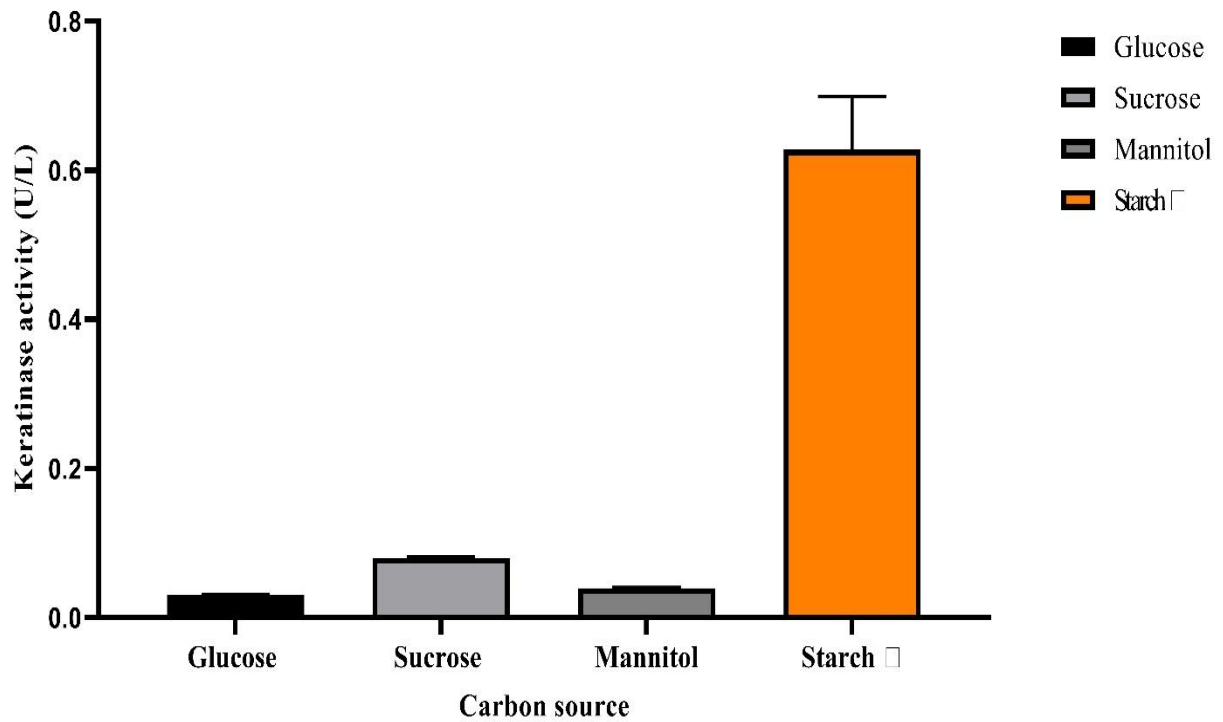


Figure 4.4: Keratinase Activity (U/L) of *B. subtilis* with Different Carbon Sources

Source: Author's Laboratory Result ,2022

4.1.9 Effect of Varied pH on keratinase Activity

Bacillus subtilis was subjected to test for production of keratinase enzyme at varied pH as shown on the Figure 4.5. Highest enzyme activity of 53 $\mu\text{l/L}$ was observed at pH7 while the least keratinase activity of 18.66 $\mu\text{l/L}$ was observed at pH 2. This implies that optimal degradation of feather by the isolate will be achieved at pH 7, a range within neutral pH. Also it was observed that keratinase activity was higher at neutral to alkaline pH range as compared to acidic pH. Increase in alkalinity has been observed to favor further rapid enzymatic attack of keratin by microorganisms resulting in higher level of keratinolysis ¹¹. This may be the reason bacteria are able to degrade feather than fungi.

