ILEAL CRUDE PROTEIN DIGESTIBILITY OF FEATHER MEAL SUPPLEMENTED WITH PROTEASE IN BROILER CHICKS

BY

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ABSTRACT

Conventional protein ingredients are expensive, thus necessitating the use of alternative sources of the nutrient. Feather Meal (FM) is high in Crude Protein (CP) but poorly digested by monogastric animals due to the presence of keratin. Poultry requires exogenous enzymes to break it down to its constituent amino acids. Information on digestibility of CP of FM in broilers is scanty. Therefore, ileal CP digestibility of FM supplemented with protease in broiler chickens was investigated.
Broiler feathers were hydrolysed to obtain FM using standard procedures. The feathers and FM were analysed for their respective chemical compositions. One hundred and forty-four 2-week old broilers weighing 223.0±11.1g were randomly allotted to four diets containing 0, 2, 4 and 6% FM and TiO_2 at 5 g/kg diet as marker for two weeks. Body Weight Gain (BWG, g/bird), Feed Intake (FI, g/bird) and Feed Conversion Ratio (FCR) were assessed. Digesta was collected from all birds to determine Apparent CP Digestibility (ACPD). Another 336 twenty-one-day old broilers were randomly assigned to eight diets containing 0, 2, 4 and 6% FM with 0 or 5 g protease/kg diet and TiO_2 in a 2x4 factorial arrangement. At day 28, digesta was collected from all birds for Digestible CP (DCP) determination in FM using standard procedures. In a growth study, 360 1-day old broilers were randomly allotted to twelve diets containing 0 or 2% FM at three CP levels (15.5, 17.5 and 23%) based on recommended matrix value of protease and two protease levels (0 and 5 g/kg). They were fed for 42 days in a 2x2x3 factorial arrangement. The BWG, FI, FCR, carcass measures and organ weights were determined. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Percentage chemical composition were 88.6 CP, 5.0 ether extract, 0.3 ash, 0.03 calcium, 0.04 phosphorus and 4.4 Kcal/g Gross Energy (GE) for the broiler feathers and 83.8 CP, 10.3 ether extract, 0.6 ash, 0.02 calcium, 0.03 phosphorus and 4.3 Kcal/g GE for FM. The ACPD (%) were 42.0±2.1, 52.0±1.9, 58.0±2.4 and 63.0±2.8; FI obtained were 358.3±9.8, 323.6±15.6, 283.9±13.1 and 307.8±1.2; BWG were 218.9±24.9, 194.6±19.0, 145.7±15.6 and 152.8±16.8 and FCR were 1.6±0.3, 1.7±0.2, 2.0±0.3 and 2.0±0.2. With protease supplementation, ACPD (%) were 58.8±2.0, 64.1±2.0, 75.8±1.5 and 85.3±2.3 for birds on diets 0, 2, 4 and 6% FM respectively. The DCP in FM was 65.9%±1.4 and 76.1%±2.3 without and with protease respectively. The functional relationship between DCP and CP intake from FM without protease supplementation was highly significant (R^2=0.98). The FM inclusion significantly decreased FI from 63.4±2.1 to 55.2±0.7 and BWG from 243.7±7.5 to 197.3±6.6 but increased FCR from 2.7±0.1 to 2.9±0.1 at 0-21days. At 22-42 days FI decreased from 1647.5±25.3 to 1302.9±23.2, BWG from 605.4±15.0 to 579.1±18.7 and FCR from 2.8±0.0 to 2.3±1.0. Protease supplementation improved live weight, percent dressed weight and significantly increased the breast meat by 2.1, 1.9 and 4.1% at 42 days.

Feather meal inclusion levels above 2% and its ileal digestibility required protease supplementation for improved broiler performance.

Keywords: Hydrolysed feather meal, Ileal digestibility, Protease supplementation, Broiler chicken performance

Word count: 492

DEDICATION

To the one who's I am, whom I serve and for whom I have suffered the loss of many things and through whom I have gained all things;

To Him who alone preserved my life in accidents at home and on the road,

My GOD in Him I trust.

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CERTIFICATION

I certify that this work was carried out by Helen Inikpi AJAYI in the Department of Animal Science, University of Ibadan. Ibadan.

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CHAPTER ONE

1.0. INTRODUCTION

The expected 2% yearly increase in livestock production worldwide (Wenk, 2000) indicates that a crisis will be precipitated in the livestock and feed industries in the near future due to increasing demand for food by humans and feed by animals and also in the vast amount of wastes that will be generated as a result. Man and animals basically feed on same foods but when there is scarcity, man is considered first. This consideration for man first will eventually lead to starvation for man as, less and less of animal protein will be available for him. Finding alternatives to the regular feedstuff being competed for by both humans and animals will on the long run save man from starvation. Globally, the demand for poultry meat is on the increase as more and more people see the health benefits of feeding on white meat instead of red meat. A survey of poultry meat production over 10 years reveals that developing countries like India, China, Brazil and Vietnam recorded the highest percentage increase between 1995 and 2005 (FAO, 2007a and b). Chicken meat production and consumption in the United States exceeded that of beef or pork (Scanes, 2007). These have led to the quest for the use of by-products with nutrient value for animal feeding and less or no value for humans. There is however, the need to ascertain the availability, affordability, accessibility and nutrient digestibility of these alternative feedstuffs. Many by-products have been investigated and a number are being used in animal feeding. Many of these are energy sources which are relatively cheaper than protein sources. They include cassava peel meal, oil sludge, brewer's dried grain (BDG) and palm kernel cake (PKC). Generally protein sources are more expensive than other nutrient sources when their percentage in diet is compared with other nutrients.

In feeding protein feedstuff however, the environmental problem of nitrogen pollution from animal wastes must be taken seriously. According to Raney *et al.* (2009) and Wenk (2000) world poultry production has the highest rate of increase due to increased demand for meat. This means the increased production of agricultural feedstock is driven by poultry production which accounts for about 70% of nitrogen in the environment from animal wastes. Development towards industrial production systems where wastes end up in the environment makes it more urgent a problem to be handled. Thus, world food production must grow without increasing the environmental waste load. This realization by animal nutritionists has forced a shift in focus from maximizing performance (i e. meeting the protein requirements of the animal, which does not take into cognizance oversupply of protein/amino acid) to meeting the requirements with the lowest nitrogen output.

In order to meet the animal's requirement for protein without oversupply, adequate knowledge of digestibility of protein or amino acids is inevitable. This is because the digestibility of amino acids cannot be simply deduced from crude protein digestibility as the data from the Dutch Bureau of Livestock Feeding (1994) shows. Amino acid digestibility is therefore a more satisfactory basis for poultry diet formulation and for monogastric animals generally. Digestibility is one of the main determinants of feeding value of feed ingredients. It is a measure of the biological availability of an ingested and digested nutrient, and the extent of absorption and its use in metabolism (Ammerman *et al*., 1995). For a feed ingredient to be effectively used in diets, it is therefore important to properly estimate the feeding value of such an ingredient. Other determinants of nutritional value of feed ingredients include: total nutrient content, contents of anti-nutritional factors, physico-chemical properties, ingredient-specific

effects on utilization of absorbed nutrients, effects of feed ingredient on voluntary feed intake and the effects on the final animal product quality (meat, eggs, milk and manure).

Determination of protein/amino acid digestibility of any dietary protein source is essential to its appropriate use with little or no detrimental effect on the environment. This approach will also reduce the cost of production as protein feedstuffs are expensive. This means that if the cost of the protein source is reduced, the cost of the feed will be reduced. Successful use of feather wastes as protein source in poultry diets comes with no competition between man and animal for it.

As the consumption of poultry meat increases so will the production of feathers; there is particular need in developing countries (where animals compete stiffly with humans for available conventional feed ingredients and there is lack of good environmental laws and or its enforcement) to find an economic and efficient use for this waste. The environment will also be better for it as the amount of nitrogen from degrading feathers would have been converted to a better use with or without good environmental laws. Finding alternatives to conventional feed ingredients is a vital key to reducing cost of animal protein which is directly proportional to the cost of production *viz-a-viz* cost of feed ingredients. Animal protein will therefore be more available and at a cheaper rate for man.

1.1. JUSTIFICATION OF STUDY

Feathers are wastes from the poultry industries which increase nitrogen in the environment; a reduction in the environmental nitrogen will therefore be of great value. With an average feather percentage of 5.9 - 8.52 for broiler chickens at 8weeks

(Ajayi, 2010) and 8 - 10 % as reported by Kim and Patterson (2000) and Karthikeyan *et al.* (2007), a poultry farm producing 30,000 birds per day with an average bird live weight of 2kg will produce about 2 tonnes of feather wastes yearly. Putting feather wastes to good use will make more lands available for use as landfills for their disposal will become vacant.

Use of proteases in animal feeds help to reduce the amount of proteins fed, which directly affects the amount of protein (nitrogen) voided (Oxenboll *et al.*, 2011).

Fish meal is expensive and a cheaper replacement will reduce the cost of poultry protein production.

The conversion of feather protein to a useful protein source will lead to increase in animal protein.

This study investigated utilization of feather meal supplemented with a mono-component protease for poultry production.

1.2.0 OBJECTIVE

To determine digestibility of crude protein of hydrolyzed feather meal with or without protease enzyme supplementation in broiler chickens.

1.2.1. THE SPECIFIC OBJECTIVES

- To produce and evaluate the proximate composition of hydrolyzed feather meal (HFM) from feathers.

- To estimate the optimum level of HFM for broiler chickens based on its apparent crude protein digestibility (ACPD).
- To determine the CP ileal digestibility of HFM and the effect of supplementation with a protease, CIBENZA DP^{100}.
- To evaluate the effect of HFM and supplementation with a protease on the growth performance and carcass measures in 42 d-old broiler chicks.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. ANIMAL FEEDS

Animal feed is very important in animal production, as it determines to a large extent the cost of production, performance and carcass quality of animals. Protein source is the second most expensive of all feed stuff for animal feeds (Agricultural Review, 2011). While a kilogramme of Fishmeal costs ₦550; a kilo of maize is ₦80; and a kilo of Soya bean meal is ₦160 (Ajayi, 2012). Feeds are therefore more expensive when more of protein feedstuffs are used. Any feedstuff that can replace or reduce the inclusion level of the conventional plant protein source in a feed will definitely reduce the cost of production. Proteins are very important for animal growth and development. As a result, many alternatives to the regular feedstuffs are being investigated for use in animal feeds. A number of food wastes as non-competitive feedstuff have been successfully used in animal feeding but most of these are energy sources. These and other materials not fully utilized are resources that can be exploited in animal feed production. Virtually all types of industries produce wastes and waste utilization is an ecologically safe and economically efficient means of waste management (Okonko *et al.*, 2006). Feathers are wastes generated from the processing of poultry birds.

Sometimes feed additives are added to animal feeds to improve digestion and nutrient availability in feedstuffs. These help to reduce the cost of production and maximize feed nutrients. Feed additives include enzymes, probiotics, prebiotics, dietary amino acids, minerals and vitamins, toxin binders and metabolic modifiers.

2.2. FEATHERS

Feathers distinguish birds from other vertebrates. There are different types of feathers; contour feathers, down feathers, semiplumes, filoplumes and powder downs. A feather has a hollow central shaft known as the rachis from which barbs branch out. The barbules branch out from the barbs (Figure 1). In down feathers, the barbs and barbules are loose and fluffy; the rachis is either missing completely or substantially reduced in length. Feathers serve to keep birds warm. Feathers are almost entirely proteins.

Feathers are made up of protein called keratin; the same tough protein fiber that makes up hair, wool, horns, scales, fingernails and hooves. Keratin monomers assemble into bundles to form intermediate filaments (Figure 2), which are tough and insoluble and form strong tissues found in reptiles, birds, amphibians, and mammals. The only other biological matter known to approximate the toughness of keratinized tissue is chitin found in insects. Feather is about 90% keratin (Karthikeyan *et al.*, 2007) which is the outstanding protein fibre structure that still lacks an interpretation generally acceptable in any detail. It has long been known that its X-ray diffraction diagram is of a β-type with the strong indication that the residue-length is only about 3110 Å (Martinez-Hernández and Velasco-Santos, 2012) but some works (Table 1) have shown a predominant α- type (Reddy and Yang, 2007).

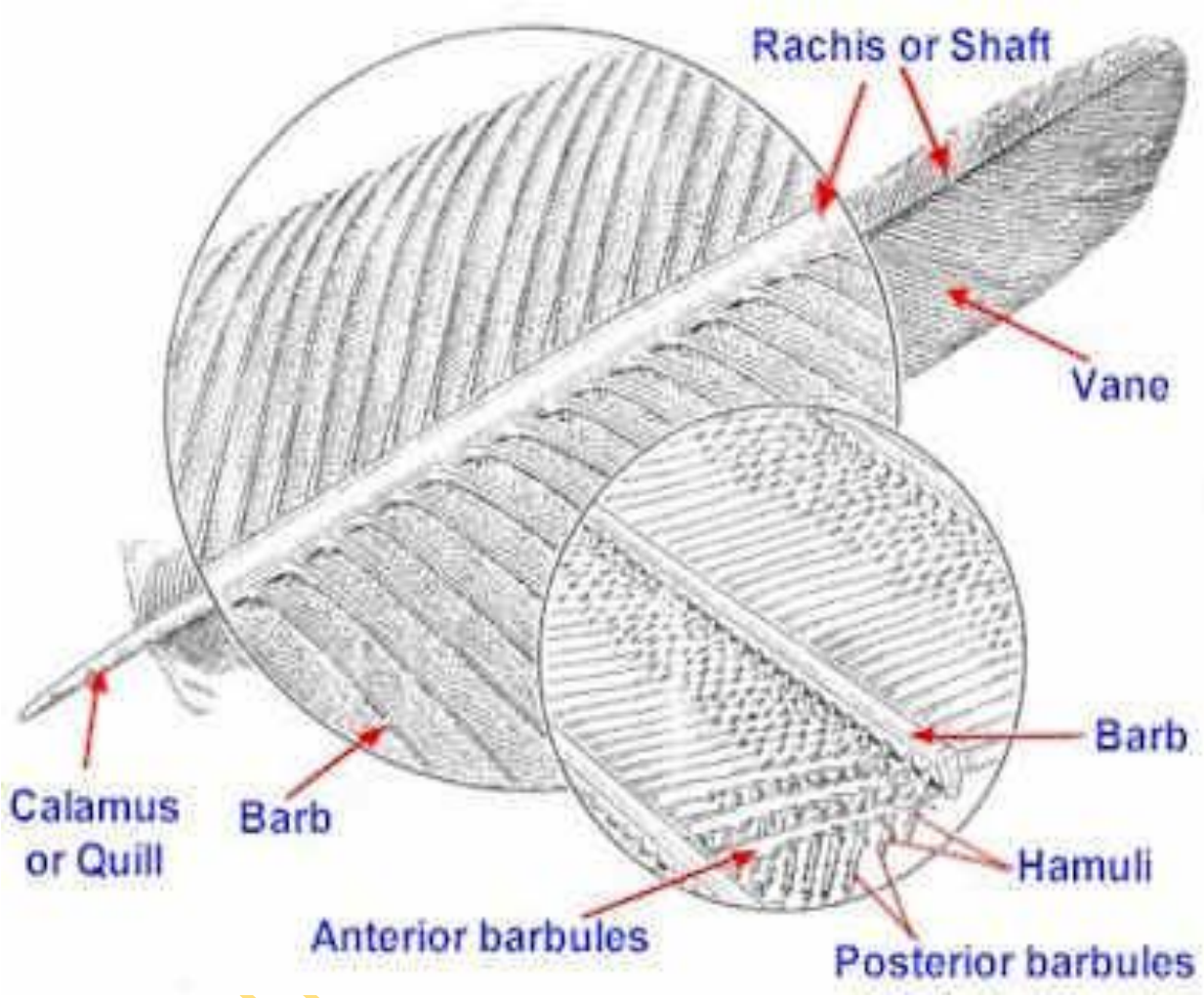


Figure 1: Structure of Feather.
Source: Fernbank Science centre, Atlanta.

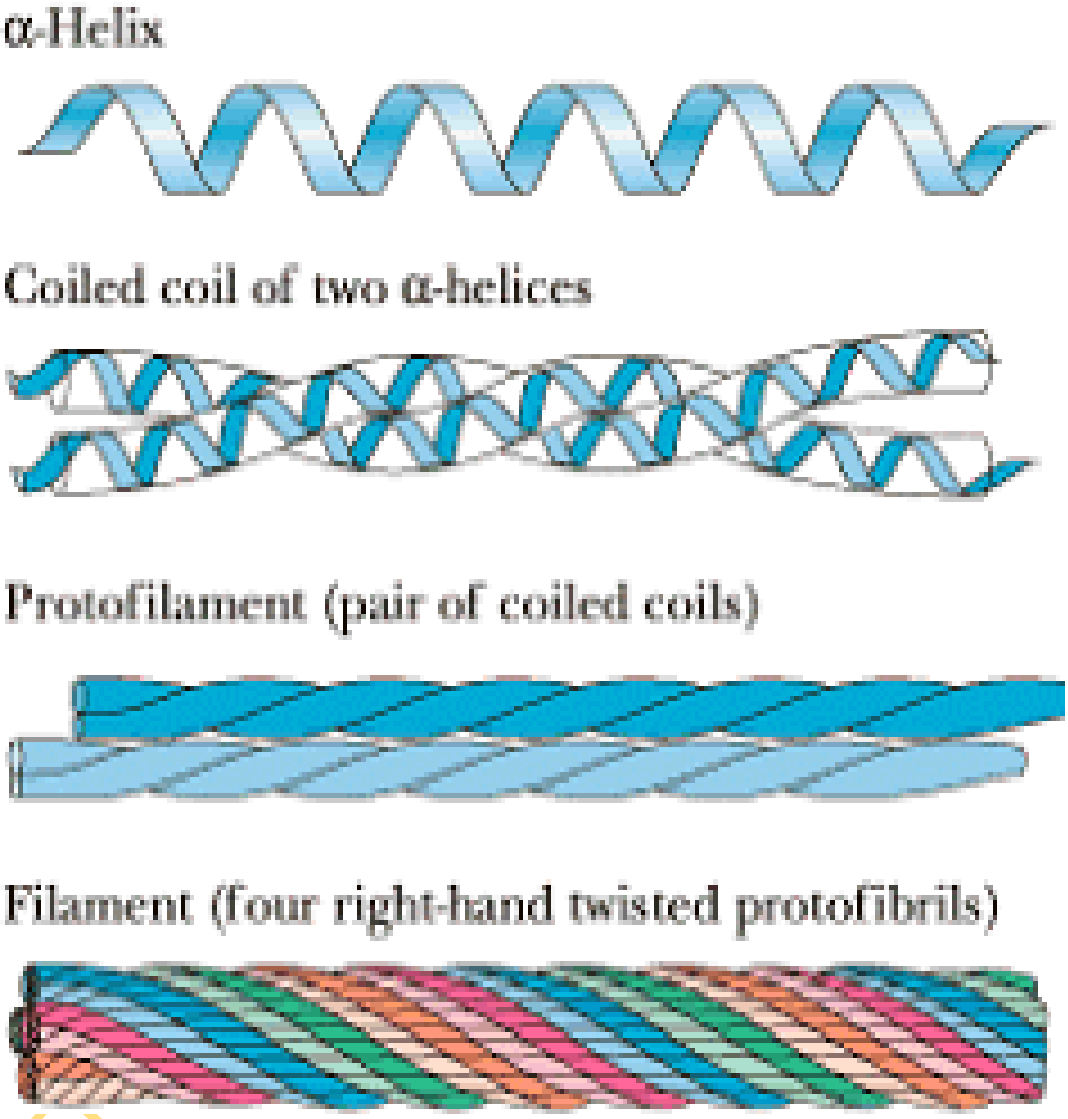


Figure 2: Filaments in keratin structure.
Source: Parry *et al.*, (1977).

Table 1: Proposed keratin secondary structure of chicken feathers

Feather part	Proposed secondary structure	Reference
Barbs	α- keratin type	Reddy and Yang, 2007
Rachis	78% β- sheet, 18% helical from twisted sheet	Schor and Krimm, 1961
Non specific	9.38% α- helix, 47.19% β- sheet, 32.25% β- turn and 11.18% random	Sun *et al*., 2009
	41% α-helix, 38% β- sheet and 21% random	Fraser *et al*., 1971

Source: Extracted from Martinez-Hernández and Velasco-Santos, 2012.

The moleculer weight of feather keratin is 10,500g/mol (Meyers *et al.*, 2008). The amino acid content of feathers depends on the breed, food and environment but

generally serine, proline, glucine, valine, and cystine are more abundant (Walker and Rogers 1976, Schmidt, 1998 and Martinez-Hernández *et al*., 2005).

Keratin contains about 10% cystine, which is formed as a result of the formation of disulphide linkages between two cysteine molecules. Keratins are classified as hard or soft depending on the percentage sulphur content. Hard keratin contains at least 5% sulphur while soft keratin contains about 1% (Karthikeyan *et al.,* 2007). These disulphide linkages can be broken by keratinases yielding cysteine and other amino acid molecules. Animals do not produce keratinase and as a result cannot breakdown any food containing keratin (El-Boushy *et al.,* 1990). Feathers are by-products of poultry processing. A poultry farm producing 30,000 birds per day will produce an average of 1,189,440kg feathers per year (Ajayi, 2010) in Nigeria if the birds are slaughtered at 8 weeks.

2.2.1. FEATHER MEAL

Feather Meal (HFM) for animal is produced by hydrolyzing feather at 40^0C temperature and pressure of 207 - 690kPa for 6 - 60minutes with moisture of 60 - 70% (El-Boushy *et al.*, 1990); this is similar to autoclaving (McGovern, 2000); the resulting HFM has a crude Protein (CP) of not less than 80%, crude fat (EE) of less than 6% and a crude fibre (CF) of not less than 3% (Poultry protein and fat council, 2009).

So far, HFM has been fed successfully to dairy cows, sheep and swine because it is an excellent source of by-pass protein and sulphur containing amino acids (Jordan and Croom, 1957; Combs *et al.*, 1958; Goedeken *et al.*, 1990; Blasi *et al.*, 1991; Thomas *et al*, 1994; Klemesrud *et al.*, 1998; Southern *et al.*, 2000, Apple *et al.*, 2003) but it has not been so in poultry production. Shiroma and Hongo (1974) stated that

methionine, lysine, histidine and tyrosine are considered deficient in HFM, while arginine, cystine, phenylalanine, threonine, valine, leucine and glycine are abundant compared to their levels in fish meal. According to Eissler and Firman (1996) HFM contains 2.09% lysine, 0.49% methionine, 5.16% cystine and 0.31% tryptophan while Wang and Parsons (1997), analyzed 6 HFM samples reported an average of 88.7% CP, 1.99% Lysine, 4.38% Cysteine and 0.71% Methionine. Cotanch *et al.*, (2007) reported HFM as having a Dry Matter (DM) content of 90.4 - 96.8%; Crude protein of 84.1 - 90.5%; Ether Extract of 6.1 - 14.8% and Ash of 1.5 - 2.8%.

2.2.2. FEATHER PROTEIN

Keratin of mammals is of the α-type while those of birds are the β-type. Beta-keratins are more insoluble as compared with the α-keratins as they form the β-sheet structure which has more disulphide linkages (Fig.3 and 4).

The disulphide linkages between cysteine residues are responsible for the inertness of β-keratin. Feather keratin is so tough that it is being investigated for use in fibre plastic production.

