CHAPTER 1 GENERAL INTRODUCTION

Feeding plays a pivotal role in poultry and especially broiler chicken production. Broilers require balanced diets for their proper nourishment, optimum growth and development. Diets for fast-growing meat chickens are complex mixtures of many feedstuffs. Different ingredients are mixed together to make this a balance mixture, which usually come from both plant and animal sources, along with the supplementation with inorganic or organic sources. The nutrient requirements of the chicken are met mostly from vegetable ingredients (predominantly cereal grains). Apart from this, the requirements for other vital nutrients such as protein, vitamins, and minerals are met by both animal and plant feed sources, and some supplementation with inorganic or synthetic feed sources.

As feed is the most expensive of all the variable inputs required for the production of broiler chickens, a cost–effective diet formulation may offer a potential beneficial effect for achieving production efficiency through reduction in feed costs. Feed ingredient is the single variable unit, and is considered as the building block of diet formulation. The ingredient quality affects the quality of balanced diet formulation. Availability of quality feedstuffs at a reasonable cost is a key to successful poultry operation (Hooge and Rowland, 1978). So, any improvement in the performance of broilers and layer birds due to their diet, can inevitably have a profound effect on profitability of poultry farming (Roy *et al.*, 2004).

However, the modern meat chicken is a fast-growing bird, and is an efficient converter of feed nutrients to meat. The key objective of rearing meat chickens is to provide premium quality of animal meat for human consumption as well as to meet the huge protein gap of the world. To achieve this goal, broiler chickens should be fed correct proportions of quality diets that are rich in quality feed proteins. To supply this, proteins recovered from animal feed sources are considered quality protein because of having all essential amino acids with higher biological value. The protein requirements of broiler chickens in their diet are met mostly by both vegetable and animal protein sources. The conventional feed ingredients of animal origin such as meat meal, meat and bone meal, fish meal are commonly used in broiler diet, and are considered as excellent protein sources for broiler chicken (Parson *et al*., 1997; Robinson and Sing, 2001; Giang *et al*., 2001).

Despite being satisfactory sources of quality protein for chickens, some inherent constraints, for example, zoonotic disease transmission, higher cost, product quality, poor shelf life, food contamination etc., discourage the extensive use of these products in diet formulation for poultry. Apart from these, the threat of new emerging zoonotic diseases like bird flu and swine flu in human populations has aggravated this situation, leading to drop in the patronage of poultry and its allied by-products, followed by destruction of millions of animals across the world leading to a great loss of income of animal farmers as well as storage of products for consumers. Furthermore, most crucial protein sources and conventional diets are more costly in some regions of the world. Oluyemi and Robert (2000) reported that, a critical cost appraisal of poultry feed formulate would show that protein, especially proteins of animal origin, are the most expensive per unit cost of production. Therefore, the poultry industry is always exploring the cheapest sources of feed ingredients to gain maximum profit with the lowest investment in feed.

Owing to above constraints of animal protein sources, poultry nutritionists, feed formulators are trying to re-evaluate the present strategy of diet formulation by excluding or reducing expensive ingredients from livestock diets. This trend can make the feed formulation strategy more complex and unsatisfactory from quality standpoint to supply the quality diets for the ruminant and non-ruminant animals. The exclusion of animal by-products from diet formulation not only reduces the nutritive value of the formulated diets but also limits the ability of the formulations to satisfy the required nutrients for the animals (Hossain *et al*., 2011a). For this reason, there is the need to explore the feasibility of developing vegetable-based protein diets as alternative for poultry. Identification of such feedstuffs would help diet formulators not only to cut down their production costs, but also to improve the efficiency of their production (Teguia and Beynen, 2005).

Considering the above perspectives, diet formulation of poultry focusing entirely on vegetable protein feedstuffs is now an important issue for the poultry industry in many parts of the world. This tendency is growing and intensifying the pressure on nutritionists how to supply organic, safe, hygienic, and quality poultry products to the consumers, through provision of quality diets, which are free from animal by-products or growth promoters.

Meats from fed on all vegetable diets are now preferable in the world market, especially in the European Union and Middle East countries (Mendes, 2003). So, vegetable-based protein diets are becoming a reference diets in research evaluating the feeding of poultry (Parson and Wang, 1998; Bellaver *et al.*, 2001). Incorporation of vegetable ingredients notably soybean meal, canola meal, sunflower meal, mustard oil cake etc., to animal proteins as alternatives would need to be studied, to supply correct amount of protein and amino acid balance. These feedstuffs are good sources of nutrients, comparatively inexpensive, readily available, easy to process with a low cost, and less risk to disease contamination.