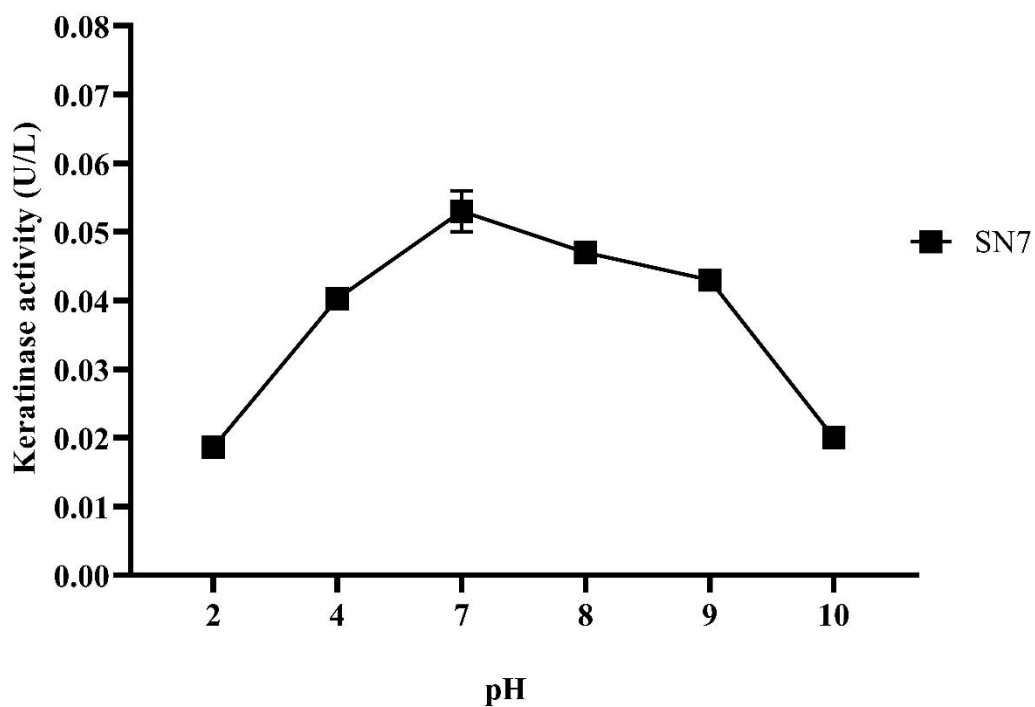


Figure 4.5: keratinase Activity (U/L) of *Bacillus subtilis* with Varied pH

Source: Author's Laboratory Result, 2022

4.1.10 Keratinase Activity (U/ μ l) of *B. subtilis* at Different Temperatures

Bacillus subtilis was subjected to test for production of keratinase at different temperatures as shown on the figure 4.6. It was observed that at 45°C the highest amount of keratinase (61 μ l/L) was produced while at temperature of 40°C the lowest amount of keratinase of 8 μ l/L was produced. This implies that *B. subtilis* produces keratinase typically at mesophilic temperature range (40–50°C) ¹⁰. However, keratinase activity reduced considerably at 50°C, an observation that time has not allowed its unravelment in this work.

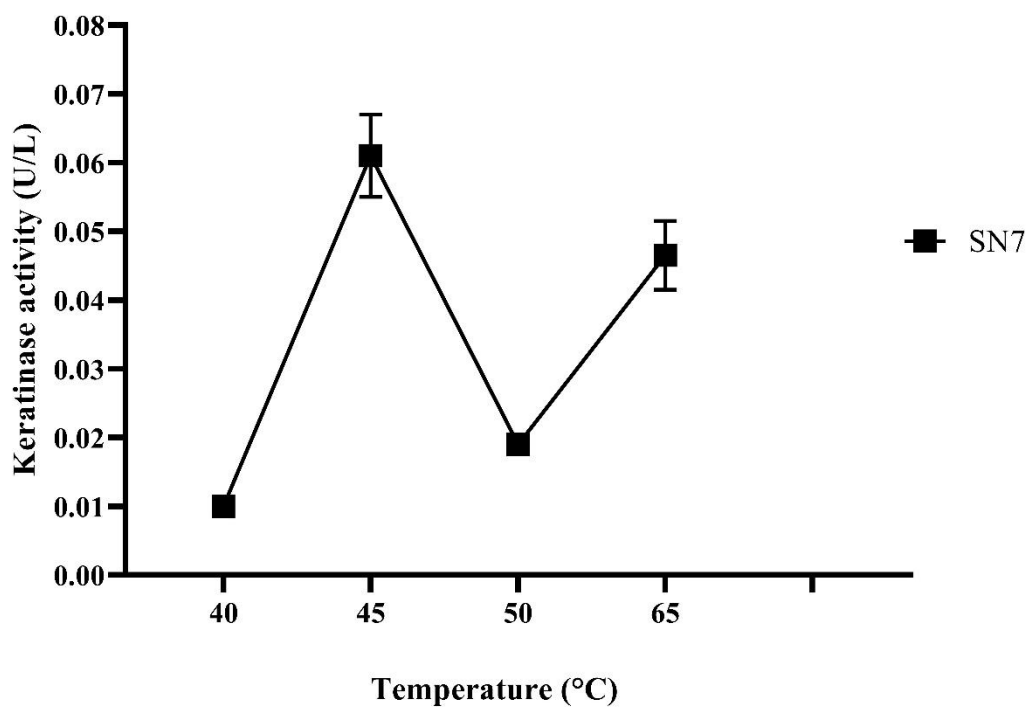


Figure 4.6: Keratinase Activity (U/ μ l) of *B. subtilis* at Different Temperatures

Source: Author's Laboratory Result, 2022

4.1.11 Application of Partially Purified Keratinase On Laboratory Scale-Sized Feathers to Achieve Feather Meal



Plate 4.3. Degradation of feathers after 48 hours

Source: Author's Laboratory Result, 2022



Plate 4.4: Feather Meal

Source: Author's Laboratory Result, 2022

4.1.12 Integration of The Recovered Feather Meal into Poultry Broiler Ration at Graded Concentration

Experimental Feed Design

- I. 100% hydrolyzed feather meal
- II. 75% feather meal, 25% fish meal
- III. 25% fish meal, 75% feather meal
- IV. 50% feather meal, 50% fish meal
- V. 100% fish meal
- VI. 100% non-hydrolyzed feather meal.