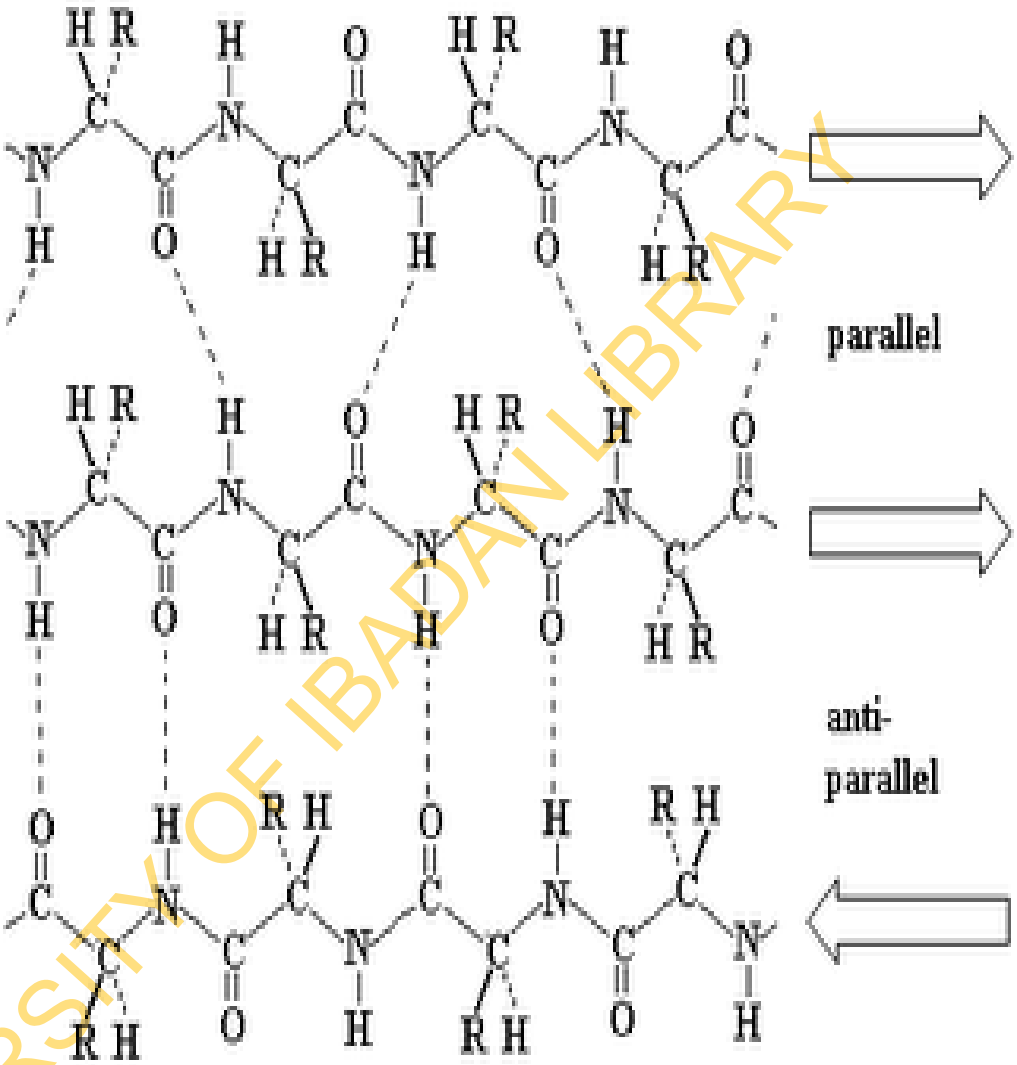


Figure 3: Molecular structure of β keratin of feathers
Source: http;//www.bird-log.blogspot.com

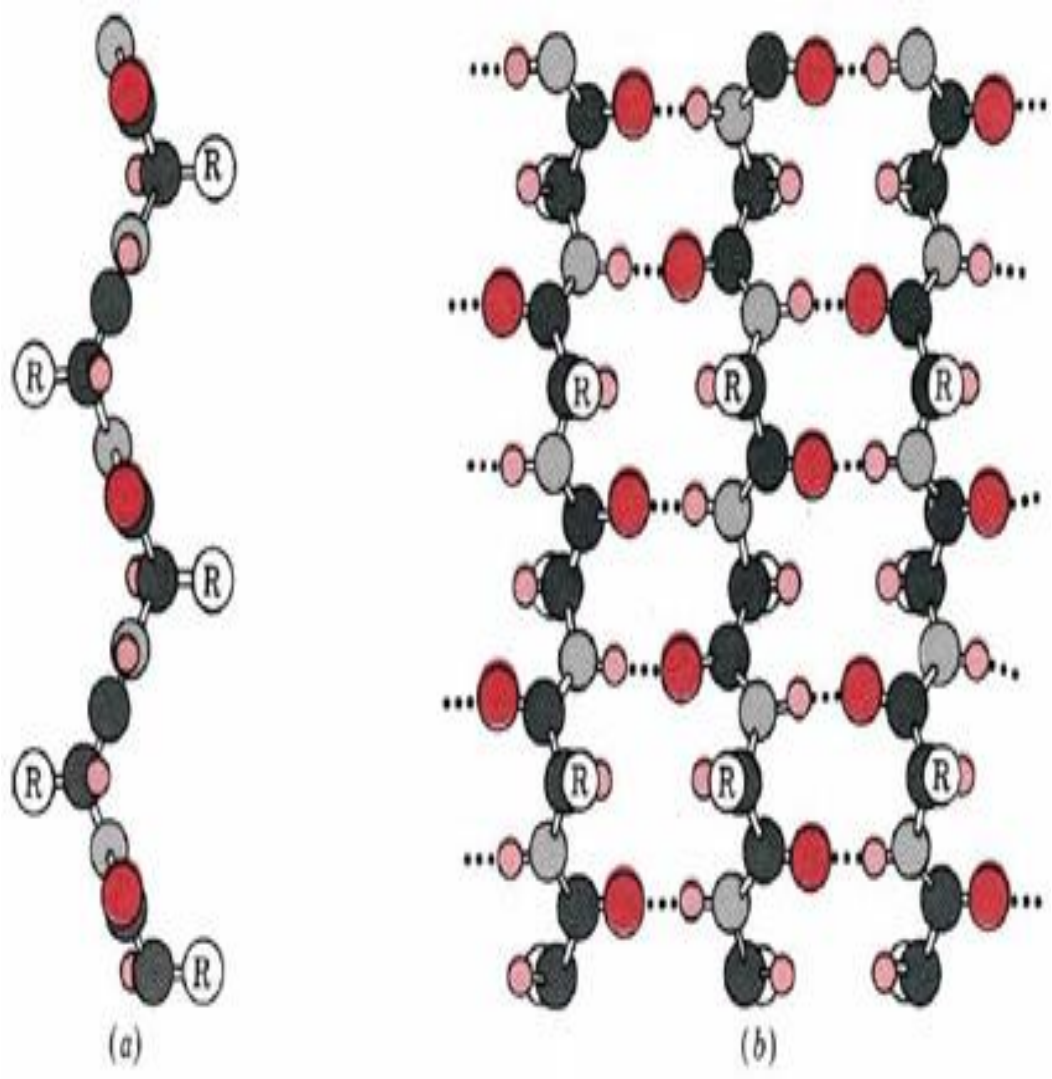


Figure 4: (a) A single strand of the primary structure. (b) The β-sheet structure showing hydrogen bonding in dots between the primary structures.
Source: Chempaths; http://www.chempaths.chemeddl.org

The toughness of feathers does not allow for easy degradation. In the environment however, it does not accumulate but is degraded by microorganisms. This ability of certain microorganisms to breakdown feather has led to the discovery of keratinase

producing microorganisms. Many workers have reported the identification, isolation and characterization of keratinase producing microorganisms (Meinhardt *et al.,* 1989; Dozie *et al.,* 1994; Lee *et al.,* 2002; Korkmaz *et al.,* 2004; Hoq *et al.,* 2005; Termignoni *et al.*, 2005; Tapia and Contiero, 2008). With the discovery of such microorganisms, enzyme preparations like Versazyme and Cibenza DP^{100} as animal feed supplements were produced with keratinase activity.

2.3. ENZYMES

Enzymes are biological catalysts, speeding up the rate of specific reactions. All living things depend on enzymes for sustenance of life. Enzymes could be digestive or metabolic. Digestive enzymes are exogenous while metabolic enzymes are endogenous. Enzymes are exogenous when they act outside of the cell that produced them; they are endogenous when they carry out their activity within the cell that produced them. Enzymes employed in animal feeds are exogenous in nature and are substrate specific with most being protein in nature. Enzymes are usually classified based on the type of reactions they catalyze e. g Transferases catalyze group transfers.

Most of the enzymes used in poultry diets are not produced in the birds or are produced in very little quantities. All enzymes used to improve nutrient availability in animals are hydrolases as they catalyze the hydrolysis of their substrates. Exogenous enzymes are used to help birds maximally utilize nutrients in feeds. There are different classes of feed enzymes as shown in Table 2. When feedstuffs that are not very natural to birds are given to them, they may require exogenous enzymes to help in the digestion of such materials. All animals cannot digest any keratin material because they do not produce keratinase that hydrolyzes (El-Boushy *et al.,* 1990)

keratin to its constituent amino acids thereby making the amino acids available to the animal. Ruminant animals are able to utilize keratin materials because their rumen contain a variety of microorganisms that produce keratinase the enzyme that breaks down keratin (Jordan and Croom, 1957; Combs *et al.*, 1958; Blasi *et al.*, 1991).

Enzymes for poultry have the greatest potential in diets containing feedstuffs with anti-nutritional factors or with structures which hinder the availability of nutrients. Fibre polysaccharides like cellulose, β-glucans, arabinoxylans and pectins are major targets of many commercial enzymes as they limit the energy content of the feedstuff (Ferket, 1993). Many grains contain these fibre polysaccharides in different percentages. This calls for enzyme mixtures when applying enzyme to diet formulations containing such grains. For example, barley contains cellulose, β-glycans and arabinoxylans but corn contains cellulose and arabinoxylans (Ferket, 1993); these grains will require different enzyme mixtures. Many works have been carried out on enzyme supplementation but few have been carried out on proteases in broiler diets (Angel *et al.*, 2011).

Table 2: General feed enzyme classifications

Substrate	Examples of enzymes
Protein (animal or plant)	Protease, Peptidase, Keratinase
Starch	Amylase
Lipids	Lipase
Phytate	Phytase
Cellulose	Cellulase, Cellobiase
Hemicellulose (grains)	Hemicellulase
Pentosans (wheat, rye)	Pentosanase, Xylanase
Beta-glucans (barley, oats)	β-glucanase
Pectins (plant protein supplements)	Pectinase
Polygalacturonans, Mannans, Galactans, Arabinans, Xyloglucans	α-galactosidase

Source: Ferket 1993; Thorpe and Beal (2001).

Enzyme cocktails are enzyme mixtures that have been produced to combat the challenge of many enzyme substrates in a diet. Proteases are employed to improve the

availability of proteins/amino acids in a diet. There are not many branded mono-component proteases with keratinase activity for use in animal feeds.

The enzyme used in these studies (Cibenza DP^{100}) is heat stable up to 105°C and recommended level of inclusion is 500g/Metric Tonne. Its enzyme activity is 600,000 proteolytic units per gram of enzyme. Each proteolytic unit is defined as the increase in absorbance of 0.01 at 410nm for 15 minutes at 37°C in 1ml reaction volume. The expected rate of enzyme in finished feed is 300units per gram of feed.

Some feed enzyme preparations could be regarded as probiotics in a sense because they contain the organisms that produce the enzyme required. Probiotics are live microbial feed supplements with beneficial effect on the host animal by improving its intestinal balance (Sang, 2011).

2.3.1. THE USE OF EXOGENOUS ENZYMES

The use of exogenous enzymes in animal feeds is not new as Lewis *et al*., (1955) and Baker *et al*., (1956) had much earlier examined the effects of adding pepsin, pancreatin, a fungal protease, a diastatic protease and papain to different soya bean diets in a number of trials using pigs. Zamora and Veum (1979) also investigated the effect of feeding whole soya beans fermented with *Aspergillos oryzae* and *Rhizopus oligosporus* on growing pigs. Most of the works on feed enzymes have been done on cereal based diets like barley and wheat (Dierick, 1989). Most of the feed enzymes available also show this trend as interest is on unlocking the nutrients in vegetable feed sources. Most feed ingredients are of vegetable origin except for fishmeal which is usually the only feed ingredient of animal origin. The production of enzymes for animal feeds and feeding has evolved from crude preparations to pure single or

combined enzyme preparations (Table 3) which has improved efficiency of enzyme use in animal nutrition.

Table 3: The evolution of feed enzyme technology

Generation	Breakthrough
1st	Crude enzyme preparations, 1950's
2nd	"pure", single activity enzymes from submerged liquid fermentation for wheat and barley diets, mid 1980's
3rd	Combinations of pure enzymes, early 1990's
4th	Phytase, early 1990's
5th	Combinations of pure enzymes for use in corn-soy diets, 1993
6th	Solid state fermentation, 2000

Source: Agricultural Review; Livestock Kenya.com. August 2011

According to Thorpe and Beal (2001) and Cmiljanic *et al*., (2005) the use of exogenous enzymes will increasingly be popular for a number of reasons:

i. The increasing shift to the use of alternate nutrient sources for animal diets.

ii. Increased availability of free amino acids which may reduce high quality dietary protein sources/supplements in feeds.

iii. Introduction of environmental pollution controls to reducing the excretion of Nitrogen and Phosphorus by animals.

iv. The increasing knowledge and availability of enzymes successfully used in animal diets.

v. The ban on the use of meat, meat-bone meals and antibiotics as growth promoters in animal feeds.

2.3.2. ENZYME PRESENTATION TO ANIMALS

Most feed enzymes are presented in feed in the dry form, with the enzyme being added during mixing preceding pelleting. This however is deleterious to enzymes that are not heat stable like amylases, proteases, phytase and β- glucanase. Such enzymes can be added post pelleting in the liquid form either in feed or sprayed on feed. (Inborr, 1990; Cowan, 1992; Bedford and Pack, 1998; Thorpe and Beal, 2001). Cibenza DP^{100} is heat stable and retains its activity even at temperatures as high as 105°C. Different enzymes have different optimum temperature for their activity and a deviation from such will inactivate them or reduce their efficacy.

2.3.3. PRE-TREATMENT WITH ENZYMES

Sometimes pre-treatment of feed ingredients is required to measure precisely exogenous enzyme activity that is expected in the animal. Pre-treatment of particular feed ingredients before mixing with the other ingredients allows for ascertaining enzyme effect before the diet is given to animals.

This is important when assessment of exogenous protease in the ileum is to be carried out. Immunochemical techniques are developed for detecting presence of an exogenous enzyme in the ileal digesta (Bennett *et al.*, 1994). Table 3 shows different pre-treatments for feedstuffs from soya bean as reported by Thorpe and Beal (2001).

Pre-treatment of soya beans has been used to improve the nutritional quality of legumes in human foods for many years but this is usually through fermentation (Campbell-Platt and Cook, 1991). Zamora and Veum (1979) reported feeding pigs with heated fermented soya beans.

Their works show that fermentation improved performance of the animals. Recently pre-treatment is used to assess enzyme activity *in vitro* for any potential benefit the enzyme may have *in vivo* (Huo *et al.*, 1993; Meijer and Spekking, 1993; Hessing *et al.*, 1996 and Kumar and Wyman, 2009).

Table 4: Summary of protease pre-treatment of Soya Bean Meal.

Vegetable substrate	Enzyme	Treatment
SBM	Acid protease (P2)	0.1% protease added to SBM (800g/kg moisture) pH 4.5. Incubated for 3hrs at 50°C, neutralized, dried at 65°C.
SBM	Acid protease (P2)	0.1% protease added to SBM (800g/kg moisture) pH 4.5. Incubated for 3hrs at 50°C, dried at 55°C.
SBM	Alkaline Protease(P1)	0.1% protease added to SBM (800g/kg moisture) pH 8.5. Incubated for 3hrs at 50°C, dried at 55°C.
SBM	Acid protease (P2)	0.1% protease added to SBM (800g/kg moisture) pH 4.5. Incubated for 2hrs at 50°C, fed as wet mash.
SBM	Alkaline Protease(P1)	0.1% protease added to SBM (800g/kg moisture) pH 8.5. Incubated for 2hrs at 50°C, fed as wet mash.
SBM	Protease (*B. subtilis: subtilisin*)	0.1% Protease added to SBM (1:2 wt:vol water) pH 4.5. Incubated for 16hrs at 50°C, freeze dried. 50ml of enzyme solution (pH 4.5) to give final enzyme concetration of 0.1% sprayed on SBM, air dried at ambient temperature for 24hrs.
FFSBM (autoclaved)	Protease (P4)	0.25% added to soymeal (1:3 wt:vol water) incubated for 24 hrs at 20°C, fed as liquid.
Raw soya beans	Protease (P4)	0.25% added to soymeal (1:3 wt:vol water) incubated for 24 hrs at 20°C, fed as liquid.
Micronized FFSBM	Protease (P3)	0.5% added to soymeal (1:3 wt:vol water) incubated for 24 hrs at 20°C, fed as liquid.
Raw soya beans	Protease (P3)	0.5% added to soymeal (1:3 wt:vol water) incubated for 24 hrs at 20°C, fed as liquid.

SBM= soya bean meal. FFSBM= full fat soya bean meal. All proteases supplied by Finnfeeds International Ltd (Marlborough, UK)

Source: Thorpe and Beal, 2001.

2.3.4. BENEFITS OF ENZYME INCLUSION IN ANIMAL DIETS.

Enzyme use in feeds allows for the use of a wide range of ingredients without compromising performance. This gives room for least-cost formulations. Exogenous enzyme use in animal feeds results in reduced environmental contamination/pollution from excreted animal wastes. It results in cleaner and better environment for humans (Oxenboll *et al.*, 2011). Production cost is lower and economic efficiency is improved.

A number of researchers have shown that enzyme inclusion in poultry diets improves the performance of both broilers and laying birds (Odetallah *et al.*, 2003; Gheorghe *et al.*, 2005; Cmiljanic *et al.*, 2005; van Krimpen *et al.*, 2010; Woyengo *et al.*, 2010).

The benefits of enzyme inclusion in poultry diets include:

i. Reduction in digesta viscosity (Dunn, 1996; Gunal *et al.*, 2004; Palander *et al.*, 2005; Piel *et al.*, 2005; Choct, 2006 and Kiarie *et al.*, 2007)

ii. Increase in available energy (Rogel *et al.*, 1987; Annison and Choct, 1993; Choct *et al.*, 1995; Slominski *et al.*, 2006; Amerah *et al.*, 2008 and Zhou *et al.*, 2009)

iii. Improvement in nutrient digestibility (Friesen *et al.*, 1992; Marquardt *et al.*, 1994; Graham, 1996; Wang *et al.*, 2005; Tibaldi *et al.*, 2006; Malayoglu *et al.*, 2010 and Liu *et al.*, 2013)

iv. Health improvement (Morgan and Bedford, 1995; Choct *et al.*, 1995 and Choct, 2006)

v. Friendly impact on the environment (Khattak *et al.*, 2006)

2.4. CRUDE PROTEIN

The crude protein in any given material is an estimate of the Nitrogen content of that material. This means that any Nitrogen containing substance in the test ingredient will add to the crude protein measure, this addition is negligible in many feedstuffs. Proteins are the only class of nutrient that contains nitrogen and it is 16% of any protein material. Nucleic acids also contain nitrogen but in very minute amounts. The crude protein analysis involves the digestion of the test ingredient in concentrated sulphuric acid with addition of a catalyst for at least 8 hours. The clear solution is then distilled to obtain the ammonia gas collected in bromine water and titrated against hydrochloric acid (Appendix 3). The crude protein value of a feedstuff helps in feed formulation for animals and gives a good idea about how much protein an animal will get from a given set of ingredients; however, this does not show how useful or available the protein is to the animal. Proteins are hydrolysed by digestive proteases into amino acids; the amino acids are then converted into products required by the animal through metabolism. If a protein source is not digested, it cannot be absorbed and used in metabolic processes. The degree to which a nutrient is digested is a reflection of how available for use it is to an animal.

2.5. NUTRIENT DIGESTIBILITY

After the ingestion of feed, digestion and absorption of nutrients take place in the alimentary canal. The degree to which feed nutrients are digested determines the degree of absorption. Nutrient digestibility is therefore important in maximizing the cost of feeds for animal production as undigested and subsequently unabsorbed nutrients are of no use to the animal.

Digestibility is the quantification of the digestive process, which gives a relative measure of the extent to which ingested food and its nutrient components have been

digested and are absorbed by an animal; digestible nutrients are not excreted (McNab, 1994). Digestibility can be expressed as a coefficient or as percentage.

Digestibility of nutrients can be apparent or true. Digestibility is termed true when the value is corrected for contributions from endogenous compounds like eroded epithelial cells, enzymes and products of bacterial fermentation. It is apparent when not corrected for endogenous loss. Endogenous loss contribution is important in protein and amino acid digestibility determinations.

Endogenous nitrogen and amino acid losses can be determined by

1. Linear regression
2. Feeding essentially protein-free diet and measuring the nitrogen amino acid flows in the digesta collected at the terminal ileum (Furuya and Kaji, 1989; Donkoh *et al.*, 1995).
3. Feeding semi-synthetic diets with the sole nitrogen source being purified amino acids such that the animal is not in negative body nitrogen balance (Darragh *et al.*, 1990 and Butts *et al.*, 1993).
4. Feeding protein-free diets accompanied with intravenous amino acid infusion (de Lange *et al.*, 1989 and Leterme *et al.*, 1996a).
5. Feeding natural proteins lacking specific amino acids as sole nitrogen source and administration of intravenous infusion of the amino acids missing in the natural protein being fed (Butts *et al.*, 1993).

6. Feeding guanidinated proteins in diets (only lysine can be determined by this method) according to Hagemeister and Erbersdobler, 1985; Rutherfurd and Moughan, 1990.

7. Feeding of enzymatically hydrolysed protein (Moughan and Rutherfurd, 1990).

8. Isotope dilution where the feed protein is labeled with a stable or radioactive tracer (Souffrant *et al*.1993 and Leterme *et al*., 1996b).

According to McNab, 1994 apparent nutrient digestibility and true digestibility can be calculated by the formulae:

$$TD, \% = \frac{N_i - [N_e - EL]}{N_i} \times 100$$

$$AD, \% = \frac{N_i - N_e}{N_i} \times 100$$

Where:
AD= Apparent Digestibility
N_i = Nutrient ingested
N_e = Nutrient excreted
EL = Endogenous losses
TD = True Digestibility

Digestibility studies can be carried out either by an *in vivo* or an *in vitro* method of estimating bioavailability of nutrients. The *in vivo* method can be direct or indirect; the direct method could be any of three techniques: balance trial, nylon-bag technique and growth assay (Agboola, 2011). Figure- 5 shows the relationship between the methods employed in digestibility studies and the techniques involved in *in vivo* estimation of nutrient digestibility.

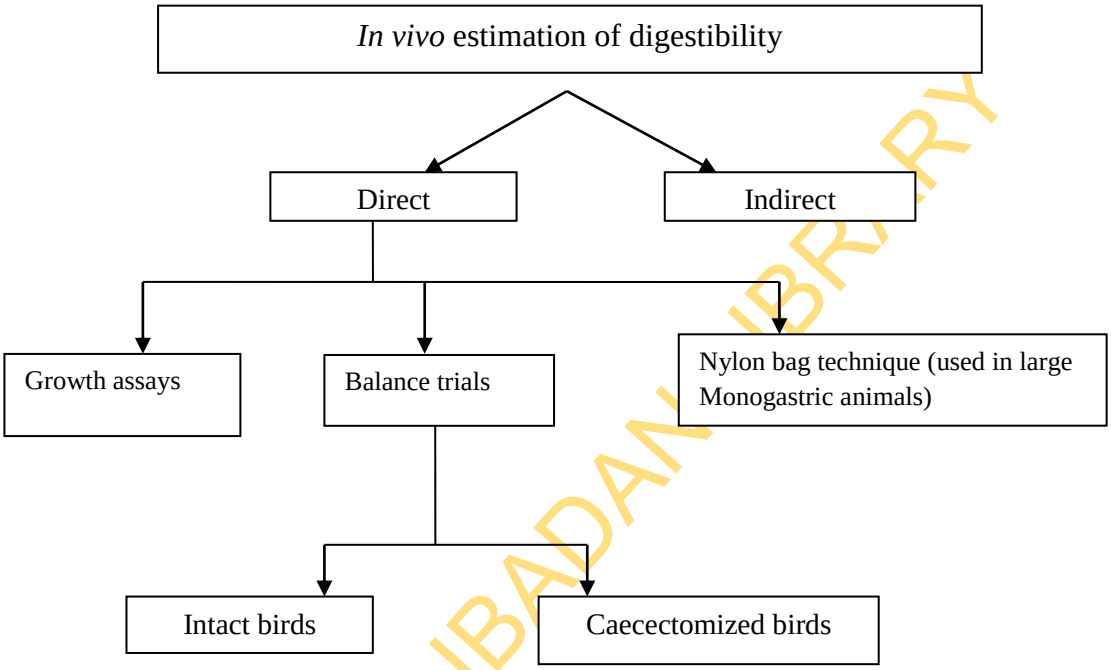


Figure 5: Relationship between methods in *in vivo* estimation of nutrient digestibility and the techniques involved.