In contrast, many studies reported that, poultry productivity on vegetable protein diets is often poor due to deficiencies in nutrients such as amino acids and minerals, imbalances in calorie to protein ratios (Dilger and Baker, 2008). Some of the sources contain deleterious factors such as NSPs, polyphenols or phytic acid (Iji *et al*., 2004; Olukosi *et al.*, 2010). However, poultry nutritionists are striving to improve the quality of vegetable protein diets for the optimum performance of the birds through various practices, including crop breeding, feed processing, supplementation with nutrients and so on. If these practices are adopted appropriately, then vegetable protein ingredients can serve as excellent nutrient sources along with cutting feed cost significantly for poultry (Cruz *et al.*, 2009)**.** Data on such formulation are still limited and the requirements by the industry differ from one region to another. For this reason, the current study has been undertaken to assess the nutritional value of vegetable protein diets for broiler chickens, and to bring about radical changes in the feed formulation strategy for economic poultry production. The study will primarily focus on the most appropriate strategies to evaluate the comparative nutritive value of vegetable and traditional feeds, and identify the ways and means to improve the value of vegetable protein diets for the poultry. The core objectives of the current study are to

- develop and compare the nutritive value of traditional diets with vegetable protein diets;
- identify factors that may limit productivity on vegetable protein diets;
- assess the preference for such diets by broiler chickens;
- explore ways by which the quality of vegetable protein diets can be improved, and improve productivity of meat chickens on vegetable protein diets.

CHAPTER 2 LITERATURE REVIEW

2.1 INTRODUCTION

Feed formulation requires adequate knowledge and ideas about the feed ingredients and type of birds to be fed. It is both a science and an art through which different ingredients are mixed together to obtain a single uniform mixture, and supply the birds to satisfy their nutrient requirement. The main objective of the poultry industry is to formulate cost-effective compound diets, and ensure optimum production through supply of well-balanced nutrients to the birds. Information on price of available ingredients, their chemical composition, nutritive value, digestibility, safety in using ingredients etc., are necessary for quality diet formulation. In recent years, authentication and objective feed information have been the main goal of producers (Monin, 1998).

However, feedstuffs undoubtedly play a pivotal role in formulation, and for nourishing modern broiler chickens to ensure their optimal growth. In commercial farming practice, modern meat chickens are supplied with balanced diet through formulation in the form of compound feed, mixing with different selected feed ingredients together, for maintaining their optimum growth, and consistent production level. Moreover, this balanced diet is necessary for the rapid body growth and development, as it contains all essential nutrients that are required by the broiler chickens. Although many changes have taken place in the nutrition and genetics of modern meat chickens, free selection, and indiscriminate uses of feedstuffs in poultry feed formulation can be risky due to disease outbreak via animal by-products. Both plant and animal ingredients are used extensively to formulate balanced diets for poultry. The inclusion of animal by-products (e.g. meat and bone meal) in practical diets, has already been banned by the European Union, as a result of the consumer concerns on diseases such as bovine spongiform encephalopathy (BSE), and the contamination of animal food products with other pathogenic agents (e.g. *Salmonella* spp. and *E. coli*) (Veldman *et al*., 1995; CEC, 2000 and Hofacre *et al*., 2001). When consumers have restrictions to change their food items, poultry producers have to reorganize their current production strategies by using alternative approaches in feed formulation, e.g. by exclusion of animal by-products. Furthermore, per unit cost of meat chicken production goes up if animal protein is used in broiler diets as compared to vegetable feedstuffs (Oluyemi and Roberts, 2000).

However, the extent of research works so far conducted on diet formulation is mainly on the basis of using both vegetable and animal feed stuffs in practical diets of broiler chickens. Therefore, plenty of literature is available regarding these diets and their effects on poultry, but research into formulation of exclusively vegetable protein diets is limited. There is much potential for using vegetable protein ingredients for supplying birds with essential nutrients in poultry diets. Apart from supplying macro-nutrients (energy, protein), the availability of micro-nutrients such as indispensable amino acids, vitamins and minerals from vegetable-based diets can be limiting.

Feed formulation using only vegetable materials can minimize the feed cost, ensure consumer's food safety and meet broiler chicken requirement for optimal growth and health. However, the application of this new strategy of diet formulation in poultry nutrition, especially in broiler chicken production, is a relatively new research area. Due to the limitations of vegetable feedstuffs in some nutrients or their bioavailability, particularly protein quality, essential amino acids, vitamins and minerals, there is a gap between actual requirements by the modern broiler chicken and supplemental levels recovered from these feeds. The exact pattern of digestion, absorption, assimilation and excretion of these feed nutrients found in vegetable feeds are not fully understood. There are not enough data to recommend optimal composition of those diets for broiler chickens. This review will sum up the literature on current state of poultry feedstuffs and the comparative value of vegetable protein and traditional diets for broiler production. The review will identify the limitations, inadequacy and gaps in knowledge of all-vegetable feedstuffs for broiler birds.