4.1.13 Effect of Feather Meal and Commercial Feed On Broilers Chicken

Eighteen Broilers were fed with experimental feeds that contain degraded feather meal and commercial fish meal at different percentages and it was observed that the weight of broilers fed with a smaller percentage of feather meal and a higher percentage of fish meal is higher than those fed with a higher percentage of feather meal. This implies that the low digestibility of protein feathers protein could affect the weight of broilers. The Control group fed with compounded feed devoid of Fish meal and feather meal had severe stunted growth.

Table 4.5 Weight of Broilers fed with 100% Fish meal

Day 1	4	7	10	14	18	21
0.50	0.60	0.80	0.95	1.15	1.35	1.55
0.50	0.60	0.80	0.95	1.10	1.35	1.50
0.50	0.60	0.80	0.95	1.10	1.35	1.50

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Table 4.6 Weight of Broilers fed with 50%fish meal and 50%feather meal

Day	1	4	7	10	14	18	21
	0.50	0.65	0.80	1.00	1.20	1.45	1.70
	0.50	0.65	0.80	1.00	1.30	1.55	1.70
	0.50	0.65	0.80	1.00	1.30	1.55	1.70

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Table 4.7: Weight of Broilers Fed with 25% Hydrolyzed Feather and 75% Fish Meal for 21 Days

Day 1	4	7	10	14	18	21
0.50	0.60	0.75	1.00	1.20	1.45	1.60
0.50	0.60	0.75	1.00	1.20	1.45	1.60
0.50	0.60	0.76	1.00	1.22	1.44	1.60

Source: Author's Laboratory Result, 2022

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Table 4.8: Weight of Broilers Fed with 75% Hydrolyzed Feather and 25% Fish Meal Feeds for 21 Days

Day 1	4	7	10	14	18	21
0.50	0.60	0.65	0.85	1.00	1.20	1.35
0.50	0.60	0.70	0.90	1.10	1.35	1.45
0.50	0.60	0.70	0.90	1.10	1.35	1.46

Source: Author's Laboratory Result, 2022

Table 4.9: Weight of Broilers Fed with Hydrolyzed Feather Meal 100%

Day 1	4	7	10	14	18	21
0.50	0.55	0.65	0.80	1.00	1.15	1.30
0.50	0.55	0.60	0.70	0.80	0.90	1.20
0.50	0.55	0.60	0.70	0.80	0.95	1.30

Source: Author's Laboratory Result, 2022

Table 5.0: Weight of Broilers Fed with Non-Hydrolyzed Feather Meal 100%

Day 1	4	7	10	14	18	21
0.50	0.55	0.60	0.70	0.70	0.80	0.90
0.50	0.55	0.65	0.70	0.60	0.70	0.75
0.50	0.55	0.65	0.70	0.65	0.75	0.75