2.5.1. GROWTH ASSAYS

This is a technique employed in direct *in vivo* studies of nutrient digestibility. Silbald (1987) enumerated the steps in basic growth assay of an available nutrient to involve:

i. A basal diet devoid or deficient in the nutrient of interest.

ii. Supplementation of the basal diet with one or more graded levels of the nutrient of interest.

iii. Feeding these diets to animals under same conditions to establish a relationship between animal response and the nutrient levels.

Animal response in growth assays are determined by measuring feed intake, body weight gain and nutrient retention as dependent variables against the known nutrient of interest level in test diets as the independent variable.

2.5.2. BALANCE TRIALS

This is an age old method of nutrient retention determination in animals. It is a balance trial because the input and output of nutrients are measured and the difference gives the amount of nutrient retained (McNab, 1994). Though an old widely accepted method, it does not give a satisfactory estimate of nutrient retention in animals (Silbald, 1987). In this method of digestibility estimation, the nutrient or feedstuff of interest is offered as part of a regular mix of feedstuff in animal diet or alone; these diets and a control diet (without the feedstuff or nutrient of interest) are fed to animals under same conditions. Indigestible markers may or may not be added in the diets. Feed intake and body weight gain are measured as performance indices while feaces

or excreta is collected over a period of 72 hours and analysed for excreted nutrient levels.

Digestibility determined by balance trials can also be called the total tract digestibility and it is expressed as:

$$TTD,\ \% = \frac{N_i - N_e}{N_i} \times 100$$

Where:
TTD = Total tract digestibility
N_i = Nutrient ingested
N_e = Nutrient excreted

Intact or Caecectomized birds are used in balance trials. When intact birds are used the excreted nutrients does not reflect satisfactorily the amount of nutrient retained or available to the animal (Norberg *et al.*, 2004). This is because the hindgut of birds contains microorganisms (Apajalahti *et al.*, 2004) that will further degrade the nutrient in the digesta and also pass their secretions into the digesta before it comes out; in addition, uric acid the metabolic waste from protein metabolism is passed out together with the digestive waste. This means that the total tract estimation of digestibility in intact birds cannot be a satisfactory estimate as both metabolic wastes and digestive wastes are inseparably excreted. This challenge can be averted by the use of caecectomized birds as proposed by Parsons (1984). The total tract digestibility balance trial is however easier and less costly when intact birds are used as the birds are not sacrificed (Angkanaporn, 1997b; Ravindran *et al.*, 1999). Though some studies showed that type of feedstuff affects hindgut micro flora activity and eventually protein digestibility, it remains a better choice for protein feed sources because of endogenous sources of protein that are added to the digesta before it comes

out as excreta. Amino acid digestibility of meat and bone meal was lower when caecectomized roosters were used by Johnson (1992) and Parsons *et al.* (1997). The use of caecectomized birds ensures the effect of hindgut microorganisms is removed but the contribution of nitrogen from uric acid remains which is important in protein digestibility determination. This led to the proposal by Ravindran *et al.* (1999); Ravindran and Bryden, (1999); Rodehutscord *et al.* (2004) that analysis of ileal content gives a more reliable digestibility coefficient when proteins are involved. These researchers took a clue from Payne *et al.* (1968 and 1971) who suggested the use of ileal content against excreta for nutrient digestibility determinations.

2.5.3. ILEAL OR PRE-CEACAL DIGESTIBILITY

In poultry protein digestion and absorption extends to the terminal ileum or pre-ceaca. If the ileal content is harvested before the ceaca and the cloaca, metabolic wastes will not increase the nitrogen content and the hindgut micro flora effect will also be eliminated. Therefore determination of digestibility using ileal content (ileal or pre-ceaca digestibility) is becoming increasingly popular for pigs and poultry (Ravindran *et al.,* 1999; Ravindran and Bryden, 1999; Rodehutscord *et al.*, 2004; Adedokun *et al.,* 2007). According to Ravindran *et al.,* (1999) and Kadim *et al.,* (2002), pre-ceacal digestibility of proteins (amino acids) is generally considered a more reliable measure for feed protein evaluation in poultry. This has led to feed formulation for broilers based on Standardized Ileal Amino Acid Digestibility (SIAAD) (Hoehler *et al.*, 2006).

Ileal digestibility is expressed as Standard Ileal Digestibility (SID) when the Apparent Ileal Digestibility (AID) is corrected for basal ileal endogenous losses (endogenous Protein/AA flow) (Stein *et al*., 2001 and Adedokun *et al.*, 2011) and True Ileal Amino Acid Digestibility (TIAAD) when the SIAAD is corrected for additional specific

endogenous losses induced by the composition of the test material (Golian *et al.*, 2008). Inclusion of specific losses can be achieved by feeding test ingredients at increasing graded levels and measuring the slope of regression that corresponds to True Ileal Digestibility (TID) (Rodehutscord *et al.*, 2004; Kluth *et al*., 2005 and Kluth and Rodehutscord, 2006). The specific losses are estimated by extrapolating the slope of protein/amino acid intake to zero. The need for endogenous amino acid losses becomes necessary as the protein/amino acids reaching the terminal ileum are not only from dietary sources. Digestive enzymes, bacteria, eroded lining cells and mucoproteins contribute to the ileal digesta protein/amino acid content. Ileal Endogenous Protein/AA flow is expressed as mg/kg of Dry Matter (DM) intake (Moughan *et al*., 1992) and represents the endogenous protein/amino acid losses.

$$\text{IEP/AA(mg/kg DMI)} = \text{PID (mg/kg DM)} \times \frac{\text{IM intake (mg/kg DM)}}{\text{Ileal IM (mg/kg DM)}}$$

Where:
IEP = Ileal Endogenous Protein/Amino acid
PID = Protein in digesta
IM = Indigestible Marker
DMI = Dry Matter Intake

The basal endogenous losses can be determined by feeding protein/nitrogen- free diet on the assumption that excreted proteins/amino acids is entirely from endogenous sources.

2.5.3.1. THE REGRESSION METHOD

For this method to be applicable the animals must be fed increasing graded levels of the protein and the intake and output measured. Assumptions of this method are

i. The amount of protein ingested is directly proportional to the amounts excreted.

ii. There are no changes in the endogenous protein/amino acid secretions.

iii. Only undigested dietary protein is responsible for the increased ileal protein flow.

The method allows for amino acid digestibility (AAD) to be measured alongside Endogenous Amino Acid. The protein digestibility of any test ingredient is the slope of the linear relationship between the protein intake and the amount digested or the rate of its disappearance (Short *et al.*, 1999; Rodehutscord *et al.*, 2004).

2.5.3.2. PRE-CEACAL SAMPLING AND DIGESTA COLLECTION

This is the method employed in ileal digestibility determinations where digesta is harvested before reaching the ileo-ceaca-colonic junction in Monogastric animals. This can be done in living animals by cannulation, anastomosis, caecectomy and in slaughtered animals. In young poultry the common technique employed is the sacrifice method. Cannulation of animals for digesta collection allows for collection over a period during which enough sample is collected for analysis. In sacrificed animals, a section of the terminal ileum is excised to obtain the content; Yap *et al.*, (1997) and Kadim *et al.*, (2002) reported the terminal 15cm of the ileum as being suitable for digesta harvesting in chickens.

Many animals are required to get enough digesta sample for analysis when small animals are involved like young birds. When the animals are slaughtered, care must

be taken not to allow contamination with the mucosa lining of the ileum (Leeuwen *et al.,* 2000) and according to Palander *et al*. (2004a) asphyxiation by carbon dioxide is preferable to neck dislocation and mechanical stunning as these techniques gave lower pre-ceaca digestibility coefficients.

Cannulation of adult birds has been achieved in cockerels and roosters using glass or silicon T-cannulas (Raharjo and Farell 1984 and Leeuwen *et al.*, 2000), it is an acceptable technique of digesta collection (Donkoh and Moughan, 1999).

2.5.4. FACTORS AFFECTING MEASUREMENTS OF DIGESTIBILITY

Many researchers have shown that many factors affect measurement of digestibility. The following affects digestibility measurement; feed processing (Amornthewaphat *et al*., 2005), feed particle size (Svihus and Hetland, 2001; Fastinger and Mahan, 2003), feed intake (Stein *et al*., 1999; Stein *et al*., 2001; Moter and Stein, 2004), feeding regime (Yap *et al.,* 1997), enzyme supplementation (Ravindran *et al*., 2001; Rutherfurd *et al*., 2002; Rodehutscord *et al*., 2004; Wang *et al*., 2005), type of indigestible marker used (Yap *et al.,* 1997; Fan and Sauer, 2003), presence of anti nutritional factors (Wiseman *et al*., 2003), species and age of poultry (Huang *et al.,* 2000; Lemme *et al*., 2004; Palander *et al.,* 2005; Ravindran and Hendriks, 2004a; Huang *et al*., 2005; Kluth and Rodehutscord, 2006).

CHAPTER THREE

3.0. ILEAL DIGESTIBILITY OF FEATHER MEAL IN BROILER CHICKENS

3.1. INTRODUCTION

Feather meal is produced for animal feeding using the physical method of hydrolysis where the feathers are subjected to high pressure and temperature. This method of hydrolysis usually does not give a product of uniform protein quality because there are variations between production batches (Wang and Parsons, 1997). Feather meal has been successfully used in dairy cows to improve their milk production (Southern *et al*., 2000). Generally, ruminant animals are able to utilize feather meal better than non-ruminant animals because of the microbial presence in their rumen, many of which help in the degradation of feather meal (Jordan and Croom, 1957; Thomas *et al*., 1994 and Klemesrud *et al*., 1998). Swine can tolerate up to 10% inclusion of feather meal (Chiba *et al*., 1995; 1996 and Van Heugten and Kempen, 2002). In poultry however, feather meal is recommended to be used at not more than 5% inclusion level because of the negative effects when inclusion level is higher (Aderibigbe and Church, 1983b). The inclusion level was determined based on performance and not on digestibility of the feather meal.

Feather Meal is a protein rich feedstuff that has not been successfully used in poultry production. It is a cheap source of protein as it is processed from feather wastes from poultry farms (Bohoua, 2008). It is known to be a protein feedstuff with poor

digestibility in poultry and of inferior quality compared to fishmeal (Aderibigbe and Church, 1983b; Kim and Patterson, 2000 and Kim *et al*., 2002). Ileal HFM digestibility is necessary to estimate the apparent digestibility which is more reliable compared to the total tract method (feacal method) (Rostagno and Pupa, 1995).

3.2. MATERIALS AND METHODS

Poultry feathers were collected, washed and boiled in pressure cooker (Masterchef pressure cooker of model MC- 11000PC, working pressure of 80Kpa± 10% Kpa and a safety pressure of 112-160Kpa) for 30 minutes (Papadopolous, 1985 and Blasi *et al.*, 1991). The hydrolyzed feathers were sundried and milled.

3.3. Experimental diets and management of animals

A total of 144 one-day-old broiler chicks were brooded for 2 weeks on a pelletized and crumbled standard commercial broiler starter feed given *ad libitum* with clean water. On the 14th day, birds were weighed individually in group and randomly allocated into 4 treatments with 6 replicates of 6 birds each. The experimental diets contained 0, 2, 4 and 6% HFM, they were pelletized and crumbled. Titanium dioxide was added to the diets as an indigestible nutrient marker. The composition of the experimental diets is shown in Table 5.

Feed intake and body weight gain were taken during the experimental period.

TABLE 5: Gross composition of experimental diets for broilers fed graded levels of HFM (0-4wks)

INGREDIENTS	0% HFM	2% HFM	4% HFM	6% HFM
Starch	30.0	28.0	26.0	24.0
Soyabean cake	30.0	30.0	30.0	30.0
Groundnut cake	17.8	17.8	17.8	17.8
Fishmeal	2.5	2.5	2.5	2.5
Ricebran	8.50	8.5	8.5	8.5
Palm oil	5.0	5.0	5.0	5.0
Feather meal	0.0	2.0	4.0	6.0
Dicalcium phosphate	1.5	1.5	1.5	1.5
Limestone	1.0	1.0	1.0	1.0
Salt	0.25	0.25	0.25	0.25
Titanium dioxide	0.5	0.5	0.5	0.5
Vitamin/mineral premix*	0.25	0.25	0.25	0.25
Methionine	0.5	0.5	0.5	0.5
Lysine	0.2	0.2	0.2	0.2
TOTAL	100.0	100.0	100.0	100.0

Calculated nutrient levels				
Parameters				
ME Kcal/Kg	2914.9	2892.3	2869.5	2846.8
% CP	23.5	24.8	26.1	27.4
% Ca	1.1	1.1	1.1	1.1
% P	0.8	0.8	0.8	0.8
% Methionine	0.8	0.8	0.9	0.9
% Lysine	1.5	1.5	1.5	1.5

* Vitamins.A 12000000iu; D3 2500000iu; E 20000mg; K3 2000mg; B1 2000mg; B2 5000mg; B6 4000mg; B12 15mg; Niacin 30000mg; Pantothenic acid 11000mg; Folic acid 1500mg; Biotin 60mg; Choline chloride 220000mg; Antioxidant 1250mg; Mn 50000mg; Zn 40000mg; Fe 20000mg; Cu 3000mg; I 1000mg; Se 200mg; Co 200mg.

3.4. ILEAL DIGESTA COLLECTION

On day 28, the birds were weighed and asphyxiated using carbon dioxide. They were immediately cut open and the section between the Meckel diverticulum and 2cm anterior to the ileo-caeco-colonic junction excised and flushed with distilled water to harvest the digesta.

Ileal digesta were pooled according to replicates, frozen, freeze-dried, milled and analysed.

3.5. DIGESTIBILITY CALCULATIONS

Crude protein digestibility (%) was calculated using the following formula:

$DC_{CP,diet} = 1- \{(TiO_{2diet} \times CP_{digesta})/(TiO_{2digesta} \times CP_{diet})\} \times 100$

Where

DC_{CPdiet} = Crude protein digestibility %
TiO_{2diet} = Amount of titanium dioxide in the diet
$CP_{digesta}$ = Crude protein of the digesta
$TiO_{2digesta}$ = Amount of titanium dioxide in digesta
CP_{diet} = Crude protein in diet

Digestibility of CP in HFM was calculated on DM basis using the regression model described by Rodehutscord *et al.* (2004) and Kluth and Rodehutscord (2006). Daily CP intake was obtained as daily feed intake multiplied by analysed CP value in the diet; amount of CP digested up to the terminal ileum for each of the diets was calculated as CP intake multiplied by the apparent CP digestibility for each diet. The slope of the linear graph of digested CP values plotted against CP intake represents the HFM CP digestibility.

3.6. CHEMICAL ANALYSIS

The Feather Meal was analyzed for its proximate composition by the methods of AOAC, (2000).

The feed and ileal digesta samples were analysed for CP, Fat, Ca and P according to AOAC, (2000). Crude protein (CP) was analysed by the Kjeldahl method; Percentage fat by ether extraction; Calcium by and Phosphorus by spectrophotometric methods (Appendix 2, 3, 4 and 5).

Titanium dioxide in feed and digesta samples were determined by digesting samples in concentrated H_2SO_4 for 2 hours, adding 30% H_2O_2 and absorbance measured at 410 nm. Titanium dioxide concentration was determined by reading values of samples from a standard linear curve derived from absorbance (at 410nm) of titanium dioxide solutions of known concentrations. All samples, standards and blanks were subjected to same treatments according to Myers *et al.*, 2004. (Appendix 1).

3.7. STATISTICAL ANALYSIS

The treatment was diets with HFM in increasing graded levels and ANOVA was used to determine the effect of HFM inclusion at the graded levels in a completely randomised design. Data were analysed using the GLM procedure in SAS (SAS, 2010) where significant differences were obtained and the means were separated by Tukeys.

3.8. RESULTS

The results of the proximate composition of the hydrolysed feather meal (HFM) and the non-hydrolysed feather meal are as presented in Table 6. Hydrolysed Feather Meal had a higher DM, Ether extract and minerals. The crude protein was however higher in the non-hydrolysed feather meal, but on DM basis the CP was slightly higher in HFM (81.09%) than in the in non-hydrolysed feather meal (79.63%).

The results of the proximate compositions of diets are shown in Table 7. The DM ranged from 98.57 to 99.87%, CP was 20.3% in the control diet, 22.8% in diet containing 2% HFM, 27.6 in diet with 4% HFM and 35% in diet with 6% HFM. Calcium level varied from 0.66 to 1.1 %.

The results of growth performance characteristics are presented in Table 8. The final weight, average weight gain and feed intake were significantly different at $\alpha_{0.05}$ but the Feed Conversion Ratio (FCR) was not significantly different between birds on the control diet and birds fed diet with 2% HFM. Birds on the control diet performed better than those on the HFM diets as weight gain decreased with increase in HFM level. With increase in HFM levels above 2% birds feed intake and body weight gain decreased. The relationship between Feed Intake, Weight gain and percentage HFM inclusion levels is shown in Figure 6.

Nutrient digestibility in birds fed graded level of HFM are as presented in Table 9.The ileal digestibility of DM, CP, Ca, and P in birds on the control diet are 49, 42, 36 and 35; for birds fed 2% HFM 65, 52, 39 and 48; for birds fed 4% HFM 62, 58, 49 and 39 while values obtained for birds fed 6% HFM were 58, 63, 45 and 42. Digestibility of nutrients in birds fed diets containing HFM was higher than in birds on the control

diet. The relationship between CP digestibility and the HFM inclusion levels is shown in Figure 7.

The Dry Matter (DM) digestibility was significantly ($\alpha_{0.05}$) increased as the HFM level increased at 2%, however values at 4% inclusion level were not different. The highest DM digestibility of 65.0% was obtained in birds on the 2% HFM diet. Birds on the control diet had significantly inferior DM digestibility. Digestibility of Phosphorus followed a similar pattern to the DM digestibility. Crude Protein digestibility on the other hand significantly ($\alpha_{0.05}$) increased with increase in the HFM level (0 - 6%). Digestibility of Calcium showed significant increase from 0 - 2% HFM inclusion level, significantly decreased at 4% HFM inclusion level but with no significant difference between the 4% and the 6% inclusion levels.

TABLE 6: Proximate composition of non-hydrolysed and hydrolysed feather

Parameter (%)	CP	EE	ASH	DM	Ca	P	NFE	GE(kcal/Kg)
Hydrolysed Feather (HFM)	83.8	10.3	0.6	96.8	0.02	0.03	5.3	4.3
Non-Hydrolysed Feather	88.6	5.0	0.3	89.9	0.03	0.04	16.1	4.4

CP= Crude protein, EE= Ether extract, DM= Dry matter, Ca= Calcium, P= Phosphorus, NFE= Nitrogen free extract and GE= Gross energy.

TABLE 7: Analysed nutrient levels of the experimental diets.

Parameters	0% HFM	2% HFM	4% HFM	6% HFM
% DM	98.6	99.7	99.9	99.6
GE (Kcal/g)	4.3	4.2	4.2	4.2
% Fat	7.0	6.5	5.1	8.8
% CP	20.3	22.8	27.6	35.0
% Ca	1.1	0.7	0.8	0.9
% P	0.6	0.6	0.5	0.6

HFM= Feather Meal, DM= Dry matter, GE= Gross energy, CP= Crude protein, Ca= Calcium and P= Phosphorus

TABLE 8: Performance of birds fed graded levels of HFM, 2 - 4 weeks. (n= 6 replicates of 6 birds each)

Parameter	0% HFM	2% HFM	4% HFM	6% HFM	SEM
Final wt. (g)	3230.0[a]	3007.5[b]	2703.3[c]	2659.2[c]	63.8
Weight gain (g)	1313.3[a]	1134.0[b]	916.7[c]	842.5[c]	57.4
Feed intake (g)	2150.0[a]	1909.2[b]	1846.9[c]	1674.2[d]	17.9
Av. Feed intake (g/bird)*	358.3[a]	318.2[b]	307.8[c]	279.0[d]	2.9
Av. Wt. gain (g/bird)*	218.9[a]	189.0[b]	152.8[c]	140.4[c]	9.6
FCR	1.6[a]	1.7[a]	2.0[c]	1.9[b]	0.02

[a,b,c,d] Values along the row with same superscript are not significantly different, SEM= Standard Error of Mean, (P= 0.05)
FCR= Feed Conversion Ratio, HFM= Feather Meal
* = Values are for the experimental period (14 days)

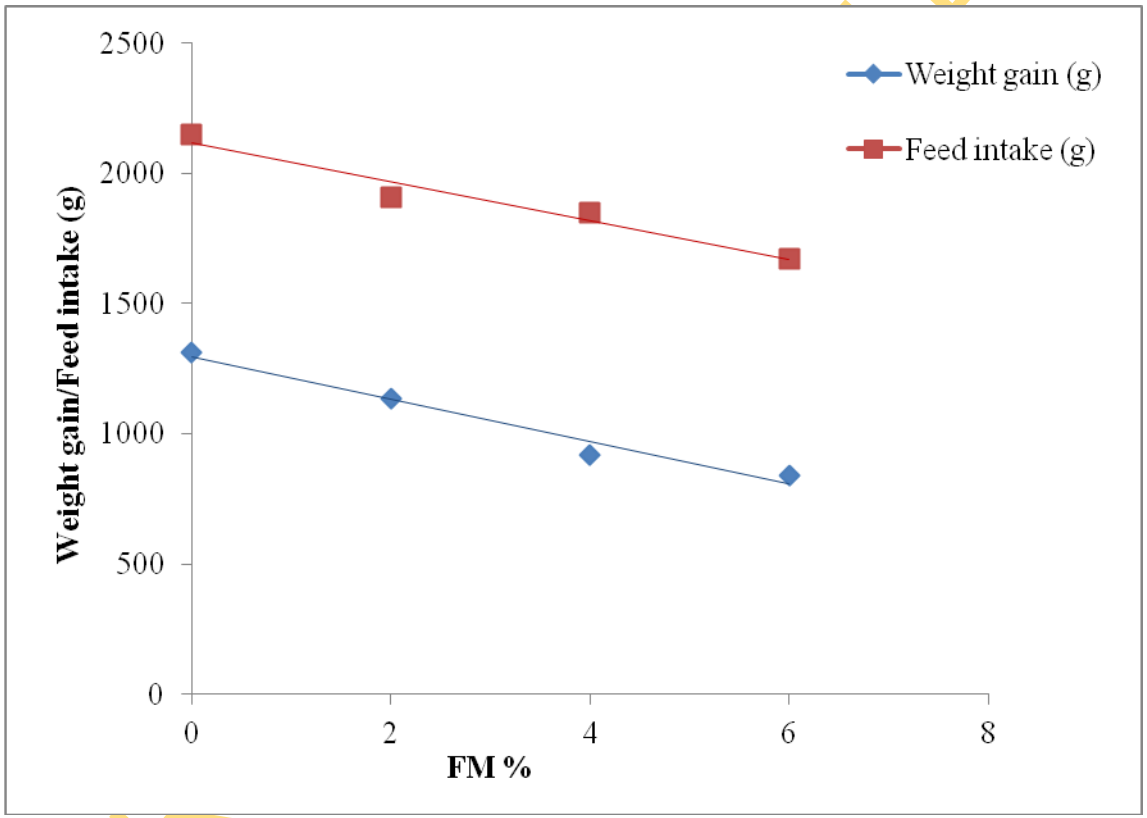


Figure 6: Relationship between feed intake, weight gain and percentage HFM

TABLE 9: Nutrient digestibility in birds fed graded levels of HFM. (n= 6 replicates of 6 birds each)