2.2 FEED INGREDIENTS FOR POULTRY

2.2.1 Energy sources

In poultry diets, energy is mainly provided by cereal grains, and their by-products (e.g. rice bran, wheat bran, rice polish etc.). Cereal grains such as maize, wheat, sorghum, barley, oat, and rice etc., are used as principal sources of energy for poultry diets, while rye, triticale, and oats etc., are also used to a lesser extent. Apart from these, fats or vegetable oils are also used as supplementary sources of energy for poultry diet. Some animal fats (e.g. lard, tallow, poultry fat, fish oil) and vegetable oils (e.g. soybean, palm, canola, sunflower and cotton) are used as supplemental sources of energy for poultry diets. Energy in feed incurs 40 % of the cost for producing poultry products i.e. meat and eggs (Sibbald, 1982). The major portion of most poultry diets is cereal grains (60-70 %), which are the main energy suppliers. The energy value of grains can vary depending on the cereal type, variety, and environment (Classen *et al.*, 1988; Jeroch and Danickel, 1996).

2.2.2 Protein sources

Both vegetable and animal feedstuffs are used as protein sources for poultry diet formulation. Oilseed meals such as soybean, canola, cottonseed, sunflower meals, mustard oil cake, and some legumes such as peas and lupins, are used mainly as vegetable protein sources. Globally oilseed production has increased consistently to meet the higher demand of human consumption (FAO, 2000), and consequently, more meals are now available for use in animal production. On the other hand, animal feedstuffs are mainly by-products, including meat meal, meat and bone meal, fish meal, blood meal, feather meal, poultry by-product meals etc., and are used as protein sources in poultry diets. Animal protein is considered as a high quality protein because of more balance in amino acids and higher biological value than vegetable protein sources.

2.2.3 Fat and oil sources

Animal fats and vegetable oils are the most concentrated sources of energy, and are used to supply some energy in the diet. These are derived from the meat processing industry, manufacture of soap, refining of vegetable oils or vegetable oils themselves. For the most part, the only nutrients these materials supply are energy and varying amounts of fatty acids such as linoleic acid; however, they impart some desirable quality to feeds such as reduction of dustiness and improvement in the palatability of diets (Singh and Panda, 1992). Fats and oils may also contain vitamins.

2.2.4 Micro-nutrients

Micronutrients, particularly vitamins and minerals, are essential, but are required in very small amounts; otherwise characteristic deficiency syndromes may develop due to their inadequacy in the practical diet. As the gut micro flora of chickens cannot synthesise optimum vitamins, birds often suffer from vitamin deficiency, and show deficiency symptoms if not supplied in the diets. Moreover, intensively-reared chickens undergo many environmental stresses such as high temperature, which necessitates the supplementation of micronutrients to such poultry. So broiler diets are very often supplemented with micro-nutrients in form of premixes, which are commercially available in the market. Apart from this, many feed ingredients also provide considerable amounts of vitamins and minerals for poultry.

Leeson and Summers (2001) have identified important nutrient inter-relationships, which are important with regard to the nutritional integrity of diets, and poultry can respond negatively if these are ignored in the formulated diets. Such relationships include various interactions between vitamins, between vitamins and minerals, and between minerals, particularly trace elements. Green vegetable feedstuffs are a rich source of vitamins, minerals, and anti-oxidants (Omenka and Anyasor, 2010). Vitamins and minerals premix is usually added in poultry diets, but its requirement may be reduced in the formulation by using vitamin-rich vegetable and plant sources such as alfalfa, soybean meal, lucern meal, canola meal, azolla meal and pasture grasses. Other plants also contain vitamins and minerals in their leaves, hulls and bran (Agbede and Aletor, 2003). Corn protein is a poor source of minerals, particularly calcium (0.03 %), potassium (0.45 %), manganese (7.3 mg/kg), and copper (5.4 mg/kg) (Giang *et al.*, 2001). Vieira *et al.* (2005) reported that corn soybean meal diets can provide more potassium than is required by the birds, and can be about 20 % greater than a conventional diet with animal by-products.

2.3 ANIMAL BY-PRODUCTS

2.3.1 Key animal by-products used in poultry feeding

Many by-products are retrieved from different live or dead animals through various processes. These include meat meal, meat and bone meal, fish meal, blood meal, feather meal, and poultry by-product meals etc., which are all rich sources of protein and other nutrients for poultry.