Source: Author's Laboratory Result, 2022

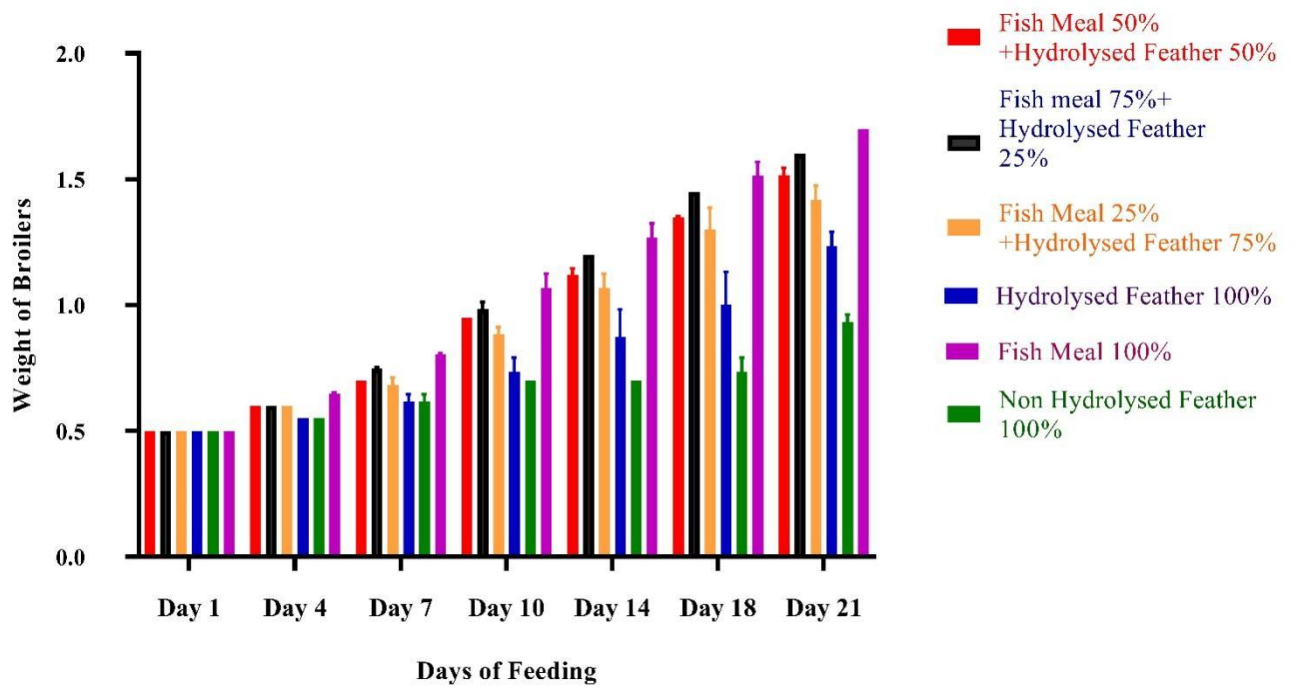


Figure 4.7: Growth Pattern of Broilers Fed with Compounded Feedstock with Feather Meal and Fish Meal at Varied Percentages

Source: Author's Laboratory Result, 2022

4.2 Discussion of Findings

The dumping sites contain large amount of feathers and native microbes may have adapted to utilize feather keratin as substrate. Various previous studies have reported the isolation of keratinolytic bacteria from dumping sites of feathers that exhibited the ability to degrade keratin¹. In this research, the keratinase enzyme producing *Bacillus* sp was isolated from feather dumps at optimum conditions for maximum enzyme production in feather minimum broth medium containing keratin feather, and degradation of feather by selected isolate was studied. These colonies were tested for their ability to grow on feather minimal isolate. Keratinase fermentation from thermophilic *Bacillus* spp. in minimal media containing feather meals at room temperature and pH 7 for 24 hours has been reported in research work by Laba et al.². The isolate SN7 was examined gram positive rod, catalase positive, oxidase negativeable to utilize Lactose, Arabinose, Sucrose and Mannitol but was unable to degrade glucose. The morphological and biochemical characters were compared with Bergey's manual of determinative bacteriology and this identified the isolate belonging to the *Bacillus* Sp. Similar result was reported by Williams and Garlich³, where a straight rod shape aerobic and endospore forming bacterium appeared in single or in chain catalase positive and gram variable classified as *Bacillus* sp was discovered. This bacterium isolated from high temperature poultry waste digest and able to degrade feather keratin when using feather as a primary source of carbon and energy. According to the results obtained from this present study, the results obtained from the 16S rDNA gene sequence analysis the isolates were conformed and identified as *Bacillus subtilis*. *Bacillus subtilis* is a Gram positive, rod-shaped, facultative anaerobic, motile and spore forming bacterium which is commonly found in soil and food and it has been discovered from studies that it possesses both proteolytic and keratinolytic potential⁴.

A number of factors play a very crucial role in enzyme production such as incubation time, temperature, pH, aeration, and composition of the fermentation medium ⁵. Temperature has great impact on enzyme production. A higher temperature generally results in an increasing enzyme production. If the temperatures rise beyond a certain point, however the enzyme production eventually leveled out than decline rapidly because the destroying of organism by high temperature. Selected isolate in this research was subjected to test for production of keratinase enzyme at temperatures of 40°C, 45°C, 50°C and 65°C. It was observed that at 45°C, the highest amount of keratinase enzyme (61 µl/L) was produced. It was previously reported that most *Bacillus* species are typically mesophilic or thermotolerant, and also demonstrated facultative growth at thermophilic temperatures (40–50°C) ⁶. Degradation of feather substrate was found to be associated with significant increase in pH of the medium to alkalinity, thus serving as an indicator for the efficiency of degradation. The tendency of the medium to turn alkaline has been attributed to deamination reaction leading to production of ammonia from protein, peptides and amino acids during keratin degradation. Increase in alkalinity has been observed to favor further rapid enzymatic attack of keratin resulting in higher level of keratinolysis ⁷. The selected isolate SN7 was subjected to test for production of keratinase enzyme at pH2, pH3, pH4, pH5, pH7, pH8, pH9 and pH10. This was done to determine the best pH for the optimization of the degradation of the feathers for this experiment. It was observed that at pH7 the highest amount of keratinase enzyme (53 µl/L) was produced. A previous research carried out by Samuel Pandian et al ⁸, shows that Maximum enzyme production was observed between pH 7 and 7.5 which is similar to the maximum pH obtained in this research. Other research work recorded neutral or alkaline pH as the suitable pH for production of keratinase from other *Bacillus* sp ⁹. Bacteria isolates were also tested for their ability to produce keratinase enzyme after 24 hours, 48 hours and 72 hours, isolate 7 was found to produce most amount of the enzyme at 24 hours (22.7µl/L) and