PARAMETER	0%HFM	2% HFM	4%HFM	6% HFM	SEM
DM	49[a]	65[c]	62[bc]	58[b]	0.02
CP	42[a]	52[b]	58[c]	63[d]	0.04
Ca	36[a]	39[a]	49[c]	45[b]	0.1
P	35[a]	48[c]	39[b]	42[b]	0.1

[a,b,c,d] Figures along the row with same superscript are not significantly different statistically, DM = Dry Matter, CP= Crude protein, Ca= Calcium, P= phosphorus, HFM= Feather Meal, SEM= Standard Error of Mean, (P= 0.05)

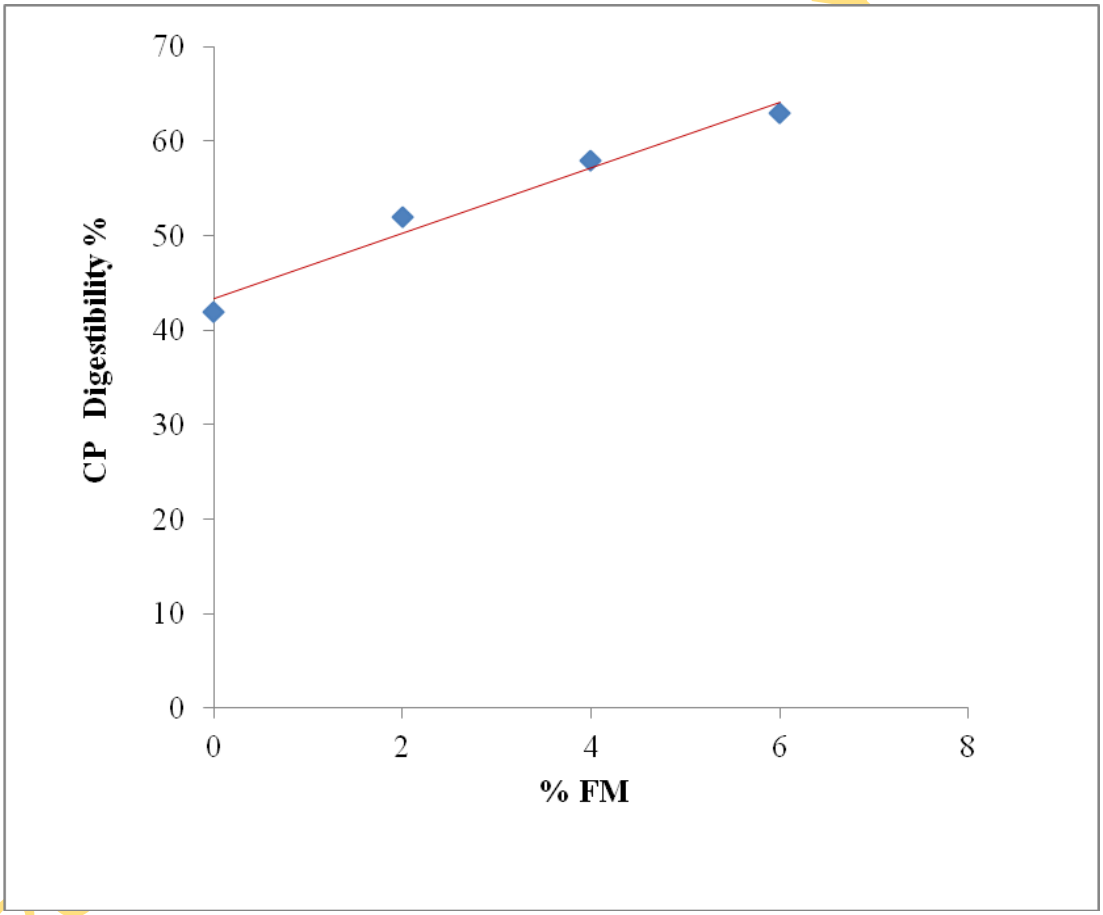


Figure 7: Apparent CP digestibility of HFM at different inclusion levels

3.9. DISCUSSION

The proximate composition of the Hydrolysed Feather and the non- Hydrolysed feather showed that the HFM had a higher DM but with lower CP content, the Gross Energy (GE), Ca and Phosphorus are similar for the two materials. These levels are consistent with reports by a number of researchers (El-Boushy *et al*., 1996; Kim and Patterson, 2000; Ayanwale, 2006). The CP level of HFM was 83% which is close to the range of earlier reports on the crude protein level of HFM. Cotanch *et al.* (2007) reported a range of 84.1 to 90.5% while Morel *et al.,* (2003) reported a range of 82.2 to 84.6% and Wang and Parsons, (1997) reported an average of 88.7%. The reported DM range for 5 samples analysed for proximate composition by Cotanch *et al.* (2007) was 91.8 - 96.8%, the DM for the HFM produced for these studies is an average of 96.6%. Earlier works also reported percentage fat range of 6.1 – 14.8 and the average value obtained for HFM in this study is 10.3. These works show the variation that exists in HFMs from plant to plant and from hydrolysis method viz-a-viz temperature and pressure combinations. They confirm the fact that all HFM do not have exactly the same nutrient composition and their digestibility may also be affected by the processing methods employed in their production (Payne, 1972; El-Boushy *et al*., 1990; Wang and Parsons, 1997; Aderibigbe and Church, 1983a). When proteins are denatured, the digestibility is decreased; HFM is produced either at a high temperature and pressure for a short period or at a lower temperature and pressure for a longer time. When the temperature of processing is raised to extract the fat, some amino acids may be lost (Aderibigbe and Church, 1983a).

Birds that were fed the control diet performed better than those on the other diets. The FI, BWG and FCR followed the same trend as they decreased with increase in HFM inclusion level. It would have been expected that since Apparent CP Digestibility increased with increase in the HFM levels, bird's performance will also show the same trend but the opposite was observed as shown in Figures 6 and 7. This may be a reflection of the poor nutrient contents of the HFM which are not as available as those of the protein source in the control diet. The low utilization of the protein in HFM is probably due to its deficiency in lysine and methionine which are limiting amino acids for broilers (MacAlpine and Payne, 1977; Apple *et al.*, 2003). This observed trend will result in more Nitrogen from diet excreted in to the environment. Kersey and Waldroup (1998) included spent hen meal (SHM) in broiler diets at 0, 5, 10 and 15%; their work showed that body weight of broilers declined significantly with increase in SHM level and it also resulted in poorer FCR, but birds on the 5% SHM performed comparably to birds on control diet at all ages. Since feathers are responsible for about 5 - 10% of a bird's weight it means that birds fed 5, 10 and 15% SHM were fed about 0.25, 0.50 and 0.75% skin and feathers; with the meat and bones being responsible for the remaining fraction. These results are similar to those obtained in this study as keratin the protein in feathers is quite inferior in quality for broilers. According to Alimuddin (2000) digestibility percent rating is good when above 70%; moderate when between 60 and 70%; low when between 40 and 60% and very low when below 40%. The values obtained in this study for CP is in the range of 52- 63% when HFM was included in diets; this falls within the range of low to slightly moderate. Perhaps if the digestibility increases to the range above 70%, performance may be better, but increasing digestibility of HFM in birds is a challenge as they do not

produce keratinase the enzyme that breaks keratin down to its constituent amino acids for utilisation.

It was observed that Apparent CP digestibility (ACPD) increased with increase in HFM inclusion level in the diet. This trend was also observed by Chiba *et al.* (1996) and Apple *et al.* (2003). ACPD was significantly different at all levels of FM inclusion.

The significant increase ($\alpha_{0.05}$) observed in crude protein digestibility suggests that the HFM may have been increasingly broken down into its constituent amino acids but due to the poor quality of the protein, it is not utilised in tissue biosynthesis (Moran *et al.*, 1966; Baker *et al.*, 1981). Feather Meal utilization has been shown to have improved with supplementation with the limiting amino acids (Combs *et al.*, 1958; Chiba *et al.*, 1995).

CHAPTER FOUR

4.0. EFFECT OF PROTEASE SUPPLEMENTATION ON THE ILEAL CRUDE PROTEIN DIGESTIBILITY OF FEATHER MEAL IN BROILERS

4.1. INTRODUCTION

Protease enzymes have been used in improving the digestibility of protein sources in poultry feeds. These enzymes as biological catalysts help to break specific peptide bonds that the animal otherwise cannot break thereby making the amino acids available for metabolic processes which is reflected in improved performance (Odetallah *et al.*, 2003; Drew *et al.*, 2005; Georghe *et al.*, 2005; Wang *et al.*, 2006; Youssef *et al.*, 2008). They are safe to use, both on the birds and indirectly on the environment (Oxenboll *et al.*, 2011). Proteases help to reduce the amount of protein inclusion level as protein availability and utilization is enhanced. Cibenza DP^{100} is a protease with keratinase activity. Its inclusion in diets containing protein sources with insoluble keratin will increase the availability of the constituent amino acids. Feathers are almost entirely keratin and this protease is added to help make the amino acids more available to the broiler chickens. Fewer studies have been carried out using single proteases in poultry feeds. Protein digestibility is influenced by but not limited to particle size of feed ingredients, passage rate of feed through the gut, age of animal, ingredients and their quality in the feed (Antipatis *et al.*, 2013). Though these factors can be controlled

substantially, a simpler alternative is the use of exogenous protease in animal feeds (Antipatis *et al*., 2013; Romero and Plumstead, 2013).

4.2. MATERIALS AND METHODS

4.2.1. Experimental diets and management of animals

Three hundred and thirty-six (336) one-day-old broiler chicks were brooded for 3 weeks in a well ventilated and illuminated poultry brooding pen. They were fed a commercial standard broiler starter diet *ad libitum*, clean water was also given as required for 3 weeks. Recommended routine medications and vaccinations were administered on the chicks. On day 21, the birds were randomly allocated to the 8 treatment diets of 6 replicates with 6 birds per replicate. The diets contained 0, 2, 4, and 6% FM; with either 0 or 0.5g protease/kg of diet. Titanium dioxide was added as an indigestible marker.

The experimental diets were fed *ad libitum* till day-26.The composition of the experimental diets is shown in Table 10.

Records of feed intake and body weight gains were taken during the experimental period.

Table 10: Gross composition of broiler diets containing graded levels of HFM (as only Nitrogen source), with and without supplementation with protease (g/kg).

	+HFM - Protease				+HFM + Protease			
Ingredients	Diet 1 0%HFM	Diet 2 2%HFM	Diet 3 4%HFM	Diet 4 6%HFM	Diet 5 0%HFM	Diet 6 2%HFM	Diet 7 4%HFM	Diet 8 6%HFM
Starch	263.0	243.0	223.0	203.0	258.0	238.0	218.0	198.0
Feather Meal	0.0	20.0	40.0	60.0	0.0	20.0	40.0	60.0
Dextrose	580.0	580.0	580.0	580.0	580.0	580.0	580.0	580.0
Maize Cob	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Soy Bean Oil	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Vitamin/Mineral.Premix*	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Dicalcium phosphate	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0
Limestone	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
NaHCO3	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0
KCl	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
K2CO3	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
MgO	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
NaCl	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Choline Chloride	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
TiO_2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Protease	0.0	0.0	0.0	0.0	5.0	5.0	5.0	5.0
TOTAL	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Calculated Nutrients:								
ME Kcal/Kg	3529.6	3507.0	3484.4	3461.8	3512.2	3489.6	3466.9	3444.4
CP (g/kg)	0.7	16.9	33.1	49.3	0.7	16.9	33.1	49.3
Ca (g/kg)	8.8	8.9	8.9	9.0	8.8	8.9	8.9	9.0
Nonphytate P (g/kg)	3.5	3.5	3.6	3.6	3.5	3.6	3.6	3.6
Lysine (g/kg)	0.0	0.5	0.9	1.4	0.0	0.5	0.9	1.4
DL-Methionine (g/kg)	0.0	0.1	0.2	0.3	0.0	0.1	0.2	0.3

*Supplied per Kg diet: Vit.A (8000IU), Vit.D 3 (1200IU), Vit.E (31IU), Vit.K (2mg), Vit.B 2 (10mg), Vit.B 5 (150mg), Mn (80mg), Zn (50mg), Cu (2mg), I (1.2mg). Co (2mg), Se (0.1mg)

4.2.2. ILEAL DIGESTA COLLECTION

On day-26, all the birds were weighed and asphyxiated using carbondioxide. The birds were immediately cut open and the section between Meckel diverticulum and 2cm anterior to the ileo-caeco-colonic junction was excised and flushed with distilled water to harvest the digesta. Ileal digesta were pooled according to replicates, frozen, freeze-dried and milled for analysis.

4.2.3. CALCULATION OF DIGESTIBILITY

Crude protein digestibility (%) was calculated using the following formula:

$DC_{CP,diet} = 1- \{(TiO_{2diet} \text{ X } CP_{digesta})/(TiO_{2digesta} \text{ X } CP_{diet})\} \text{ X } 100$

Where:

DC_{CPdiet} = Crude protein digestibility (%)
TiO_{2diet} = Amount of titanium dioxide in the diet
$CP_{digesta}$ = Crude protein of the digesta
$TiO_{2digesta}$ = Amount of titanium dioxide in digesta
CP_{diet} = Crude protein in diet

Digestibility of CP in HFM was calculated on DM basis using the regression model described by Rodehutscord *et al.* (2004) and Kluth and Rodehutscord (2006). Daily CP intake was obtained as daily feed intake multiplied by analysed CP value in the diet; amount of CP digested up to the terminal ileum for each of the diets was calculated as CP intake multiplied by the apparent CP digestibility for each diet. The slope of the linear graph of digested CP values plotted against CP intake represents the digestibility CP of HFM.

4.2.4. CHEMICAL ANALYSIS

The feed and freeze-dried ileal digesta samples were analysed for percentage CP, percentage Fat, percentage Ca and percentage P according to AOAC (2000); details of procedures are as presented in Appendix 2, 3, 4 and 5.

Titanium dioxide in all samples were determined by digesting samples in concentrated H_2SO_4 for 2 hours, adding 30% H_2O_2 and absorbance measured at 410 nm. Titanium dioxide concentration was determined by reading values of samples from a standard linear curve derived from absorbance (at 410nm) of titanium dioxide solutions of known concentrations. All samples, standards and blanks were subjected to same treatments according to Myers *et al.* 2004. (Appendix 1).

4.2.5 STATISTICAL ANALYSIS

A 2-way ANOVA was used to determine the main effects of the enzyme and HFM inclusion in the diet in a completely randomized design. Data were analysed using the GLM procedure in SAS (SAS, 2000) where significant differences were obtained and means were separated by Tukeys.

4.3. RESULTS

Results of analysed nutrient concentration in the experimental diets are shown in Table 11. Control diets contained 1% CP or less. Phosphorus concentration in the diets was similar. The DM concentration ranged from 99.67 to 99.79%.

Results of digestibility of DM, CP and Ca are shown in Table 12. Protease, HFM and their interaction significantly ($\alpha_{0.05}$) increased CP digestibility (Table 13). Feather Meal significantly ($\alpha_{0.05}$) reduced DM and Ca digestibility. There was a numerical increase though not significant of the effect of protease on these response criteria. Interaction of protease and HFM significantly ($\alpha_{0.05}$) affected digestibility of Ca but not of DM.

The relationship between amount of crude protein intake (g/kg DMI) and amount of digested CP (g/kg DM) without protease supplementation is shown in Figure 8 and with protease supplementation in Figure 9.

Being a linear regression relationship, the slopes of the curves represent the digestibility of CP in the HFM without protease (Figure 8) and with protease (Figure 9). Thus, digestibility of CP in HFM was increased by 1.47% at 56.71% without protease supplementation to 58.18% with protease addition.

TABLE 11: Analysed nutrient composition of experimental diets.

	Minus Protease				Plus Protease			
HFM, %	0	2	4	6	0	2	4	6
Diet No.	1	2	3	4	5	6	7	8
Parameters								
DM, %	99.7	99.7	99.7	99.8	99.8	99.8	99.8	99.8
CP, %	0.7	1.8	3.5	5.1	1.0	2.2	4.3	6.2
P, %	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.3
Ca, %	1.2	1.4	1.2	1.6	2.0	1.5	1.4	1.3
GE(Kcal/g)	4.4	4.4	4.3	4.6	4.4	4.6	4.6	4.5

DM= Dry matter, CP= Crude protein, P= Phosphorus, Ca= Calcium and GE= Gross energy

TABLE 12: Effect of protease, HFM and their interaction on apparent nutrient digestibility (%)

PARAMETER	Protease			HFM					*P-Anova*		
	0	5	SEM	0	2	4	6	SEM	Protease	HFM	HFM*Protease
CP digestibility	65.9[a]	76.1[b]	2.3	58.8[c]	64.1[cd]	75.8[de]	85.3[e]	3.3	0.01	<0.0001	0.03
DM digestibility	69.6	69.9	2.8	83.2[c]	69.5[cd]	58.5[d]	67.7[d]	3.9	0.9	0.002	0.8
Ca digestibility	59.6	63.7	3.8	40.1[c]	62.9[d]	78.5[d]	65.0[d]	5.3	0.5	0.0004	0.011

[a,b,c] Values along the row for a specific factor with different superscript are significantly different (P <0.05). HFM= Feather Meal, DM= Dry Matter, CP= Crude Protein, Ca = Calcium, SEM= Standard Error of Mean.

Table 13: Interaction of main effects on nutrient digestibility of broilers fed HFM as only source of nitrogen with or without protease supplementation.

Interactions	*P-Anova*		
Parameters	HFM	Protease	HFM*Protease
DM	0.1	0.0001	0.01
CP	0.1	0.1	0.03
Ca	0.2	0.02	0.1

HFM= Feather Meal, DM= Dry matter, CP= Crude Protein and Ca= Calcium

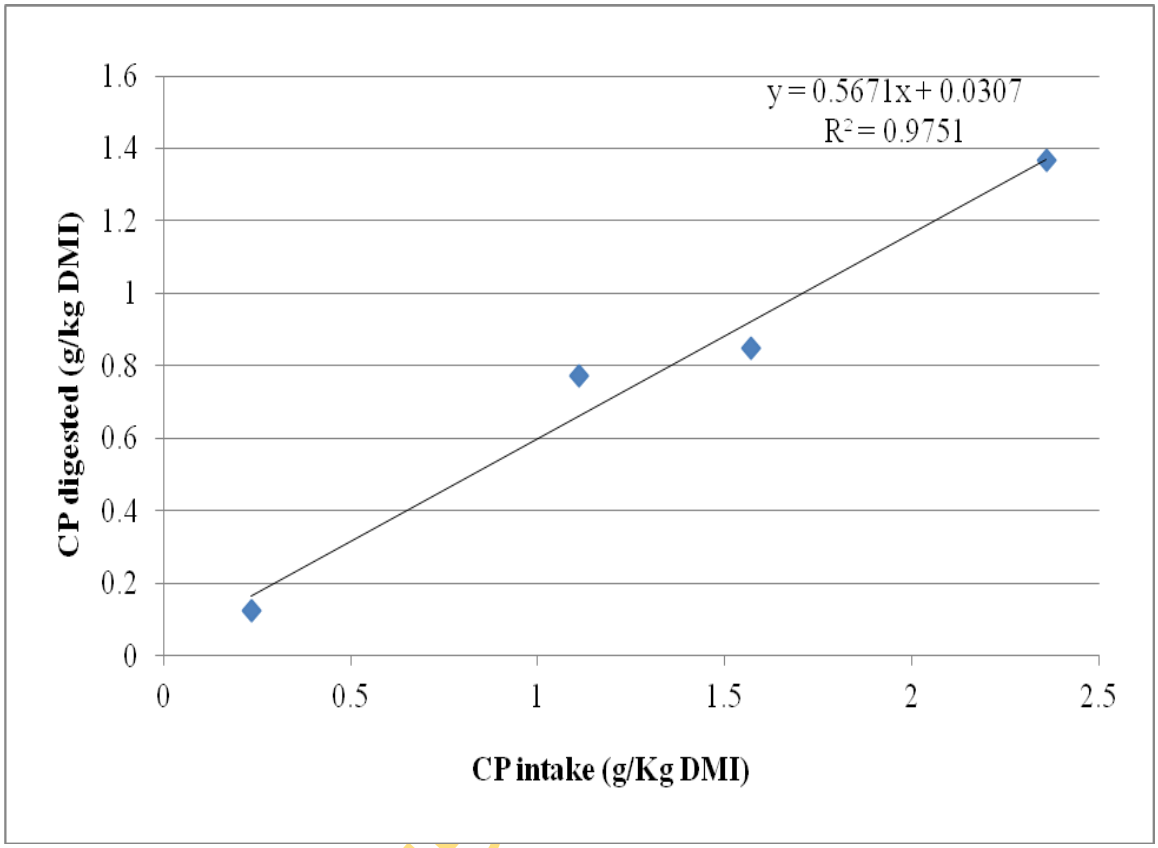


Figure 8: Relationship between crude protein ingested and amount digested without protease supplementation in broilers fed HFM on dry matter basis.

Crude Protein in HFM as analysed and used in these studies is 83.79%.

To calculate the amount of digestible CP in HFM without protease addition, the formula

Digestible CP_{FM} = Amount of CP in FM x digestibility coefficient of HFM; was used.

$$y = mx + c$$

(where y = amount of CP digestible in HFM, m = regression slope, c = intercept and X = analysed CP level in HFM).