at 72 hours (181.7 μ l/. Keratinase production beyond the optimum time result rapid decline in the enzyme yield. The optimum incubation period in this study was found similar to the results of *Bacillus subtilis* carried out by Singh et al ⁶. Therefore, bacillus isolate 7 was selected for further degradation of the feathers to be used for this study. The selected isolate which is *Bacillus subtilis* was subjected to test for production of keratinase enzyme using different nitrogen sources using Casein, Peptone, KNO₃, NaNO₃ and yeast extract. It was observed that when casein was introduced into the medium, the highest amount of keratinase enzyme (86.33 μ l/L) was produced. Similar result was found in a previous research for *Bacillus cereus* SKH1 when grown in medium containing keratin as the major nitrogen sources ¹⁰.

The *Bacillus sp* was also subjected to test for production of keratinase enzyme using different carbon sources such as glucose, sucrose, mannitol and starch. This was done to determine the best temperature for the optimization of the degradation of the feathers for this experiment. It was observed that introduction of starch into the medium, the highest amount of keratinase enzyme (581.33 μ l/L) was produced.

Raw feathers are relatively insoluble and have a very low digestibility of 5% hence; a great deal of research interest has been aroused over the possibility of processing feather to enhance its digestibility ⁴. In this research work, when the partially purified keratinase was applied on laboratory scale-sized feathers to achieve feather meal for nine days, the feathers were degraded and washed to integrate the recovered feather meal into poultry broiler ration at graded concentration and give as feed to broilers, Result showed that degradation of feathers for nine days occurred at optimum condition(temp 45⁰c,Ph 7.addition of yeast extract and starch).After the degradation process, shape of the feather in the shake bottle was no longer visible with the naked eye. indicating the feather had been completely degraded by *B.*

subtilis This is similar to experimental work carried out by Zhoufeng, et al ¹¹. Feather meal is among the most protein rich by-products. In contrast to fishmeal, soybean meal and groundnut cake, broiler feathers are cheaper and has no competing demands between humans and livestock. Up to this time, there is no documented report about any major mycotoxic agent associated with broiler feathers which could affect humans when fed to livestock ¹².

Broilers production is gaining high momentum in Nigeria because of its quick returns on investment, short production period and provision of highly palatable and good quality meat. The popularity of poultry has resulted from the comparative advantages of poultry over other livestock ¹³. However, there are challenges faced in the production of poultry meat and one of the pending issues concerning poultry production is feed, as a result of the competition involved for usage of the conventional feed ingredients by human, animal and industry which has resulted to high cost of conventional feed ingredients and in turn made feed cost to account for high range in the overall production cost ¹⁴.

Conventional protein and the energy involved in their production have contributed to the high price of animal feed which consequently lead to high price of the animal at the end of production. Hence, it becomes necessary to source for an alternative economical protein sources for animal production most especially from the poultry wastes which are abundantly available in Production and evaluation of biodegraded feather meal ¹⁵.

At day 4, the weight of the broilers that were fed with 100% Fishmeal was 0.65kg which was the highest having significant difference from the result obtained from the other treatments. The broilers fed with non-hydrolyzed feather meal had the lowest weight of 0.55kg and was significantly different from the 100% Fishmeal and also significantly different from that of the other treatments.

At day 7, the weight of broilers fed with 100% Fish meal was 0.8kg (having the highest value) also significantly different from that of the other treatments. Non- hydrolyzed feather had a weight of 0.575 (lowest) and was significantly different from that of the other treatments. The birds fed with 100% hydrolyzed feather meal and 25%fishmeal with 75% hydrolyzed feather had of 0.675, while those fed with 50% fishmeal with 50% hydrolyzed feather and 75% fishmeal with 25% hydrolyzed feather had a growth weight of 0.7g and 0.75g respectively.

At day 10, birds fed with 100% fishmeal and the 75%fishmealwith 25% hydrolyzed feather had a growth weight of 1kg. The broilers fed with 100% non-hydrolyzed feather meal had a growth weight of 0.625 while the 25% fishmeal with 75% hydrolyzed meal and the 100% hydrolyzed feather meal had a weight of 0.7125 and 0.875 respectively. Significant difference was found between the treatments and between the hydrolyzed feather meal and the non-hydrolyzed feather meal (this was observed for all the days).