$y = 0.5671x + 0.0307$

$y = 0.5671(83.79) + 0.0307$

$y = 47.55$

Therefore the digestible CP of HFM when protease is not added in broilers is 47.55% and the true digestibility is 56.71%

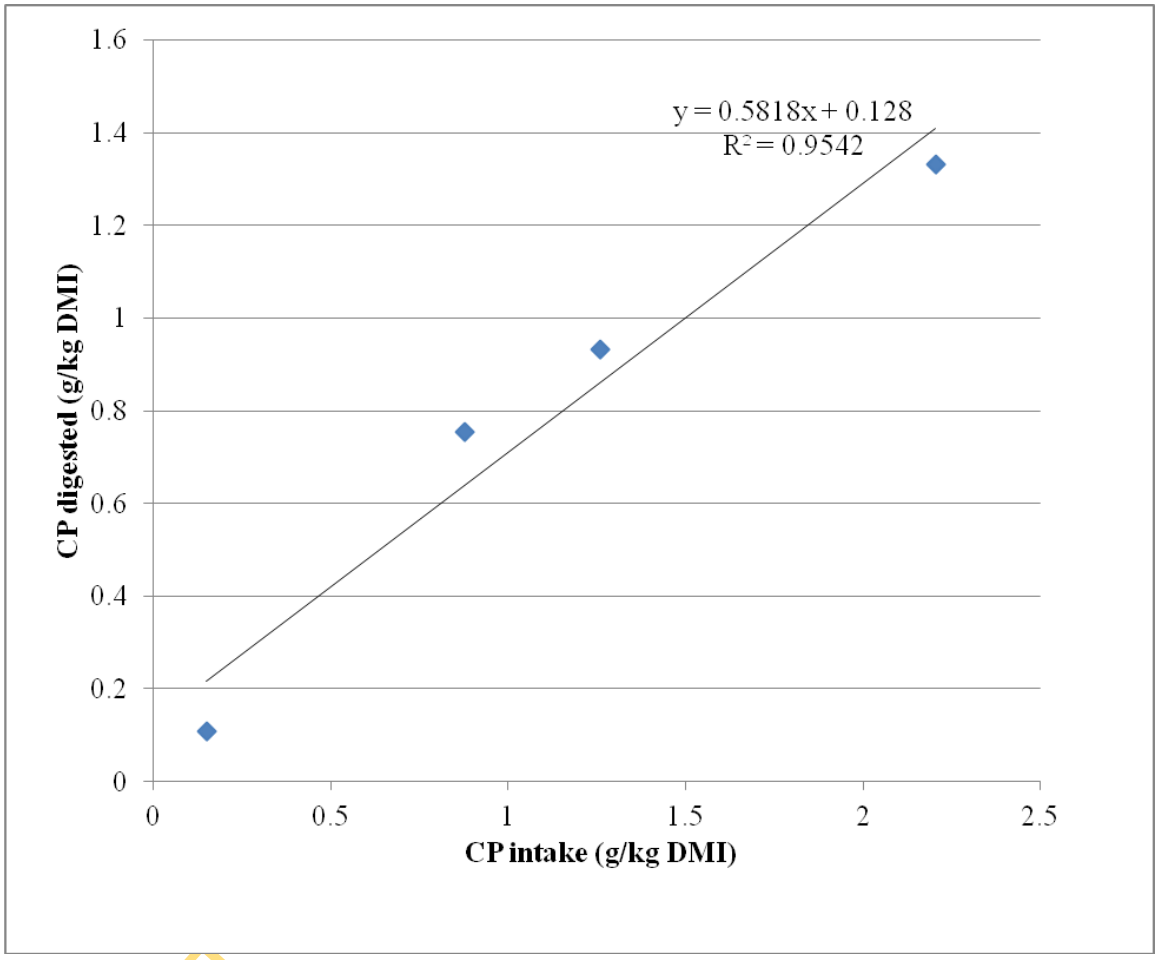


Figure 9: Relationship between crude protein ingested and amount digested with protease supplementation in broilers fed HFM on dry matter basis.

Crude Protein in HFM as analysed and used in these studies is 83.79%.

To calculate the amount of digestible CP in HFM with protease addition, the formula

Digestible CP_{FM} = Amount of CP in HFM x digestibility coefficient of HFM; was used.

$$y = mx + c$$

(where y = amount of CP digestible in HFM, m = regression slope, c = intercept and x = analysed CP level in HFM).

$Y = 0.5818x + 0.128$

$Y = 0.5818(83.79) + 0.128$

$Y = 48.87$

Therefore the digestible CP of HFM when protease is added in broilers is 48.87% and the true digestibility is 58.18%.

Protease supplementation increased the amount of digestible CP in HFM by 2.78%.

At 5.5% inclusion level of HFM, CP digestibility is same with and without protease supplementation. Figure 10 shows apparent CP digestibility of HFM with and without protease supplementation.

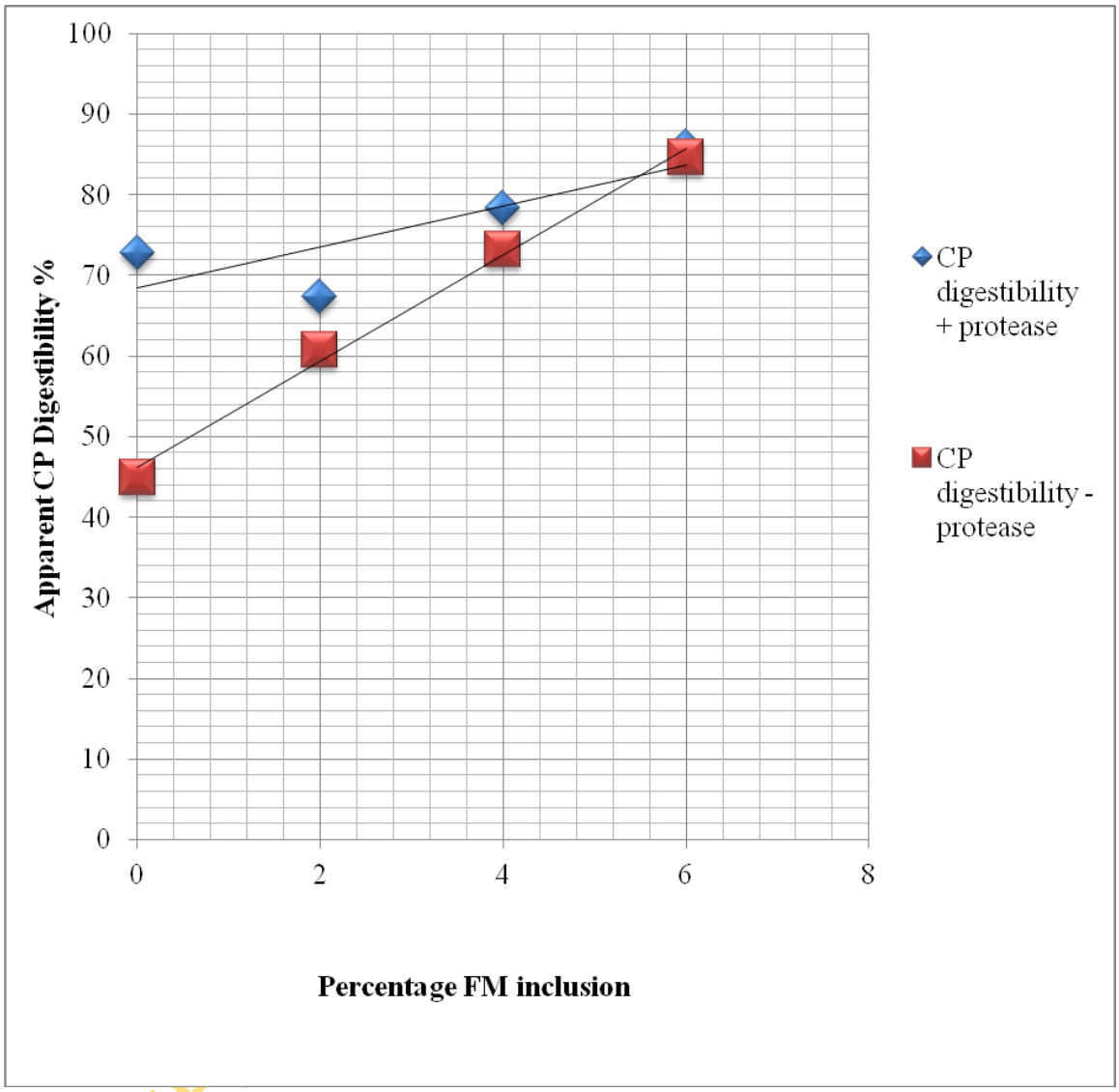


Figure 10: Apparent CP digestibility of HFM with or without protease supplementation

4.4. DISCUSSION

The Apparent CP Digestibility (ACPD) for the diets was determined to be 58.8, 64.1, 75.8 and 85.3% in sequential diets to HFM inclusion. These values are slightly higher than those obtained in chapter three where HFM was included in broiler starter phase diets at the same inclusion level but as part of a normal feed in which conventional feedstuffs were used for formulation. Steiner *et al.* (1983) reported a CP digestibility of 66.1% of HFM in broilers while Aderibigbe and Church, (1983a) reported 73.6% in lambs. These authors incorporated HFM as part of a regular feed with HFM supplying part of the protein requirement. The feeding of HFM as the only nitrogen source may have been responsible for the observed increase in HFM Crude Protein digestibility as animals tend to maximize the available nutrient in their diets while trying to make up for the short fall. It could also be because the proteolytic enzymes in the gut were adequately challenged (Aderibigbe and Church, 1983b).

Apparent CP digestibility (ACPD) increased from 8.4 to 29.1% with enzyme supplementation. The work of Aderibigbe and Church (1983b); Freitas *et al*. (2011) also showed that CP digestibility in HFM increased with increase in enzyme to nitrogen ratio in five in vitro studies where four protein sources were used and when low protein diets were fed to broilers. They also showed that the enzyme levels as observed in this study did not affect the DM. Since enzymes are specific in the reactions they catalyze and on specific substrates, this means that the protease used in this study was able to cleave some of the peptide bonds in the keratin of the HFM used in addition to the action of the

digestive proteases of the bird which resulted in the higher ACPD observed in enzyme supplemented diets.

The effect of protease on HFM digestibility was significant ($p< 0.05$) as it increased from moderate digestibility to good (64.1- 85.3%) with increase in HFM level. However, from the graph of Apparent CP digestibility (with or without protease) against HFM inclusion levels (Figure 10); HFM inclusion level of about 5.5% will give same digestibility with or without protease addition. This means that protease supplementation at 5g/kg is beneficial when HFM inclusion level is below 5.5%. Since enzyme and substrate concentrations affect enzyme activity, it may be that the increase in substrate concentration (HFM) without an increase in the enzyme concentration is responsible for this observation. With increase in substrate concentration, enzyme activity is enhanced up to a level when the enzyme becomes saturated with the substrate at which point the activity stabilizes regardless of increase in substrate level. Bharathidhasan *et al.* (2010) in their study, used 0, 0.25, 0.50, 0.75 and 1.00g/kg enzyme levels and reported an increase in BWG and FI with increasing enzyme level. Momtazan *et al.* (2011) obtained similar results with 0, 0.25 and 0.50g/kg enzyme inclusion levels. Ani *et al*. (2012) reported increase in BWG and nitrogen retention with increase in substrate (raw bambara nut waste) level when broilers were fed from 14 - 42days.

CHAPTER FIVE

5.0. PERFORMANCE AND CARCASS MEASURES IN BROILERS FED FM WITH OR WITHOUT PROTEASE SUPPLEMENTATION AT DIFFERENT CP LEVELS

5.1. INTRODUCTION

It has been reported that protease supplementation, and indeed enzyme supplementation in diets improve performance and body weight gain of broiler chickens fed low or inadequate dietary crude protein (Odetallah *et al*. 2003; Cowieson and Adeola, 2005; Wang *et al*. 2006; Agricultural Review, 2011). Feathers though entirely protein, require addition of a suitable protease to help make it more available to birds. Odetallah *et al.* (2003) included a keratinase protease into broiler diets and reported improvement in the bird's performance. Wang *et al.*, (2006) supplemented broiler diets with a keratinase enzyme and reported an improvement in amino acid utilization of diets formulated to commercial specifications.

5.2.0. MATERIALS AND METHODS

5.2.1. Management of animals and experimental diets

A total of three hundred and sixty (360) one-day-old broiler chicks were weighed in group and randomly allocated to 12 treatments of 3 replicates with 10 birds each. The diets were formulated using the matrix approach as follows:

DIETS 1-6: WITH 2% HFM

Diet 1: 23% CP; positive control (standard formulation)

Diet 2: (23-7.5%) 15.5%CP; negative control 1, full matrix

Diet 3: (23-5.6%) 17.4%CP; negative control 2; 75% of full matrix

Diet 4 = diet 1+ 0.05% protease

Diet 5 = diet 2+ 0.05% protease

Diet 6 = diet 3+ 0.05% protease

DIETS 7-12: WITHOUT HFM

Diet 7: 23% CP; positive control (standard formulation)

Diet 8: (23-7.5%) 15.5%CP; negative control 1, full matrix

Diet 9: (23-5.6%) 17.4%CP; negative control 2; 75% of full matrix

Diet 10 = diet 1+ 0.05% protease

Diet 11 = diet 2+ 0.05% protease

Diet 12 = diet 3+ 0.05% protease

Same approach was used for the finisher phase diets using the recommended 20% CP level for the control diet formulation.

The birds were fed the experimental diets from 0 - 42 days, consisting of 0 - 21 days (starter phase) and 22 - 42 days (finisher phase). Tables 14 and 15 show the experimental diet composition and calculated nutrient concentrations respectively for the starter phase, while Tables 16 and 17 show the diet composition and calculated nutrient concentrations for the finisher phase.

Records of feed intake and body weight gain were taken throughout the experimental period; at the starter and finisher phases. Clean water was given to birds *ad libitum*. On day 42, all birds were weighed, slaughtered, de-feathered and cut into parts for carcass measures.

TABLE 14: Gross composition (g/kg) of starter diets for broilers with three levels of crude protein with or without HFM and protease supplementation

	DIETS + 2% HFM						DIETS - HFM					
	Without Protease			With Protease			Without Protease			With Protease		
Diet	1	2	3	4	5	6	7	8	9	10	11	12
CP (%)	23.0	17.4	15.5	23.0	17.4	15.5	23.0	17.4	15.5	23.0	17.4	15.5
Ingredients												
Maize	510.0	677.0	742.0	510.0	677.0	742.0	500.0	662.0	726.0	500.0	662.0	726.0
Soyabean Meal	322.5	156.0	100.5	322.5	155.5	100.5	352.5	190.5	136.5	352.5	190.5	136.5
Fishmeal	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Feather Meal	20.0	20.0	20.0	20.0	20.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0
Cassava Starch	5.0	5.0	5.0	0.0	0.0	0.0	5.0	5.0	5.0	0.0	0.0	0.0
Palm oil	45.0	45.0	35.0	45.0	45.0	35.0	45.0	45.0	35.0	45.0	45.0	35.0
Limestone	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Dicalcium Phosphate	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
NaCl	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mineral/Vitamin premix*	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Methionine	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Lysine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Protease	0.0	0.0	0.0	5.0	5.0	5.0	0.0	0.0	0.0	5.0	5.0	5.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0

*Supplied per Kg diet: Vit.A (8000IU), Vit.D 3 (1200IU), Vit.E (31IU), Vit.K (2mg), Vit.B 2 (10mg), Vit.B 5 (150mg), Mn (80mg), Zn (50mg), Cu (2mg), I (1.2mg). Co (2mg), Se (0.1mg)

TABLE 15: Calculated nutrient composition of experimental diets (0-21d)

	Diets plus 2% HFM						Diets minus HFM					
	With Protease			Without Protease			With Protease			Without Protease		
DIETS	1	2	3	4	5	6	7	8	9	10	11	12
Parameters												
ME Kcal/Kg	3108.0	3277.0	3285.0	3090.0	3259.0	3267.0	3099.0	3263.0	3270.0	3081.0	3245.4.0	3252.6
CP (g/kg)	235.1	176.4	158.0	235.0	176.5	158.1	231.2	174.3	156.2	231.2	174.3	156.3
Ca (g/kg)	12.1	11.8	11.7	12.1	11.8	11.7	12.1	11.8	11.7	11.7	11.8	11.7
Total P (g/kg)	8.1	7.6	7.5	8.1	7.6	7.5	8.3	7.8	7.6	8.3	7.8	7.6
Ca:P	1.5	1.6	1.6	1.5	1.6	1.6	1.5	1.5	1.5	1.4	1.5	1.5
Lysine (g/kg)	14.7	10.6	9.3	14.7	10.6	9.3	15.0	11.1	9.8	15.0	11.1	9.8
DL-Methionine (g/kg)	7.2	6.5	6.3	7.2	6.5	6.3	7.2	6.6	6.4	7.2	6.6	6.4
Threonine (g/kg)	0.8	0.8	0.8	0.8	0.8	0.8	0.0	0.0	0.0	0.0	0.0	0.0
Tryptophan (g/kg)	0.6	0.7	0.8	0.6	0.7	0.8	0.5	0.6	0.7	0.5	0.6	0.7

TABLE 16: Gross composition (g/kg) of broiler diets with or without HFM, protease and 3 CP levels based on the protease matrix value (22 - 42d).

	Diets + 2% HFM						Diets - HFM					
	Without Protease			With Protease			Without Protease			With Protease		
Diets	1	2	3	4	5	6	7	8	9	10	11	12
CP (%)	20.0	14.4	12.5	20.0	14.4	12.5	20.0	14.4	12.5	20.0	14.4	12.5
Ingredients												
Maize	590.0	755.0	813.0	590.0	755.0	813.0	580.0	743.0	802.0	580.0	743.0	802.0
Soyabean Meal	242.5	92.5	39.5	242.5	92.5	39.5	272.5	124.5	70.5	272.5	124.5	70.5
Fishmeal	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Feather Meal	20.0	20.0	20.0	20.0	20.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0
Cassava Starch	5.0	5.0	5.0	0.0	0.0	0.0	5.0	5.0	5.0	0.0	0.0	0.0
Palm oil	45.0	30.0	25.0	45.0	30.0	25.0	45.0	30.0	25.0	45.0	30.0	25.0
Limestone	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Dicalcium Phosphate	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
NaCl	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix*	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Methionine	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Lysine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Protease	0.0	0.0	0.0	5.0	5.0	5.0	0.0	0.0	0.0	5.0	5.0	5.0
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

*Supplied per Kg diet: Vit.A (8000IU), Vit.D 3 (1200IU), Vit.E (31IU), Vit.K (2mg), Vit.B 2 (10mg), Vit.B 5 (150mg), Mn (80mg), Zn (50mg), Cu (2mg), I (1.2mg). Co (2mg), Se (0.1mg)

TABLE 17: Calculated nutrient composition of experimental diets (22 -42d)

	Diets + 2%HFM						Diets - HFM					
	Without Protease			With Protease			Without Protease			With Protease		
DIETS	1	2	3	4	5	6	7	8	9	10	11	12
Parameters												
ME Kcal/Kg	3188.8	3269.3	3299.2	3171.3	3251.8	3281.7	3179.8	3258.3	3289.2	3162.4	3240.9	3271.8
CP (g/kg)	206.9	155.7	137.5	207.0	155.7	137.5	203.1	152.5	133.9	203.1	152.5	134.0
Ca (g/kg)	11.9	11.7	11.6	11.9	11.7	11.6	11.9	11.7	11.6	11.6	11.7	11.6
Total P (g/kg)	7.9	7.5	7.3	7.9	7.5	7.3	8.0	7.6	7.5	8.0	7.6	7.5
Ca:P	1.5	1.6	1.6	1.5	1.6	1.6	1.5	1.5	1.6	1.4	1.5	1.6
Lysine (g/kg)	12.7	9.1	7.8	12.7	9.1	7.8	13.1	9.5	8.2	13.1	9.5	8.2
DL-Methionine (g/kg)	6.9	6.3	6.1	6.7	6.3	6.1	6.9	6.4	6.2	6.9	6.4	6.2
Threonine (g/kg)	0.8	0.8	0.8	0.8	0.8	0.8	0.0	0.0	0.0	0.0	0.0	0.0
Tryptophan (g/kg)	0.6	0.8	0.8	0.6	0.8	0.8	0.5	0.7	0.7	0.5	0.7	0.7
Valine (g/kg)	1.2	1.2	1.2	1.2	1.2	1.2	0.0	0.0	0.0	0.0	0.0	0.0

5.2.2. CHEMICAL ANALYSIS

Samples of experimental feeds were analysed for their proximate composition according to AOAC (2000) Appendix 2, 3, 4 and 5.

5.2.3 STATISTICAL ANALYSIS

There were two levels of enzyme inclusion, two levels of HFM inclusion and three levels of CP. The experiment was set in a 2 x 2 x 3 Completely Randomised Design (CRD) with factorial arrangement. All data were analyzed using GLM procedure in SAS (SAS, 2000) statistical package and the means separated with Tukeys.

Probability level was set at $\alpha_{0.05}$.

5.3.0. RESULTS

Analysed composition of starter and the finisher diets are as presented in Tables 18 and 19, respectively.

At the starter phase (Table 20), level of CP in feed affected birds positively as their Body Weight Gain was improved with increase in CP though not significantly. Supplementation of feeds with protease also resulted in a numerical increase in FI, BWG and FW but the increase was not significant at $\alpha_{0.05}$. Figure 11 shows the effect of protease supplementation on BWG with increase in CP level. However, the inclusion of HFM depressed FW, FI and BWG significantly ($\alpha_{0.05}$) as shown in Table 20. The effect of interaction between HFM and Protease; CP and HFM; CP and Protease, and between

HFM, CP and Protease were not significant as shown in Table 21 at the starter phase. The matrix value approach and HFM inclusion had significant effect on FI, BWG, FW and FCR when 100% matrix value was used in diet formulation (Table 22). Birds fed 75% matrix value without HFM performed better than those on the control in terms of the FCR. Birds fed HFM irrespective of the matrix value consumed about the same amount of feed. Inclusion of HFM significantly decreased FI with no significant difference with respect to the percentage matrix value of diet formulation.

At the finisher phase, increase in CP resulted in significant ($\alpha_{0.05}$) increase in BWG and a significant decrease in FI; protease supplementation significantly decreased FI but did not significantly increased BWG. Inclusion of HFM in the broiler diets decreased BWG, FI and FCR significantly at $\alpha_{0.05}$as shown in Table 23. The effect of interaction between HFM and Protease; CP and HFM; CP and Protease; and between HFM, CP and Protease were not significant as shown in Table 24 at the finisher phase. Birds fed diets with 0% matrix value (control diet) with enzyme supplementation and HFM inclusion performed better than all others as shown in Table 25. Birds fed diets with 100% matrix value formulation performed as good as birds fed 75% and 0% matrix values without HFM inclusion. Birds fed 100% matrix value formulation with HFM performed as good as those fed the control diet without HFM in terms of BWG and FCR.

TABLE 18: Analysed nutrient levels of broiler diets (0-21d)

	Plus HFM						Minus HFM					
	Minus Protease			Plus Protease			Minus Protease			Plus Protease		
CP (%)	23.0	17.4	15.5	23.0	17.4	15.5	23.0	17.4	15.5	23.0	17.4	15.5
Diet	1	2	3	4	5	6	7	8	9	10	11	12
Parameters												
% CP	22.6	17.5	14.7	21.2	17.3	14.5	22.9	17.3	15.0	22.9	17.5	14.2
% DM	99.6	99.8	99.7	99.7	99.7	99.6	99.7	99.6	99.5	99.6	99.7	99.7
%P	0.7	0.7	0.6	0.6	0.6	0.6	0.8	0.5	0.6	0.7	0.5	0.5
%Ca	1.5	1.7	1.5	2.1	1.8	1.7	1.4	1.0	1.6	1.9	1.3	1.2
GE(kcal/g	4.8	4.7	4.7	4.8	4.8	4.7	4.9	4.8	4.8	4.8	4.8	4.8

CP= Crude protein, DM= Dry matter, P= Phosphorus, Ca= Calcium and HFM= Feather Meal

TABLE 19: Analysed nutrient levels of broiler diets (22-42d)

	Plus HFM						Minus HFM					
	Minus Protease			Plus Protease			Minus Protease			Plus Protease		
CP (%)	20	14.4	12.5	20	14.4	12.5	20	14.4	12.5	20	14.4	12.5
Diet	1	2	3	4	5	6	7	8	9	10	11	12
Parameters												
% DM	99.7	99.7	99.6	99.7	99.6	99.6	99.7	99.7	99.6	99.6	99.6	99.6
% CP	18.3	16.3	15.0	19.6	14.9	12.6	20.9	16.3	13.9	21.9	15.3	13.8
% Ca	1.2	1.7	1.2	1.6	1.5	1.7	2.6	1.3	1.1	2.2	1.9	1.1
% P	0.4	0.7	0.5	0.6	0.6	0.5	0.7	0.5	0.7	0.56	0.7	0.7
GE (Kcal/g)	4.7	4.7	4.7	4.8	4.7	4.7	4.7	4.6	4.5	4.8	4.6	4.4

DM= Dry matter, CP= Crude protein, HFM= Feather Meal, P= phosphorus, Ca= Calcium and GE= Gross energy

TABLE 20: Growth performance of broilers fed HFM with protease supplementation at different CP levels (0-21d)

	CP (%)			SEM	Protease (%)		SEM	HFM (%)		SEM	*P-Anova*		
	15.5	17.5	23		0	5		0	2		CP	Protease	HFM
Parameters													
FW(g/bird)	233.3	277.5	278.3	14.5	249.4	276.7	11.9	285.6[a]	240.6[b]	11.9	0.1	0.1	0.01
BWG (g/bird)	190.5	235.2	235.8	14.2	206.6	234.4	1.2	243.7[a]	197.3[b]	11.6	0.1	0.1	0.01
FI(g/bird)	59.3	58.7	59.9	0.8	58.7	59.8	0.7	63.4[a]	55.2[b]	0.7	0.6	0.2	<0.0001
FCR	3.2	2.6	2.7	0.2	2.9	2.7	0.1	2.7	2.9	0.1	0.1	0.1	0.3

[a,b,c] Values in the same row for a specific factor with different superscripts are significantly different (P <0.05). FW= Final weight, BWG= Body weight gain, FI= Feed intake, FCR= Feed conversion ratio, CP = Crude protein and HFM= Feather Meal and SEM= Standard error of mean.