At day 14, birds fed with 100% fishmeal was 1.25 and was the highest while with those fed with the non-hydrolyzed feather meal had a weight of 0.75 and treatments were significantly different from each other. At day 18 and 21, similar results were obtained as that obtained for day 14 but the growth weight values increased after the 18th day and the 21st day. At day 21, 25% fish meal with 75% hydrolyzed feather meal and the 75% fishmeal with 25% hydrolyzed had a growth weight of 1.6 showing there was no significant difference between them but they showed significant difference from the other treatments.

Endnotes

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Chapter Five

Conclusion

5.1 Summary of Findings

Microorganisms isolated from the feather sample obtained from feather dump sites in Ibadan were found to be positive for proteolytic and keratinolytic activity on casein and feather medium respectively. The optimum condition for feather degradation were observed at pH 7.0 and at 45⁰c.

Eighteen broilers were fed with experimental feeds of the degraded feather at different percentage and it observed that the weight of broilers fed with smaller percentage of feather meal and those fed with the feed that contains fish meal as source of protein had recorded weight higher than those that are fed with higher percentage of feather meal.

5.2 Conclusion

Birds fed with 100% fish meal-based diet performed best, those fed with 50% fish meal combined with 50% feather meal performed a little lower than the first group mentioned. The isolated keratinolytic microorganism could be a potential candidate for degradation and utilization of feather keratin to produce animal feed protein.

It is an indication that with proper pretreatment of feathers with microbes like *B. subtilis*, the recalcitrant nature of this waste can be impaired making the protein locked within it available for broiler birds as nutrient when incorporated into their feed in combination with fish meal at proper ratio. This approach will also indirectly contribute to getting rid of waste in our environment.

5.3 Recommendations

1. Feather meal can be used as a protein source for broilers with no noticeable negative effect thereby reducing the cost of feed and providing a means of reducing the cost of disposal of agricultural products such as feathers.
2. The results from the feeding trial revealed that birds fed with the microbially degraded feather meal performed slightly better than the unhydrolyzed feather meal. The effect may be clearer with further studies with a longer duration of hydrolysis and feeding manipulations.
3. It can also be suggested that further studies on feeding modification may enhance the growth performance of broilers that will be fed with keratinase-degraded feather meal-based diets.
4. It will also be useful in biotechnological applications since the isolated microorganism(s) that will degrade feathers from this work can be bioengineered to improve specific gene that encodes keratin degradation.

5.4 Contribution to Knowledge

This work has proved that microorganisms with keratinolytic capacity could be obtained from feather waste and that microbiologically degraded feather meal could be used as a source of protein or supplement in broiler compounded feeds without a noticeable negative effect on the birds.

The chief anti-nutrients in feathers are keratins which can be effectively degraded by several processing methods such as boiling or steaming, chemical treatment and enzymatic degradation. Of all these techniques, enzymatic degradation of keratins through the use of microorganisms is the most economical because it is cheaper and does not involve the use of hazardous chemicals nor does it involve generation of excess heat to the environment.

Incorporation of broiler feathers into livestock feed particularly the feeding of broiler chickens, is an effective means of reducing its environmental nuisance.

Also from this research it can be deduced that Feather meal should be introduced into the feed ration gradually because the protein in feather meal is degraded slowly in the rumen compared to most other protein sources.

Considering the high cost of conventional protein feedstuffs such as groundnut cake, soybean meal and fishmeal, feather meal can be effectively used as a cheaper replacement for these expensive protein feedstuffs. This is because broiler feathers are easy to obtain and does not cost much to process into edible meal for feeding poultry birds.

Broiler feathers are a rich protein feedstuff that can be potentially utilized across all facets of livestock feeding for proper growth and development in order to produce wholesome animals and animal products such as meat, milk and eggs.

5.5 Suggested Areas for Further Research

- The microorganism isolated from this work could be bioengineered to improve the specific gene that encodes keratin hydrolysis for better degradation of feathers and also make it readily available for commercial purpose.
- Continuous studies are needed to find the optimal inclusion levels of feather meal in diets of broiler chickens generally to further reduce cost and improve growth performance.
- Digestibility of the hydrolyzed feather meal by the broilers may be low and this may have affected the weight of the broilers therefore there is need to carry out further studies on the duration of hydrolysis that will enhance feather meal digestibility by poultry birds

- More feeding trials could help to provide useful information on the effects of feather meal on performance and carcass attributes of broiler chickens. This will increase awareness on the usefulness of broiler chickens feather as a cheaper animal protein source thereby reducing environmental nuisance posed by indiscriminate disposal of broiler feathers and hence contribute to effective recycling of this protein-rich poultry by-product.