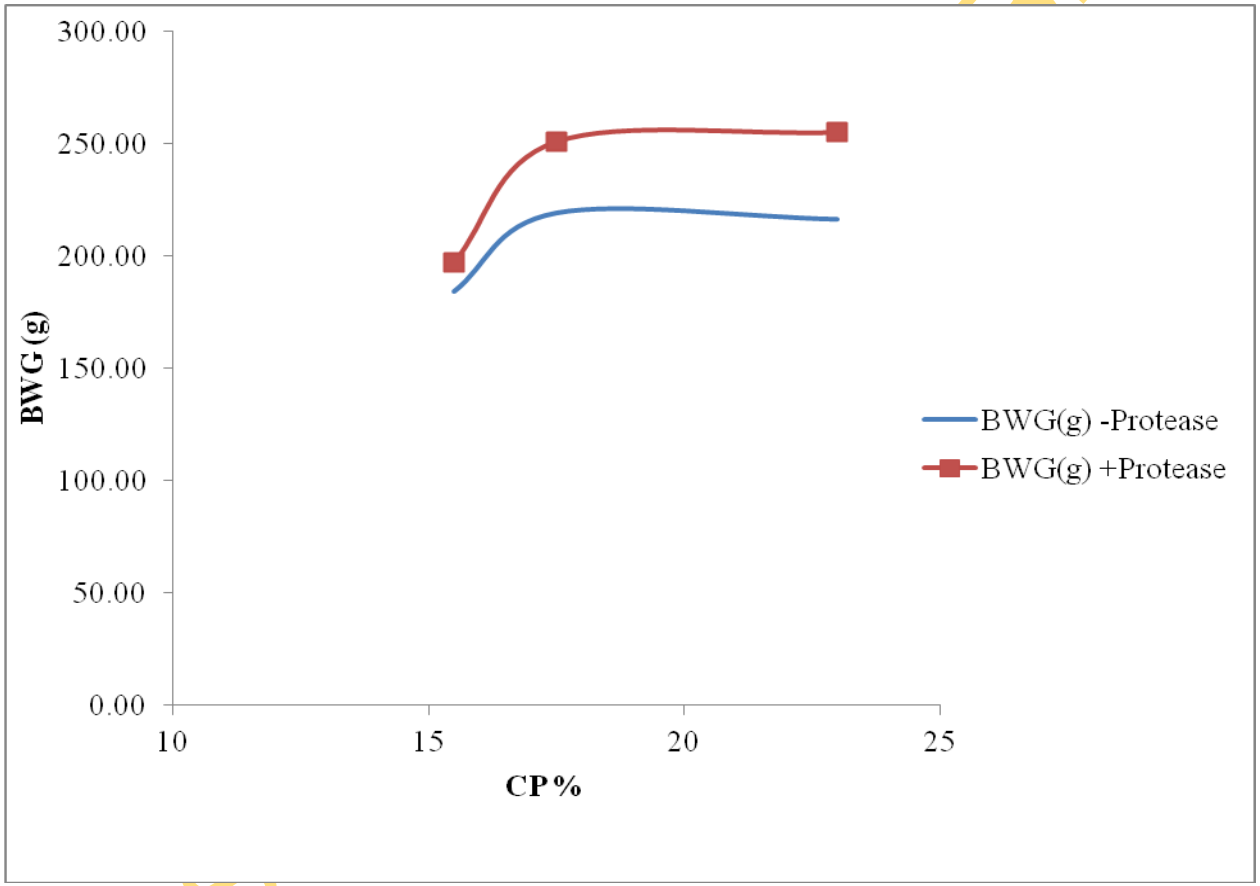


Figure 11: Effect of protease supplementation on body weight gain (BWG) in broilers fed diets containing HFM at the starter phase

Table 21: Main effect interaction for performance characteristics of broilers fed HFM with protease supplementation and different CP levels (0-21d)

	P-Anova			
Interactions	CP*HFM	CP*Pro	HFM*Pro	CP*HFM*Pro
Parameters				
F W(g/bird)	0.9	0.8	0.4	0.4
BWG(g/bird)	0.9	0.8	0.3	0.4
FI(g/bird)	0.8	0.2	0.2	0.8
FCR	0.8	0.6	0.6	0.4

CP= Crude Protein, HFM= Feather Meal, FW= Final Weight, BWG= Bird weight gain, Pro= Protease, FI= Feed Intake, FCR= Feed Conversion Ratio

Table 22: Effect of protease matrix value on performance characteristics of broilers fed diets with and without HFM (0-21d)

Matrix value (%)	CP (%)	HFM (%)	Protease (%)	FW(g)	BWG(g)	FI(g)	FCR
100	15.5	2	5.0	215.0^{a}	170.7^{a}	544.8^{a}	3.2^{d}
100	15.5	0	5.0	263.3^{bc}	223.3^{c}	648.4^{bc}	2.9^{cd}
75	17.5	2	5.0	246.7^{b}	203.7^{b}	555.4^{a}	2.8^{bc}
75	17.5	0	5.0	340.0^{d}	298.3^{d}	654.0^{c}	2.2^{a}
0	23.0	2	5.0	278.3^{c}	235.3^{c}	551.9^{a}	2.5^{ab}
0	23.0	0	5.0	316.7^{d}	275.0^{d}	635.9^{b}	2.4^{a}
SEM				29.0	28.3	16.1	0.3
P-value				<0.0001	<0.0001	<0.0001	<0.0001

^{a,b,c} Figures along the column with same superscript are not significantly different statistically. HFM= Feather Meal, FW= Final Weight, BWG= Bird weight gain , FI= Feed Intake, FCR= Feed Conversion Ratio, SEM= Standard Error of Mean, (P= 0.05)

Table 23: Growth performance of broilers fed HFM with protease supplementation at different CP levels (22-42d)

	CP (%)			SEM	Protease (%)		SEM	HFM (%)		SEM
	12.5	14.4	20		0	5		0	2	
Parameters										
BWG g/bird	538.8[a]	573.3[b]	664.7[c]	34.9	585.3	599.2	28.5	605.4[d]	579.1[e]	28.6
FI g/bird	1484.8[a]	1441.8[b]	1499.1[a]	40.7	1520.8[c]	1429.7[d]	33.2	1647.5[e]	1302.9[f]	33.2
FCR	2.8[a]	2.6[b]	2.4[c]	0.1	2.7[d]	2.4[e]	0.1	2.8[f]	2.3[g]	0.1

[a,b,c] Values in the same row for a specific factor with different superscripts are significantly different (P <0.05). FW= Final weight, BWG= Body weight gain, FI= Feed intake, FCR= Feed conversion ratio, CP== Crude protein and HFM= Feather Meal and SEM= Standard error of mean

TABLE 24: Interaction of main effects on growth performance of broilers fed HFM with protease supplementation at different CP levels (22-42d)

	CP	Protease	HFM	CP* HFM	CP* Protease	HFM* Protease	CP*HFM* Protease
	P-anova						
Parameters							
BWG (g/bird)	0.1	0.7	0.5	0.3	0.6	1.0	0.5
FI (g/bird)	0.6	0.1	< 0.0001	0.9	0.9	0.7	0.2
FCR	0.1	0.1	0.003	0.3	0.4	0.9	0.2

CP= Crude protein, HFM= Feather Meal, BWG= Body weight gain, FI= Feed Intake, FCR= Feed conversion Ratio

Table 25: Effect of protease matrix value on performance characteristics of broilers fed diets with and without HFM (22-42d)

Matrix value (%)	CP (%)	HFM (%)	Protease (%)	BWG(g)	FI(g)	FCR
100	12.5	0	5.0	614.0^{c}	1545.4^{c}	2.5^{bc}
100	12.5	2	5.0	531.7^{ab}	1331.8^{b}	2.5^{bc}
75	14.4	0	5.0	620.3^{c}	1652.5^{d}	2.7^{c}
75	14.4	2	5.0	495.0^{a}	1163.2^{a}	2.3^{b}
0	20.0	0	5.0	600.3^{bc}	1633.9^{d}	2.8^{c}
0	20.0	2	5.0	734.0^{d}	1251.2^{ab}	1.7^{a}
SEM	SEM			69.9	81.3	0.2
P-value	P			< 0.0001	< 0.0001	< 0.0001

^{a,b,c,d,e} Figures along the column with same superscript are not significantly different statistically. HFM= Feather Meal, BWG= Bird weight gain , FI= Feed Intake, FCR= Feed Conversion Ratio, SEM= Standard Error of Mean, (P= 0.05)

The result of the carcass and organ weights is presented in Table 26. Level of CP had significant effect on live weight, dressed weight, drumstick and gizzard as they increased with increase in CP. Feather Meal inclusion and protease supplementation did not have a significant effect on the carcass and organ weights generally but there was significant effect of protease on breast as it increased with decrease in CP level. There was no significant effect of the interaction of CP, HFM and protease on the response criteria measured as shown in Table 27. With 75% matrix value formulation containing HFM, Live weight and dressed weight of birds were not significantly different from those of birds fed 100% matrix value diet without HFM as shown in Table 28. All birds fed diets containing HFM were not significantly different from birds on the control diet without HFM. Breast and drumstick did not significantly differ in birds fed control diet without HFM and those fed 75% matrix value formulation with HFM. Thigh of birds fed the control diets (with and without HFM) were not significantly different.

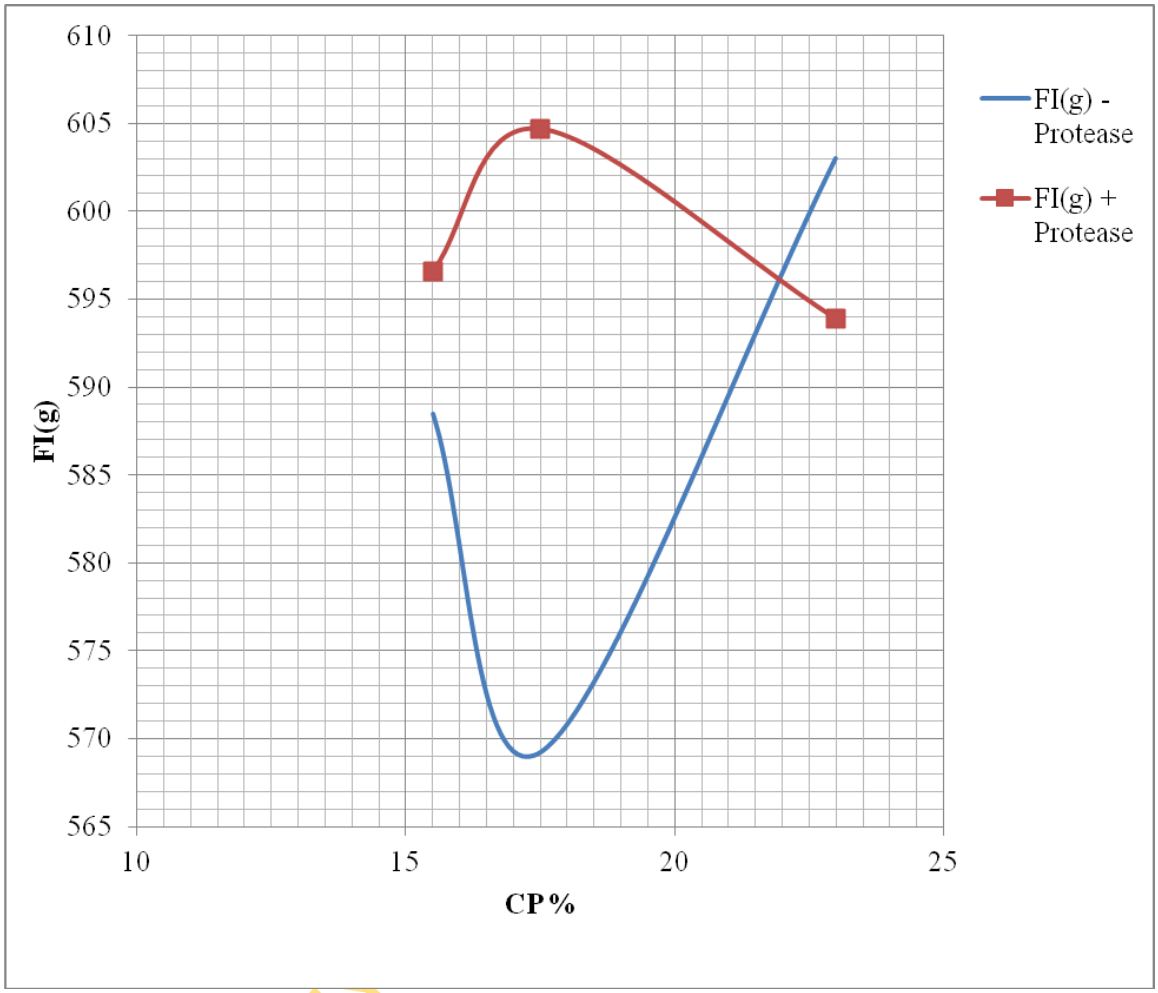


Figure 12: Effect of protease supplementation on Feed Intake (FI) in broilers fed diets containing HFM at the starter phase

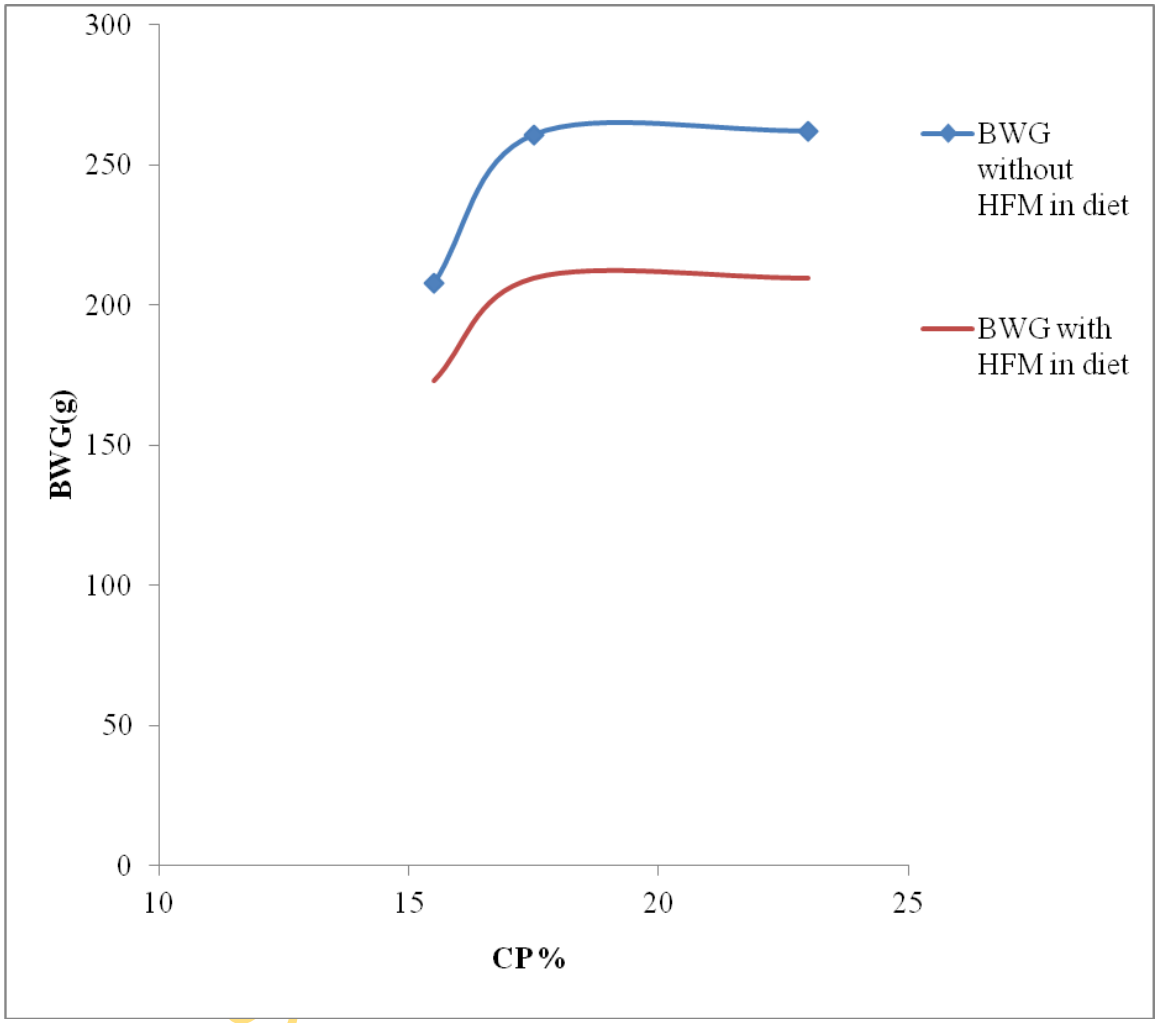


Figure 13: Effect of HFM inclusion on Body Weight Gain (BWG) in broilers at 0-21d

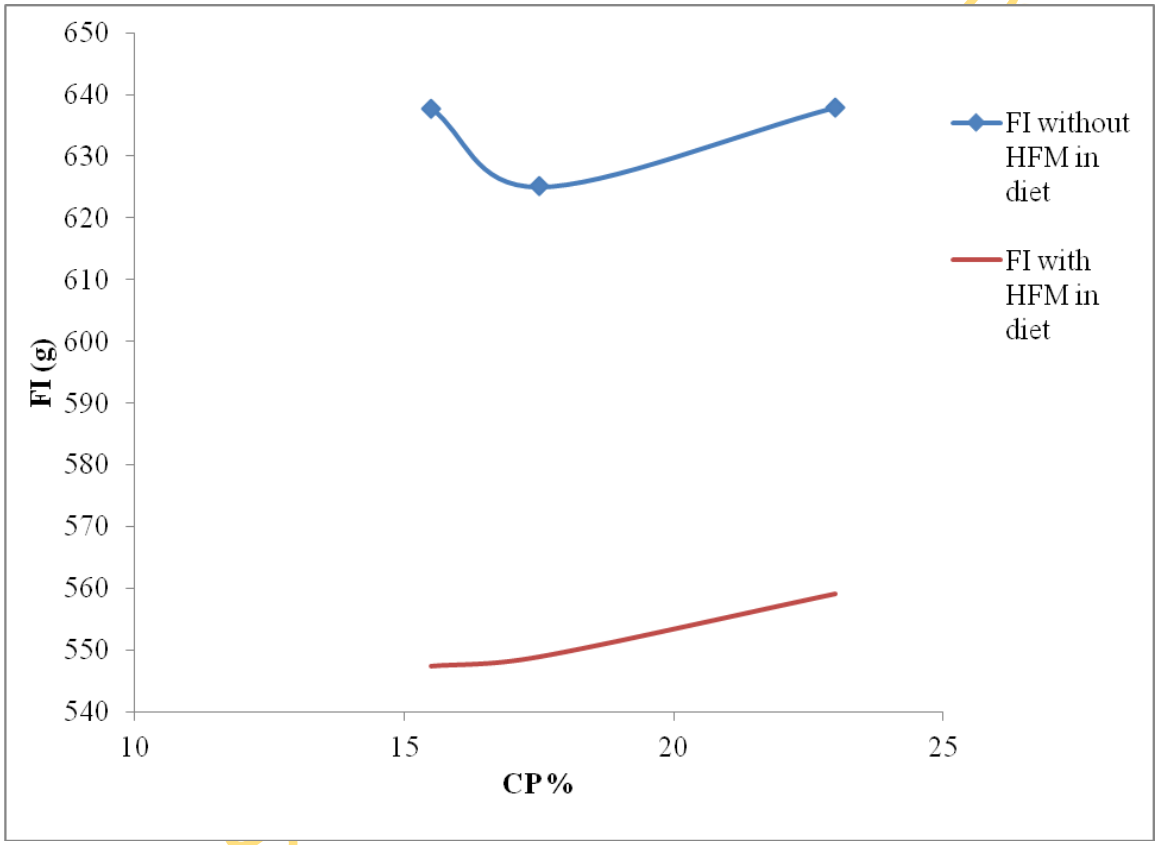


Figure 14: Effect of HFM inclusion on Feed Intake (FI) in broilers at 0-21d

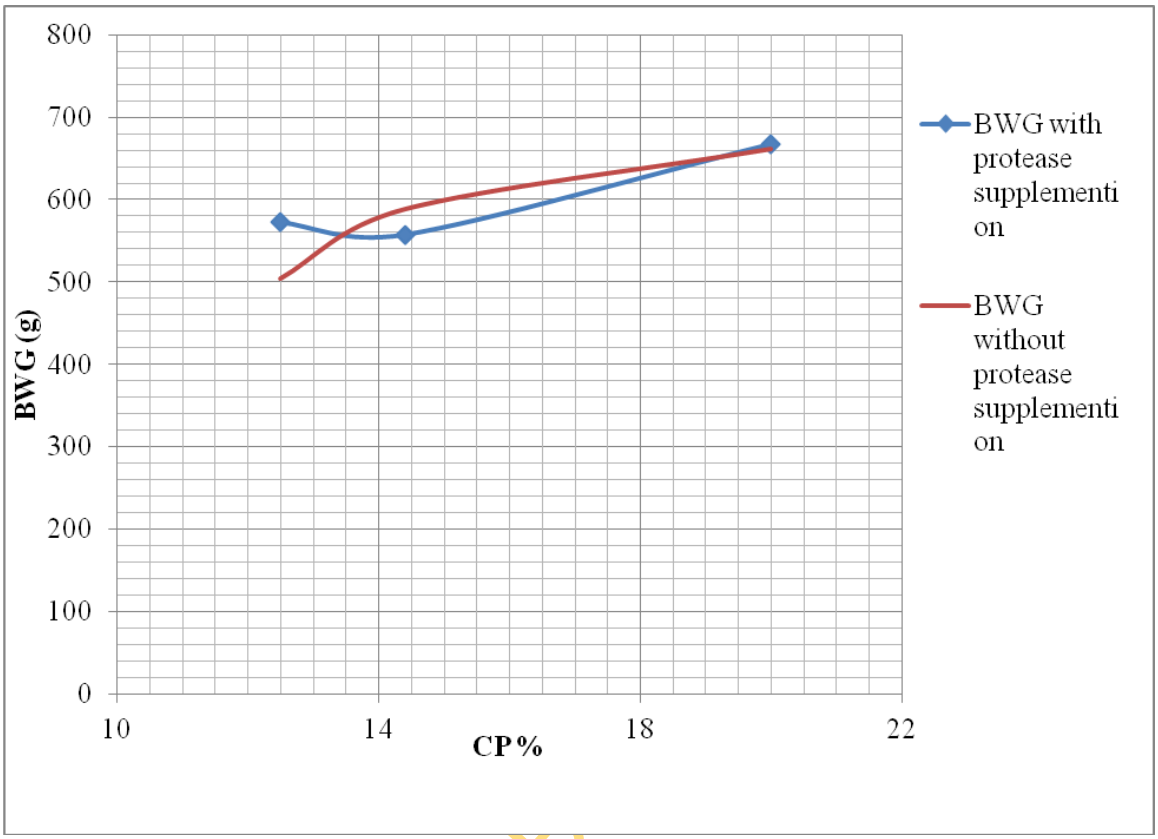


Figure 15: Effect of protease supplementation on body weight gain (BWG) in broilers at 22 - 42days