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<https://afs.ca.uky.edu/poultry/poultry-feathers-what-can-they-be-used>

Appendix I

Effect of Nitrogen Sources on Keratinase Activity

	Rep 1	Rep 2	Rep 3	Average	Standard Deviation
Casein	0.034	0.104	0.121	0.086333	0.046112
Peptone	0.019	0.091	0.041	0.049278	0.036896
KNO ₃	0.014	0.015	0.028	0.019	0.00781
NaNO ₃	0.001	0.007	0.002	0.003333	0.003215
Yeast Extract	0.068	0.052	0.066	0.062	0.008718
Control	0.017	0.016	0.015	0.016	0.001
TCA	0.06			0.06	

Source: Author's Laboratory Result,2022

Appendix II

Effect of Carbon Sources on keratinase Activity

	Rep 1	Rep 2	Rep 3	Average	Standard Deviation
Glucose	0.013	0.031	0.03	0.024667	0.010116
Sucrose	0.004	0.081	0.079	0.043529	0.04389
Mannitol	0.079	0.039	0.04	0.052667	0.022811
Starch	0.678	0.489	0.577	0.581333	0.094574
Control	0.011	0.008	0.009	0.009333	0.001528
TCA	0.033	0.027	0.028	0.029333	0.003215

Source: Author's Laboratory Result,2022

Appendix III

Effect of pH on keratinase Activity

	Rep 1	Rep 2	Rep 3	Average	Standard Deviation
pH2	0.007	0.009	0.04	0.018667	0.018502
pH4	0.03	0.076	0.037	0.040376	0.024786
pH5	0.006	0.021	0.02	0.015667	0.008386
pH6	0.033	0.029	0.043	0.035	0.007211
pH7	0.019	0.09	0.05	0.053	0.035595
pH8	0.026	0.035	0.08	0.047	0.028931
pH9	0.042	0.072	0.043	0.128	0.017039
pH10	0.042	0.014	0.02	0.0623	0.014742

Source: Author's Laboratory Result,2022

Appendix IV

Changes in Keratinase Activity of Bacteria Isolates (*Bacillus subtilis*) with Time

Number of
Hours

24

Rep1	Rep2	Rep3	Average	Standard Deviation
0.01	0.028	0.026	0.02133333	0.009866
0.005	0.014	0.009	0.00933333	0.004509
0.017	0.025	0.026	0.02266667	0.004933
0.003	0.001	0.001	0.00166667	0.001155

Source: Author's Laboratory Result,2022

Appendix V

Changes in Keratinase Activity of Bacteria Isolates (*Bacillus subtilis*) with Time

Number of

Hours

48

Rep 1	Rep 2	Rep 3	Average	Standard deviation
0.2	0.011	0.001	0.070667	0.076515
0.008	0.001	0.002	0.003667	0.003648
0.069	0.037	0.025	0.043667	0.021399
0.005	0.01	0.001	0.005333	0.004509

Source: Author's Laboratory Result,2022

Appendix VI

Changes in Keratinase Activity of Bacteria Isolates (*Bacillus subtilis*) with Time

Number of Hours		72		
Rep 1	Rep2	Rep3	Average	Standard
0.031	0.028	0.019	0.026	0.004726
0.002	0.014	0.013	0.009667	0.002269
0.116	0.236	0.193	0.181667	0.028663
0.008	0.110	0.001	0.003	0.001528