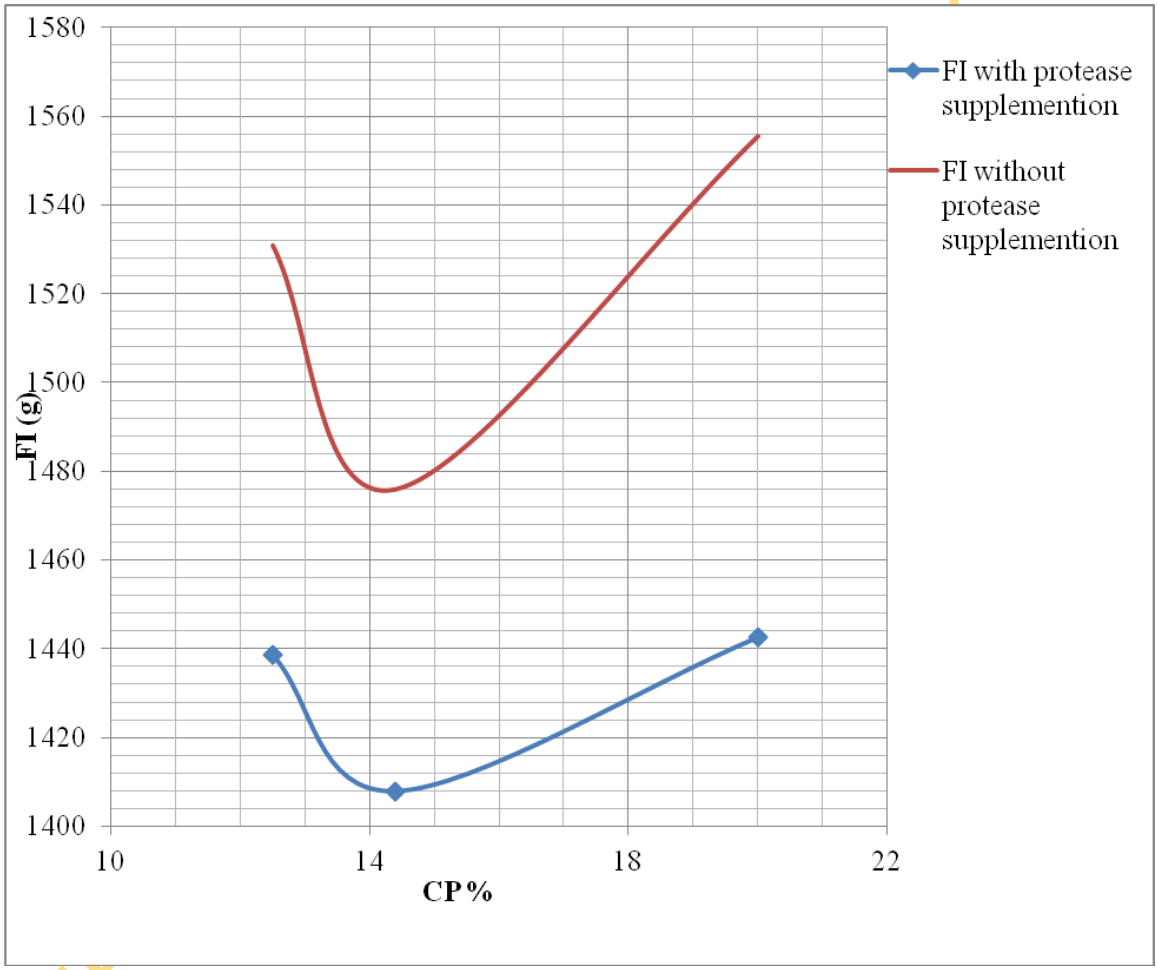


Figure 16: Effect of protease supplementation on feed intake (FI) in broilers at 22-42d

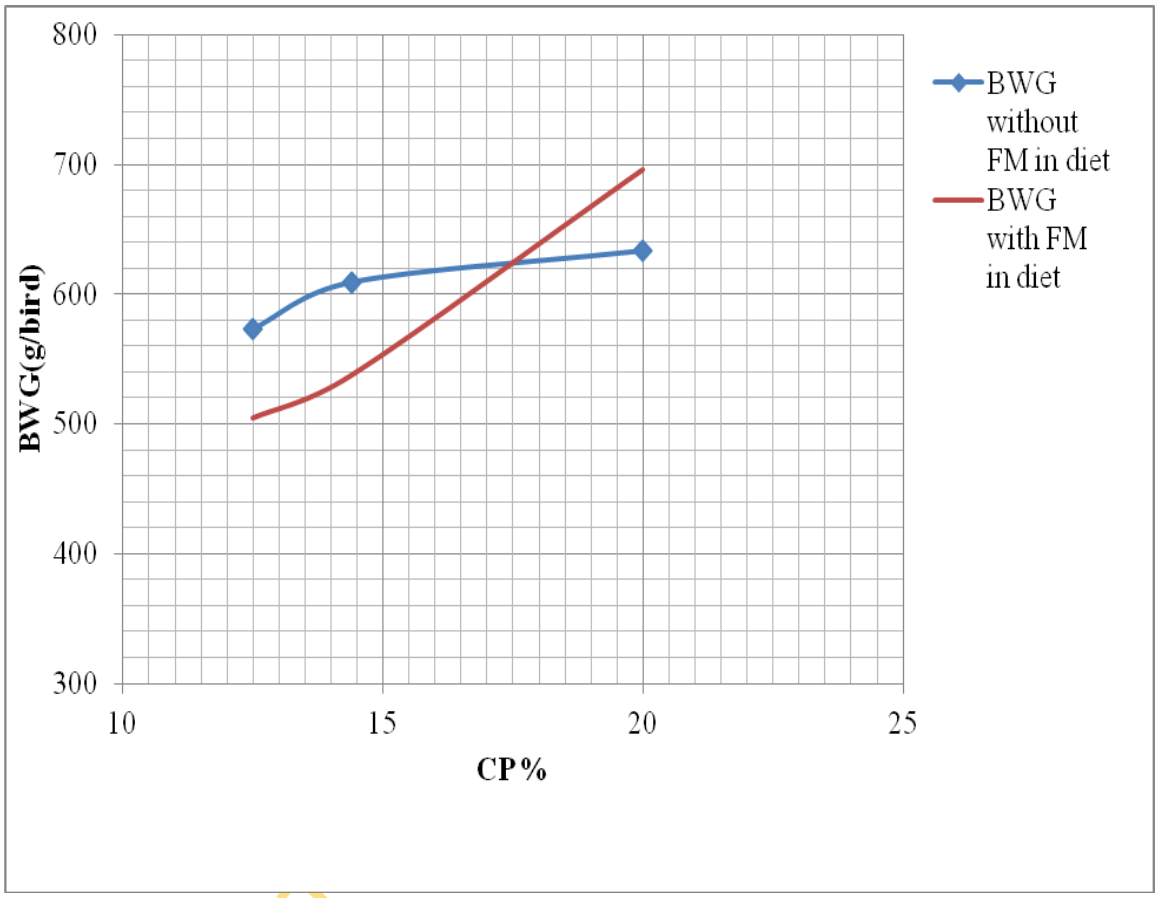


Figure 17: Effect of HFM inclusion on body weight gain (BWG) at 22 - 42days

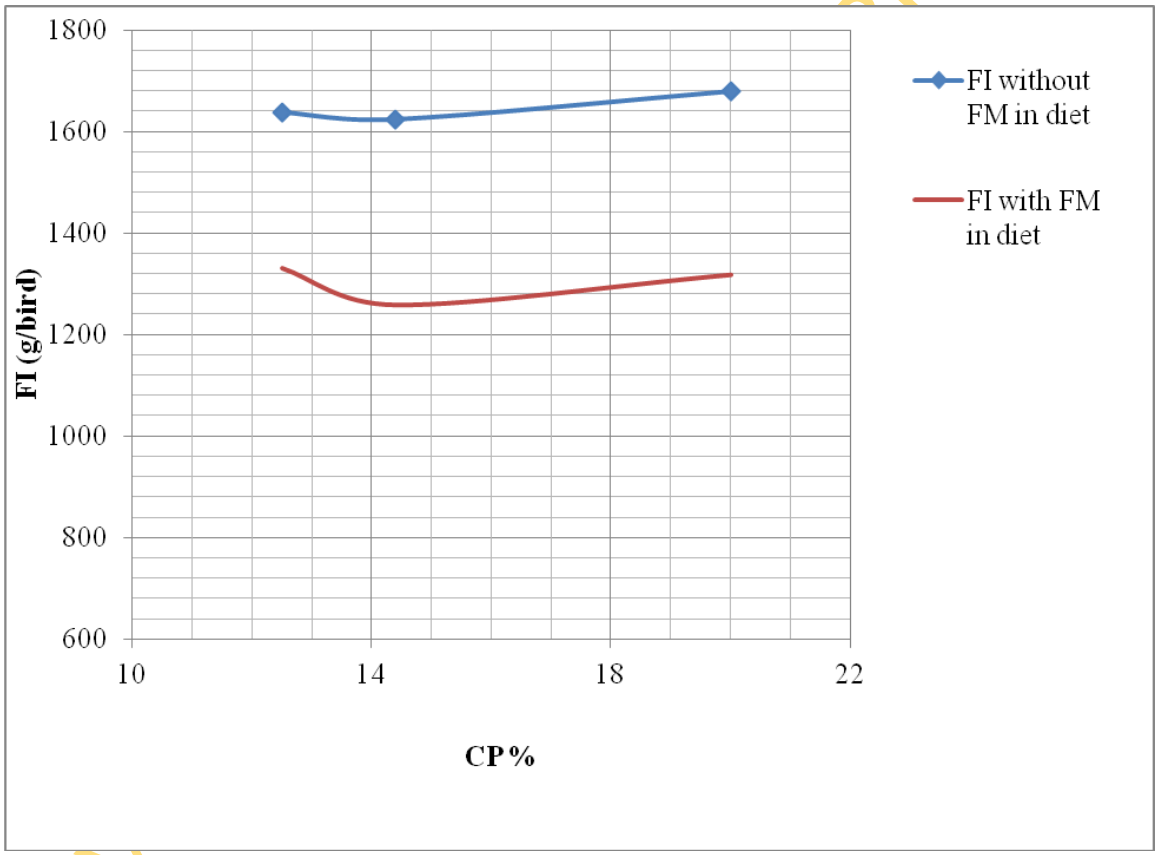


Figure 18:Effect of HFM inclusion on feed intake (FI) of broilers at 22 - 42d

TABLE 26: Carcass measures and organ weights (g/100g BW) of broilers fed HFM with protease supplementation at different CP levels (at 42days)

	CP (%)				Protease (%)			HFM (%)			P-Anova		
	12.5	14.4	20.0	SEM	0	5	SEM	0	2	SEM	CP	Protease	HFM
Parameters													
LW(g)	620.8[a]	694.2[a]	795.8[b]	29.9	696.1	711.1	24.4	725.0	682.2	24.4	0.002	0.7	0.2
D.wt (g)	446.8[a]	507.9[a]	585.7[b]	21.3	503.5	523.4	17.4	527.5	499.4	17.4	0.001	0.4	0.3
DP (%)	72.1	73.2	73.6	0.7	72.3	73.7	0.5	72.8	73.2	0.5	0.3	0.1	0.6
Breast *	22.3	23.9	23.8	0.6	22.6	24.1	0.5	23.7	22.9	0.5	0.1	0.03	0.3
Thigh*	13.6	13.9	13.8	0.3	13.8	13.8	0.3	13.8	13.7	0.3	0.7	0.9	0.8
Drumstick*	12.8[a]	12.9[a]	13.7[b]	0.3	13.2	13.1	0.2	13.1	13.2	0.2	0.02	0.6	0.6
Liver*	3.2	3.0	3.2	0.2	3.3	3.0	0.2	3.3	3.0	0.2	0.8	0.4	0.3
Kidney*	0.2	0.2	0.2	0.02	0.2	0.2	0.01	0.2	0.2	0.01	0.2	0.1	1.0
Gizzard*	4.6[a]	4.4[a]	3.9[b]	0.1	4.4	4.2	0.1	4.2	4.4	0.1	0.01	0.4	0.4

[a,b] Values within a row and for specific parameter with same superscript are not significantly different (P< 0.05). HFM= Hydrolysed Feather Meal, LW= Live weight, D.wt= Dressed weight , DP= Dressed Percent, SEM= Standard Error of Mean
*Values expressed as percentage of dressed weight.

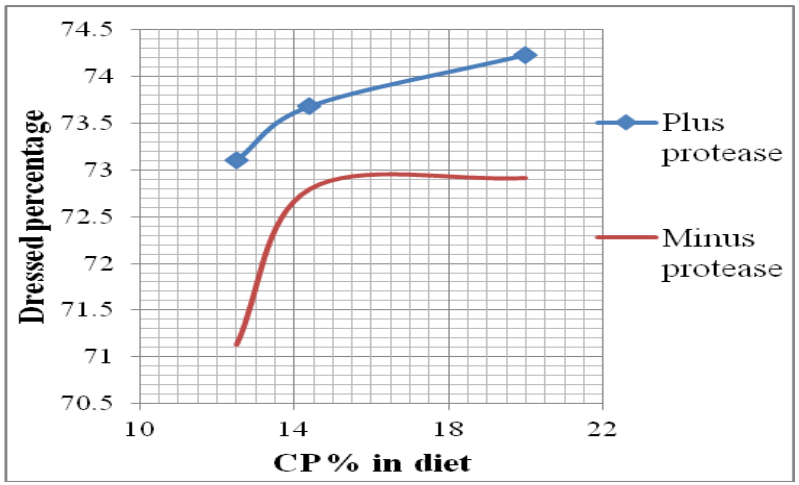
TABLE 27: Interaction of main effects on carcass measures and organ weights of broilers fed HFM with protease supplementation at different CP levels (22-42d)

	P-*Anova*			
Parameters	CP*HFM	CP*Protease	HFM*Protease	CP*HFM*Protease
Live Weight	0.8	0.9	0.7	0.9
Dressed weight	0.8	0.9	0.8	0.9
Dressed Percent	0.9	0.8	0.6	0.7
Breast	0.8	0.5	0.2	0.4
Thigh	0.8	0.2	0.5	0.9
Drumstick	0.6	0.1	0.3	0.5
Liver	0.9	0.4	0.9	0.3
Kidney	0.5	0.5	0.1	0.8
Gizzard	0.2	0.4	0.3	0.2

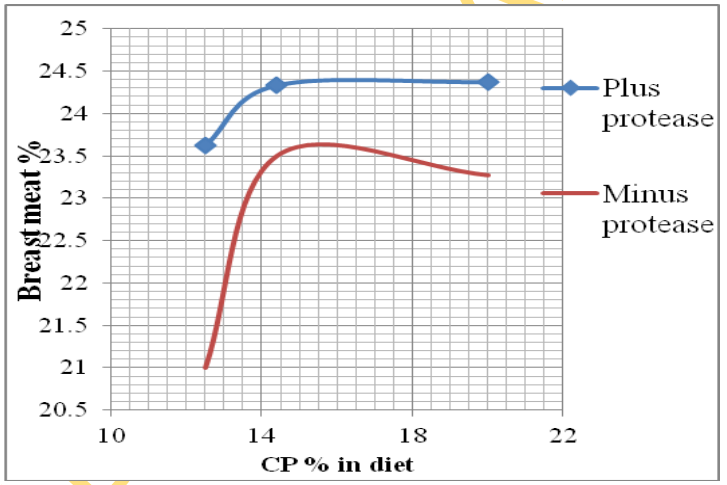
Table 28: Effect of matrix value of protease with and without HFM inclusion on carcass weights of broilers (at 42d)

Matrix value (%)	CP (%)	HFM (%)	Protease (%)	LW(g)	DW (g)	DP (%)	Breast	Thigh	Drumstick
100	12.5	0	5.0	676.7^{b}	491.2^{bc}	72.8^{a}	23.4^{a}	13.9^{b}	12.5^{a}
100	12.5	2	5.0	583.3^{a}	426.5^{a}	73.4^{ab}	23.8^{a}	14.0^{b}	12.1^{a}
75	14.4	0	5.0	726.7^{c}	527.5^{c}	72.7^{a}	23.8^{a}	13.9^{b}	12.5^{a}
75	14.4	2	5.0	656.7^{b}	490.6^{bc}	74.7^{b}	24.9^{b}	13.3^{a}	13.5^{b}
0	20.0	0	5.0	813.3^{d}	603.4^{d}	74.3^{b}	24.9^{b}	14.2^{b}	13.6^{b}
0	20.0	2	5.0	810.0^{d}	601.3^{d}	74.2^{b}	23.8^{a}	13.5^{ab}	14.3^{c}
SEM				59.7	42.6	1.3	1.1	0.7	0.5
P				< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

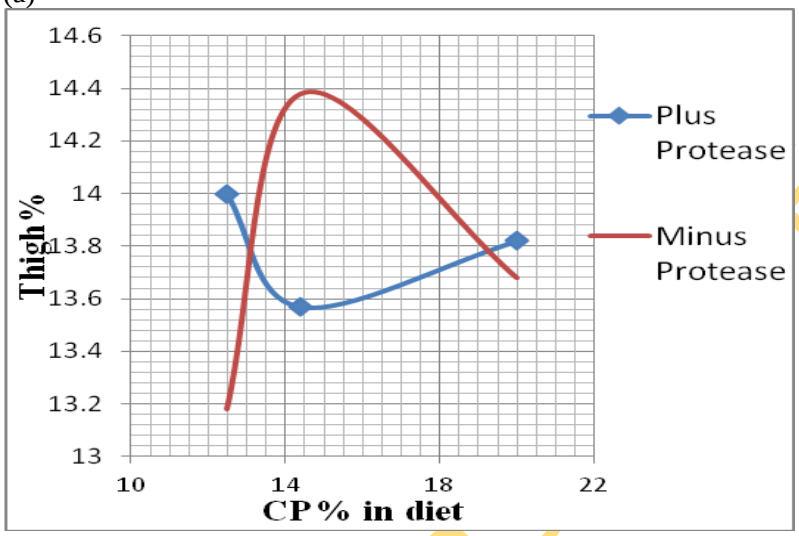
^{a,b,c,d} Values within a column and for specific parameter with different superscripts are significantly different (P < 0.05).
HFM= Feather Meal, LW= Live weight, DW= Dressed weight, DP= Dressed percent, SEM= Standard Error of Mean



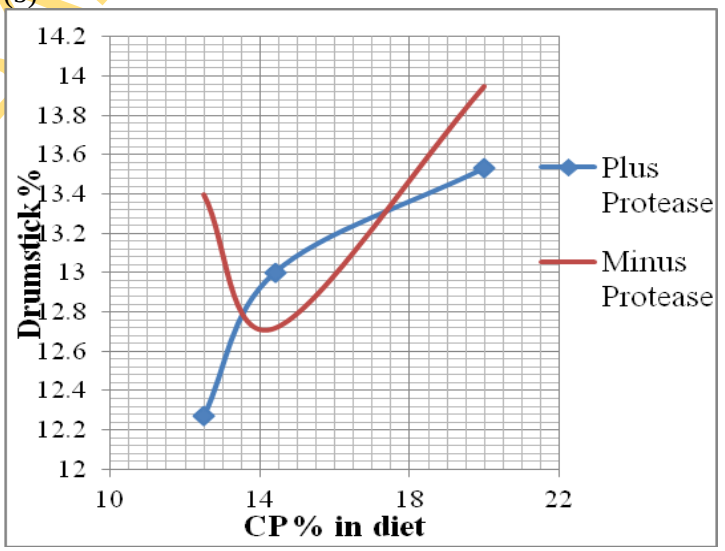
(a)



(b)

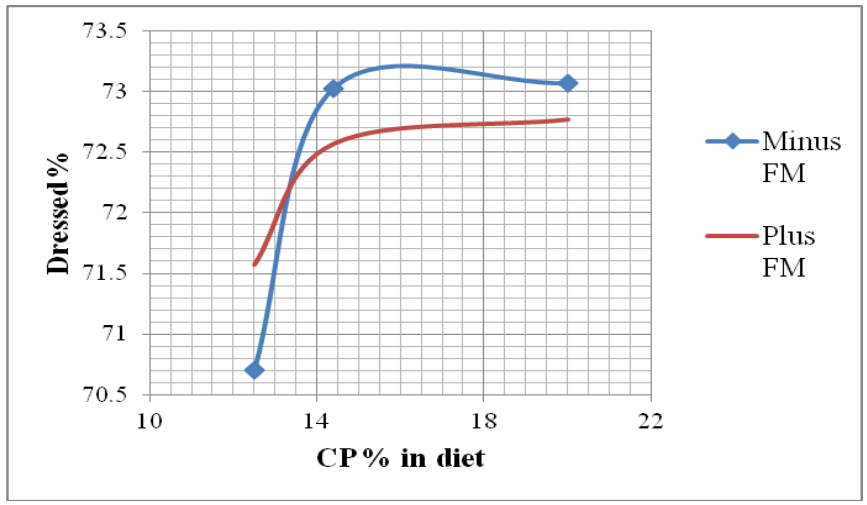


(c)

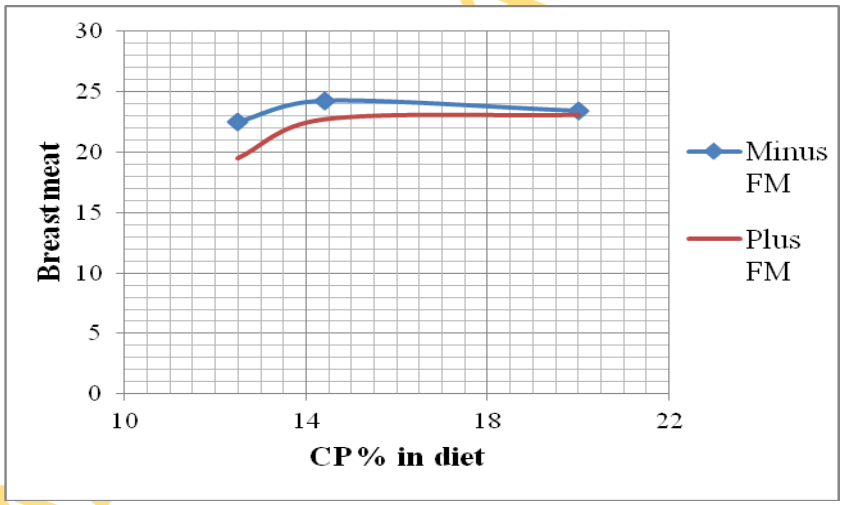


(d)

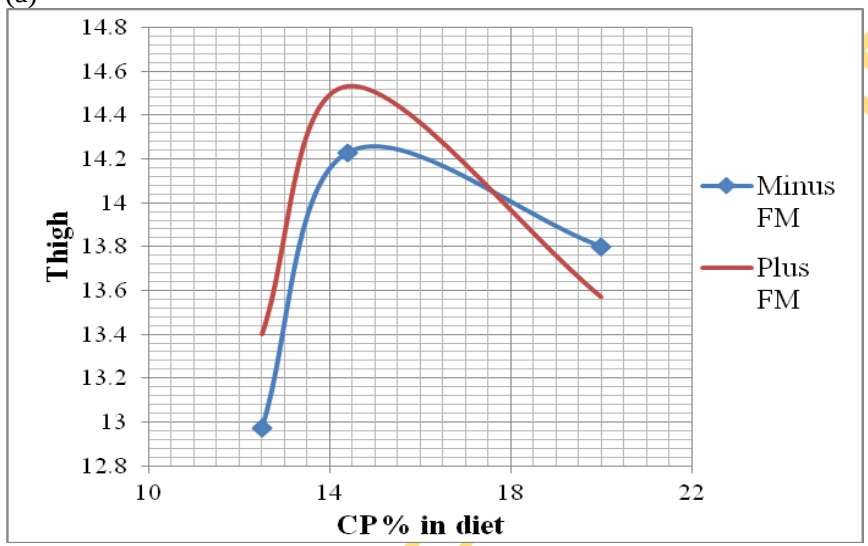
Figure 19: Effect of protease supplementation on dressed percent (a), breast meat (b), thigh(c) and drumstick (d) as percentage of live weight at different CP levels.



(a)



(b)



(c)

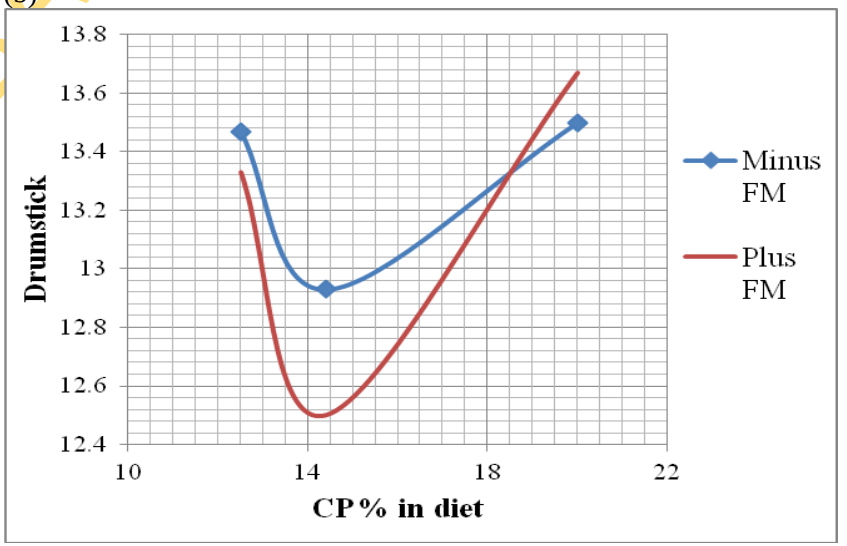


Figure 20: Effect of HFM inclusion on dressed percent (a), breast meat (b), thigh (c) and drumstick as percentage of live weight at different CP levels.

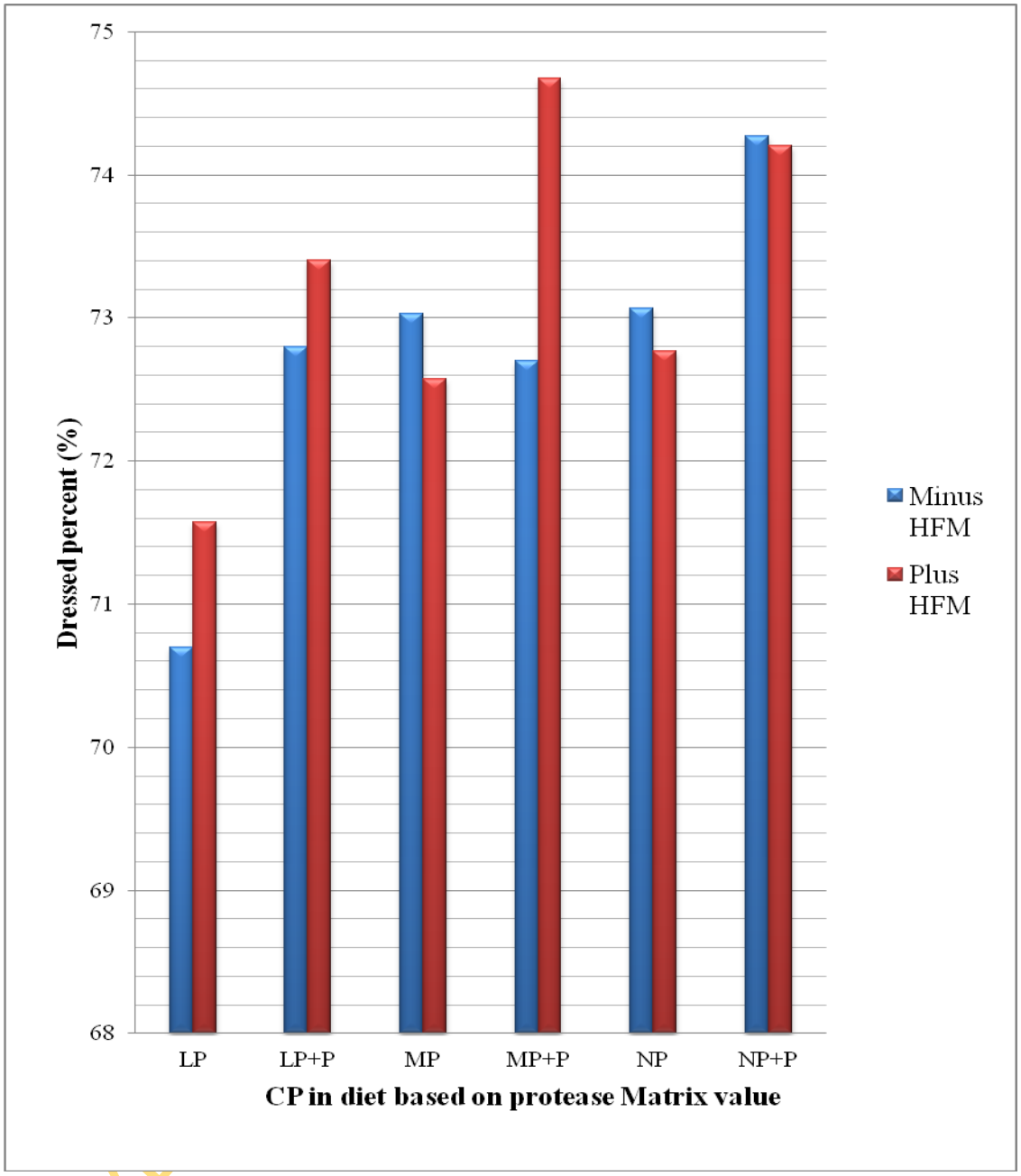


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Figure 21: Protease matrix value effect and HFM inclusion on dressed percent at 42days

KEY: LP= Diet containing 12.5%CP, LP+P= Diet containing 12.5%CP+protease; MP= Diet containing 14.4% CP, MP+P= Diet containing 14.4%CP+protease; NP= Control Diet containing 20% CP, NP+P= Control Diet containing 20%CP+protease

5.4.0. DISCUSSION

Growth performance of broiler birds fed diets containing HFM at the starter phase showed that FM inclusion had significant decreasing effect on the BWG, FW and FI. Protease supplementation did not have a significant effect on these parameters. BWG with or without HFM inclusion and BWG with or without protease followed a similar trends having a peak at about 17.5% CP level as seen in Figures 11 and 13. Though CP level did not have a significant effect on the growth performance indices, birds on the 17.5% CP performed comparably well to birds on the control diets. This is in agreement with the work of Drew *et al.* (2005) where a protease was added to rainbow trout diet and concluded that protease addition resulted in an improvement in feed efficiency and nutrient digestibility but had no significant effect on performance. Feed Intake at a CP of about 22% was same irrespective of protease addition but peaked at about 17.5% CP level when protease was added to diets and lowest at same CP when diet was without protease (Figure 12). This shows that the protease is most beneficial to the broiler birds at a 17.5% CP level at the starter phase. Odetallah *et al.* (2003) used a keratinase enzyme in broiler diets though HFM was not a component of the diets fed, and reported no significant effect on the BW but there was numerical increase in the birds fed enzyme supplemented diets. Birds fed the control diets also consumed more than the others in line with results obtained in this study. Effect of CP levels on FI for all birds were about the same as the CP level did not affect the feed consumed. Wang *et al.* (2006) fed broilers with 21, 22 and 23% CP and reported that birds on the high protein diet (23% CP) did not benefit more than those on the lower CP levels which is comparable to the results obtained in this study. The recommended CP for broiler starter phase is 23% because any

level above is not efficient and below will impair performance. When enzymes are used in diets, the nutrient level is boosted by increased availability of the constituent basic units and as a result a diet with lower nutrient level is of more benefit to the animal as the otherwise unavailable nutrients are made available for utilization (Greenwood *et al.*, 2002; Short *et al.*, 2002).

There was no significant interactive effect between the fixed factors on the dependent variables measured at the starter phase.

Feather Meal generally decreased FI and BWG resulting in higher FCR values showing low efficiency of its utilisation for birds fed the lowest CP level corresponding to 100% matrix value deduction. This could be due to the poor amino acid profile of HFM and the stage of development of the broiler chickens, at the starter phase the lysine and methionine requirement of broiler chickens is critical as they are required for tissue and organ growth and development. However all birds fed diets without HFM fared better in line with the reports of Antipatis *et al.* (2013), when a mono-component protease was used to supplement feedstuffs like soybean meal, corn/maize; these ingredients were also used in the feeds used in this study. The improved performance could be as a result of the improvement in the amino acid digestibility of the other ingredients in the feed. Fru-Nji *et al*. (2011) demonstrated that a mono-component protease improved both protein and energy digestibility which led to significant performance of birds. Though the report of Antipatis *et al*. (2013) was based on a different mono-component protease, they demonstrated a significant improvement in the amino acid digestibility in corn, soybean

meal, meat and bone meal and canola meal either on a standard feed formulation or in low protein diets.

At the finisher phase (22 - 42d), protease supplementation significantly depressed the FI and FCR which is an efficiency index, though it did not significantly increase BWG. Feather Meal inclusion significantly decreased BWG, FI and FCR at $\alpha_{0.05}$. Crude Protein levels had significant effect on the BWG, FI and FCR as they increased with increase in CP. The interaction between protease supplementation and CP level had significant effect on the BWG such that birds on the 12.5% CP benefited most; Wang *et al.* (2006) also observed this tendency at 29 - 48d with birds on a low CP (19%) diet. Manufacturers of the protease used in these studies declared a 7.5% increase in protein availability when used in poultry; this informed the matrix approach used. This means that if the enzyme improves protein availability by a 7.5%, lower CP levels up to 7.5% less should lead to performance as good as for the recommended level. Protease effect can only be appreciated when protein in the diet is reduced, the enzyme will enhance its availability (Wenk, 2000). Though the BWG for the 12.5% CP diet (7.5% less) was ($\alpha_{0.05}$) lower than for birds on the control diet, enzyme supplementation increased the BWG significantly to a level comparable to birds on the (5.6% less) 14.4% CP. This observation could be due to the additional supply of amino acids from HFM degradation by the activity of the protease used, making more amino acids available for metabolism and tissue formation. Birds on the control diet however, did not benefit from enzyme supplementation as the BWG was not significantly different with supplementation. This is expected as the birds on control diets have their CP requirement met and they require no increase in amino

acids to maintain nitrogen balance. Figure 13 indicates that broiler birds fed a diet of about 15% CP will benefit most from supplementation with the protease used in this study. The feed efficiency index (FCR) for birds on the lowest CP is comparable to those on the control with protease supplementation. Birds fed the 12.5% CP consumed as much feed as birds on the control diet without addition of protease but consumed less with protease addition (Figure 14). Eissler and Firman, (1996), Ayanwale, (2006) observed that HFM decreases feed intake which was also observed in this study (Figure 16); this could be responsible for the low BWG of birds on the 12.5% CP diet (with a good FCR) as the amount of feed consumed is directly related to weight gain when digestibility is good. The effect of protease on HFM is significant as BWG improved with significant effect on FCR. Protease supplementation generally decreased FI; this trend was observed by Freitas *et al*. (2011) in an experiment where a protease was fed at different levels (0.1, 0.2, 0.4, 0.8 and 1.0g/kg) and in another where the same protease was fed at a constant level in all diets but with varied CP and energy levels.

Freitas *et al.* (2011) used a mono-component protease which seemed to have an interactive effect with energy on the CP digestibility; though the protease used in this study is a mono-component serine endopeptidase.

At the finisher phase, the effect of the protease used was evident as birds fed control diet with FM performed better than the other birds. This means that the enzyme has keratinase activity and the amino acids made available by the protease activity was utilised by the birds at this stage of growth which is characterized with increase in already developed organs and tissues.

The carcass and organ weights showed the same trend as the growth performance. Birds fed 12.5% CP diet had live weights, dressed weights, drumsticks and gizzards comparable to those fed the 14.4% CP diet but were significantly reduced when compared to those on the control diet. Dressed percent and thigh were not different for all the treatments with or without protease and regardless of CP level. In the present study, protease supplementation resulted in significant increase in the breast meat. This is contrary to the report of Freitas *et al.* (2011) where protease supplementation had no significant effect on the deboned breast meat. The interactive effect of HFM and protease was not significant on the carcass and organ weights at 22 - 42 days of age.

Figure 19 shows the effect of protease supplementation on carcass measures. Addition of protease improved breast meat yield and the dressed percent compared to other carcass yields. It can be said that protease supplementation favours breast meat yield. Figure 20 shows that inclusion of HFM affects the dressed weight, this could mean that feeding HFM leads to the conversion of its contributed amino acids to more of feather formation. The thigh yield improved with HFM at about 14.5% CP level. The trend observed for dressed percent and breast meat yield in Figures 19 and 20 suggests that feeding HFM is not negative to breast meat deposition and that the protease CIBENZA DP^{100}, was able to make available more amino acids in the HFM for utilization. There is therefore evidence that CIBENZA DP^{100} has keratinase properties as shown in Figure 21 where birds fed a 75% matrix value formulation containing HFM had the highest dressed percent.

CHAPTER SIX

6.0. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1. SUMMARY OF FINDINGS

The use of feather meal for broiler chickens has not been satisfactory because the level of inclusion is low compared with the amount of feathers generated annually by poultry meat producers. The problem of low digestibility or nutrient availability of HFM has also been low compared to its percentage protein which is very high. Previous works on HFM digestibility employed the total tract method which has not been so acceptable in recent times; this work employed the ileal digestibility method.

The Apparent Crude Protein Digestibility (ACPD) for HFM was determined to be 59%. HFM ACPD was also improved from 8.4 – 29.1% with enzyme supplementation. Ileal digestibility of HFM in broilers is higher at 4% inclusion but 2% inclusion returned better FCR. Protease supplementation of a 17.5% CP diet containing 2% HFM is more beneficial to broilers at the starter phase than at the finisher phase.

There was no significant difference between digestibility of the control and 2% HFM diets. Birds on the 2% HFM diet had similar growth performance as those on the control diet. There was no significant difference in the carcass weight and organs due to HFM

inclusion, protease supplementation and the interaction between these independent variables.

6.2. CONCLUSION

Protease improved ileal Digestible Crude Protein of HFM, growth performance and carcass measures. A 2% dietary HFM with no protease is beneficial to broilers but inclusion levels above 2% should be with protease supplementation for improved CP digestibility and carcass measures. Digestibility of Crude protein of HFM is improved with supplementation of diets with 5% protease which is a thermophilic Bacillus derived serine endopeptidase (CIBENZA DP ^{100} Novus, USA).

6.3. RECOMMENDATIONS

In the course of this work a number of challenges were encountered which when surmounted would improve and impact on research in the use of HFM from feather wastes for animal feeding. These challenges are:

i. Grinding HFM for incorporation into other feed ingredients.

ii. Analyzing for Amino Acids in all the samples generated, to determine the individual amino acid digestibility.

Further work is required to investigate performance of broiler chickens fed 17.5%CP diets with or without 2% FM inclusion because results from these studies suggest that

birds in the two groups should perform similarly. Effect of HFM inclusion and protease supplementation as source of stress in broiler birds in the tropics should be investigated as this could be a factor in broiler production using unconventional feedstuff.

Further investigation on HFM ileal digestibility and enzyme supplementation in other animals of economic and healthy meat value should be carried out.

Other animal wastes can also be investigated for their digestibility and use in broiler feeding to reduce the competition between man and animal for food and make healthy white meat more available and more affordable.

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APPENDIX 1

ANALYTICAL PROCEDURE FOR RAPID DETERMINATION OF TiO_2

(Myer *et al.*, 2004)

1. Weigh all the macro-Kjeldahl digestion tubes to be used.
2. Weigh duplicate 0.5 g samples into 250-ml macro-Kjeldahl digestion tubes. Include a baseline sample of feces (or duodenal, ileal digesta, or forage) devoid of TiO_2 for background correction.
3. Add a reaction catalyst containing 3.5 g of K_2SO_4 and 0.4 g of $CuSO_4$ to each tube.
4. Add 13 ml of concentrated H_2SO_4 to each digestion tube; digest samples at 420°C for 2 hours. Remove from heat; allow digested sample to cool for a minimum of 30 minutes.
5. Add 10 ml of 30% H_2O_2 to each tube; allow mixture to cool for 30 minutes.
6. Bring the total liquid weight up to 100 g using distilled water. Filter through Whatman (No. 541) filter paper to remove any precipitate.
7. Measure absorbance at 410 nm. Calibrate spectrophotometer with standards, prepared by adding 0, 2, 4, 6, 8, and 10 mg of TiO2 to blank tubes (no organic matter) that are prepared as described above. Use the 0 mg standard to zero the instrument.

APPENDIX 2

PHOSPHORUS DETERMINATION BY THE MOLYBDOVANADATE METHOD

(FAO, 2011)

IS 14828 (2000): Animal Feeding Stuff - Determination of Total Phosphorus Content - Spectrophotometric Method [FAD 5: Livestock Feeds, Equipment and Systems]

Weigh 1 g or more of sample to the nearest 1 mg. Place sample in a Kjeldahl flask. Add 20.ml of concentrated sulfuric acid. Shake to impregnate the substance completely with acid and to prevent it from sticking to the wall of the flask. Heat and keep at boiling point for 10 min.

Leave to cool slightly and add 2 ml of nitric acid; heat gently then leave to cool slightly. Add a little more nitric acid and bring back to the boiling point. Repeat this procedure until a colorless solution is obtained.

Cool, add a little water and decant the liquid into a 500 ml volumetric flask, rinsing the Kjeldahl flask with hot water. Leave to cool, dilute to the mark with water, mix and filter.

To develop colour and measurement of absorbance; dilute an aliquot portion of the filtrate obtained with water, to obtain a phosphorus content not exceeding 40 µg/ml. Transfer using a pipette, 10 ml of this solution to a test tube. Add, using a pipette 10 ml of the molybdovanadate reagent. Mix and leave to stand for at least 10 min at 20 ºC.

Transfer a portion of the obtained solution to a measuring cell and measure the absorbance in the spectrometer at a wavelength of 430 nm against the reference solution.

Preparation of the calibration curve

Using the phosphorus standard solution, and by means of graduated pipettes, prepare solutions with phosphorus content of 5µg/ml, 10 µg/ml, 20 µg/ml, 30 µg/ml and 40 µg/ml respectively. Transfer, by means of pipettes, 10 ml of each of these solutions to a series of five test tubes Add to each using different pipettes, 10 ml of the molybdovanadate reagent. Mix and leave to stand for at least 10 min at 20 ºC.

Measure the absorbance of each solution at 430nm. Draw the calibration curve by plotting the absorbance against the corresponding phosporus concentrations, in micrograms per millilitre, of the phosphorus standard solutions. For phosphorus contents between 0 µg/ml and 40 µg/ml, the curve is linear.

Prepare the blank using the same procedure and the same quantities of the reagents, but omitting the test portion.

APPENDIX 3

NITROGEN/ CRUDE PROTEIN DETERMINATION (FAO, 2011)

AOAC 984.13. 2000. Protein (crude) in animal feed and pet food, copper catalyst Kjeldahl method. Gaithersburg, MD, USA.
AOAC 988.05. 2000. Protein (crude) in animal feed and pet food: CuSO4/TiO2 mixed catalyst Kjeldahl method. Gaithersburg, MD, USA

Digestion of sample

Weigh approximately 1 g sample recording to the nearest 0.1 mg (W) and transfer to the digestion tube. In each batch use a tube without sample as blank test. Add two Kjeldahl tablets* and 20 ml sulphuric acid. If fuming is a problem, add a few drops of anti-foaming agent. Place the tubes in a digestion unit and connect to the fume removal manifold or place in a fume chamber. Digest the sample for at least 1 hour at 420 ± 20 °C. Turn the digestion off, remove the tubes and allow to cool for 10 –20 minutes. Add distilled water to each tube to a total volume of approximately 80 ml.

Distillation and titration

Place a conical flask containing 25–30 ml of the concentrated boric acid under the outlet of the condenser of the distillation unit in such a way that the delivery tube is below the surface of the boric acid solution. Add 50 ml NaOH and distill the ammonium.

Titrate the content of the conical flask with hydrochloric acid standard solution after adding a few droplets of indicator solution using a titration unit and read the amount of titrant used. The endpoint is reached at the first trace of pink colour in the contents.

Record the amount of acid used to the nearest 0.05 ml for the blank test (Vb) and for each sample (Vs).

Calculations

Percent Nitrogen (% N) = (Vs – Vb) x M (HCl) x 1 x 14.007 / (W x 10)

Where:

Vs = ml HCl needed to titrate sample

Vb = ml HCl needed for the blank test

M(HCl) = molarity of HCl,

1 = the acid factor,

14.007 = molecular weight of N,

10 = conversion from mg/g to %, and

W = weight of the sample (g).

Calculation percentage Crude Protein (% CP) = % N x F

Where:

F = 6.25 for all forages, feeds and mixed feeds,

F = 5.70 for wheat grains, and

F = 6.38 for milk and milk products.

*3.5 g of potassium sulphate and 0.4 g of copper (II) sulphate pentahydrate can be used as catalyst for sample digestion.

APPENDIX 4

FAT DETERMINATION (ETHER EXTRACT) (FAO, 2011)

AOAC 920.39. 2000. Fat (crude) or ether extract in animal feed. Gaithersburg, MD, USA.

Weigh at least 5 g of the sample to the nearest 0.1 mg (W1) into the extraction thimble and cover with a fat-free wad of cotton wool or filter paper.

Transfer some silicon carbide chips to a dry flask and weigh to the nearest 0.1mg (W2), add 95 ml petroleum ether. Place the thimble in the extractor and connect it to the dry flask and reflux unit. Extract for 6 hours with petroleum ether and regulate the heating apparatus to obtain at least 10 syphonings per hour. Or follow the manufacturer's guidelines. Distill the solvent until the flask is nearly free from the solvent, leave overnight in a fume chamber to ensure all solvent is evaporated. Dry the flask with residue for 1.5 hour in a vacuum oven at 80 ± 2 °C. Cool in a desiccator and weigh to the nearest 0.1 mg (W3).

Calculation

% Crude Fat = (W3 – W2) x 100 / W1

Where:

W1 = initial sample weight in grams,
W2 = weight of flask (+carbide chips if used) in grams, and
W3 = weight of flask and fat residue in grams.

APPENDIX 5

SPECTROPHOTOMETRIC DETERMINATION OF CALCIUM (FAO, 2011)

AOAC 968.08d. 2000. Acid digestion. Gaithersburg, MD, USA

Weigh approximately 1 g to the nearest 0.2 mg (W) in a beaker and place in cold Muffle furnace. Close the furnace and gradually raise the temperature to 550 °C over about 90 minutes. Maintain this temperature for 16 hours (e.g. overnight) to remove carbonaceous material and then open the furnace and allow to cool. Add 10 ml 6 M hydrochloric acid to each beaker and place on a preheated hot plate (approximately 250 °C), cover the beakers with a glass plate, digest for 20 minutes. Allow the beakers to cool and remove from the hot plate. Transfer quantitatively the content of the beakers to a 25 ml volumetric flask, make up to the mark with distilled water and mix well. Measure calcium in the solutions and standards by measuring absorbance at 578 nm.

Calculation

Calculate the calcium content in the measured solution by linear regression.

Percent of calcium is calculated as:

% Calcium = (C x V x DF) / (W x 10)

Where:

C = concentration calcium in measure solution (mg/litre),

V = volume of solution (in litres, i.e. 0.025 (L)),

DF = dilution factor (normally, i.e. 1),

W = weight of the sample (g), and

10 = factor to convert g/kg to %.