Source: Author's Laboratory Result,2022

Appendix VII

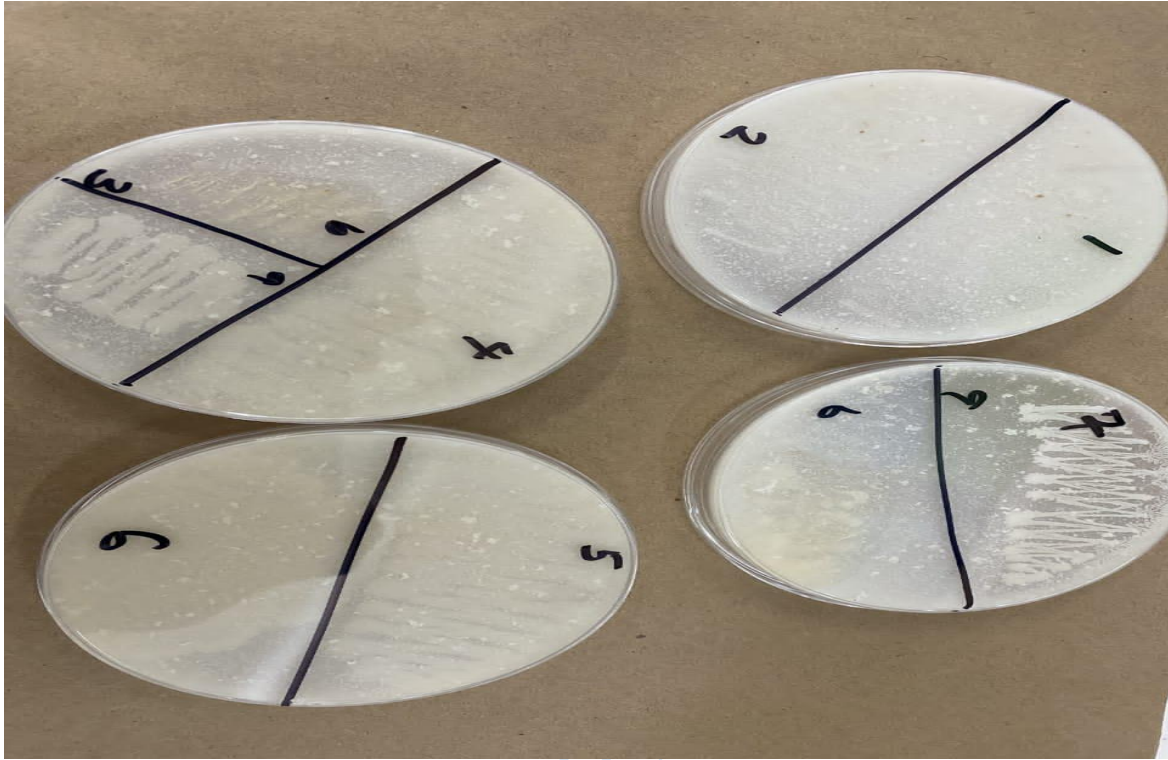
Sample SN7

GYYYCYMRRRCGGRTTGCKKTWATGCGTTWGCTGCAGCACTRARGGGCGGAAA
CCCYCYAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATC
CTGTTYGCTCCCCACGCTTTCGMTCCTCAGCGTCAGTTACAGACCAGAGAGYCGC
CTTCGCCACTGGTGTTCTCCAYATMTCTACGCATTTACCGCTACACRTGGAAT
TCCACTCTCCTCTTCTGCACTCAAGTTYCCCAGTTTCCAATGACCCTCCCCGGTTG
AGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAKCCCTTTACGCCCA
ATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGT
TAGCCGTGGCTTTCTGGTTAGRTACCGTCAAGGKACCGCCCTATTCKAACGGTAC
TTGTTCTTCCCTAACAACARAGCTTTACRATCCRAAAACCTTCATCACTCACGCG
GSGTTGYTCCGTCARAYTTTCGTCCATTGCGGAARATTCCTACTGYTGCCTCCCG
WAGGAGTYTGGGCCGKGTCTCAGTCCCAGKKGKGGCCGATCACCTCTCAGGTGCG
GYTACGCWTCGKTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGW
TCAAACRACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAG
GCAGGTTA CAAACTCTG

Isolate7- Bacillus

Source: Result derived from the Amplified Fragments That Were Sequenced Using Genetic Analyzer 3130xl Sequence

Appendix VIII



Picture showing microorganisms cultured on plates (Skimmed milk agar).

Source: My Personal phone camera

Appendix IX



Picture of Feather Meal Mixed with commercial feed

Source: My Personal phone camera

Appendix X



Picture Showing Fish Meal Used as Part of Feed for Broilers.

Source: My Personal phone camera

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Appendix XI



Picture of 2 weeks old Broilers

Source: My Personal phone camera

Appendix XII



Picture of 3 weeks old Broilers

Source: My Personal phone camera

Appendix XIII



Picture of 2 weeks old Broilers

Source: My Personal phone camera

Appendix XIV



Picture of 3 weeks old Broilers

Source: My Personal phone camera

Appendix XV



Picture of 4 weeks old Broilers

Source: My Personal phone camera

Appendix XVI



Picture of 4 weeks old Broilers

Source: My Personal phone camera

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Picture of 5 weeks old Broilers

Source: My Personal phone camera

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Bio – Data

A. Personal Data:

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GRA Ibadan

B. Educational Background

Educational Institution Attended with Dates and Qualification:

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• St Louis Grammar School, Mokola Ibadan	1995-2001	West African Exam
• The Polytechnic Ibadan	2008-2010	HND Micro Biology
• Lead City University Ibadan	2019-2022	M.S.C in view

C. Working Experience with Dates:

- Personal Business

.....
Signature

.....
Date

University Compliance Certification

This is to certify that the thesis by **Joseph, Olamide Adenike** with Matric no LCU/PG/001236 in the department of **Food Microbiology**, Faculty of Basic Medical and Applied Sciences, Lead City University, is in full compliance with the approved university format and style.

.....

Signature

.....

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