2.3.2 Nutritional properties

Animal by-products contain higher amounts of protein, fat and other nutrients than the vegetable protein ingredients. No two single protein sources that are available in nature are similar in characteristics. So the nutritional properties, physical or chemical nature, pattern of digestibility, biological value etc., of one animal by-product varies widely from another by-product. However, amongst animal by-products, certain properties of fishmeal such as higher level of protein, better amino acid profile, high protein digestibility etc., make this product more attractive than others for use in diets for non-ruminant animals.

Besides, it is an important source of micro-nutrients such as vitamins and minerals as well as varying amounts of highly digestible energy (Windsor and Barlow, 1981). The nutritive value of fish meal varies over a wide range of areas, for instance, CP, 35 to 65 %, and ash 20 to 35 %, as reported by Giang *et al.* (2001). Furthermore, fish meal, apart from being fairly rich in all amino acids, also contains some growth factors such as sulphate, polypeptide, and some others yet to be identified (Pierson *et al*., 1979; Barlow and Windsor, 1984; EI Boushy and van der Poel, 1994), along with certain micro-nutrients, particularly vitamin B_{12}, selenium (Grastilleur, 2003), carnitine, and taurine (Comb, 1998), which might enrich the quality of this ingredient. This meal has become a standard ingredient in pig and poultry diets to make up for deficiencies of essential amino acids (NRC, 1994; Pike, 1999).

Meat meal is a good source of protein (50-56.6 %) containing sufficient lysine (3.6-4 %) to supplement grain protein, but compared to fish meal it is relatively low in methionine (0.84 %) and cystine (0.6-0.7 %). It is an important source of calcium (8.0 %), and phosphorus (4.0 %). In poultry diets, it can be included at up to 10.0 % (Singh and Panda, 1992; Leeson and Summers, 1997). If meat meal contains phosphorus more than 4.5 %, then it is termed as meat and bone meal (MBM), which is also a rich source of animal protein as well as minerals, particularly calcium and phosphorus (Shirley and Parsons, 2001). The average calcium, phosphorus, crude protein, and metabolic energy values derived from a large number of MBM samples were not less than 39, 60, 500 g/kg, and 10.2 MJ/kg, respectively (Chandler, 1994;

Parsons *et al.*, 1997; Wang and Persons, 1998). Blood meal is a by-product of the abattoir. Hamilton (2002) reported that blood meal contains high protein (88.9 %), high lysine (7.1 %), and low sulphur containing amino acids (e.g. methionine 0.6 % and cystine 0.5 %). Blood meal, if used in association with other protein sources in poultry diets can offer higher nutritional value. Blood meal is very rich in limiting amino acids such as lysine, and is a fair source of arginine, methionine, cystine and leucine, but very poor sources of isoleucine, and contains less glycine than either fish meal or bone meal (NRC, 1994).

The characteristic smell of blood meal reduces its palatability, and then a 5.0 % limit is a usual recommendation for its usage in diets. There are some reports indicating that inclusion of 1 to 4 % blood meal in diets can improve poultry performance (Donkoh *et al*., 2001). Feather meal is a rich source of protein (81-85 %), contains a large amount of cystine (about 4.1-10 %), but mostly insoluble and indigestible because of its keratinous protein, and low palatability. So, proper care is needed to use this meal (protein) in broiler diets.

2.3.3 Constraints to use of animal protein sources

Consumer and animal health, food safety, quality meat product, cost issues, etc., have been major barriers to use of animal protein sources in livestock and poultry feeding. Globally the poultry industry is seeking alternative feed protein due to rising cost of animal protein and cross-contamination of food products with pathogenic agents (e.g. BSE, *Salmonella spp.*, *E. coli*), which are derived mostly from animal protein sources. Apart from these, the spread of other diseases like bird flu and swine flu in human beings has aggravated the situation, leading to a decline in use of animal by-products.

The feeding of an animal species with transformed animal proteins derived from bodies, or parts of bodies, of animals of the same species is prohibited in the European Union (Consleg, 2004). This concern has impacted greatly on protein supply not only in Europe but also across the world. The EU and some Arab countries have also restricted the importation of poultry meats from some area. So, broiler meat raised exclusively on vegetable diets by exclusion of animal protein are now mostly preferred in these countries due to health concern.

Besides these, the cost of animal proteins is comparatively higher than that of vegetable protein per unit cost of production. Ahmad *et al*. (2006) reported that protein requirements account for 45 % of total cost of poultry production. Ingredient costs might affect the profitability of poultry production. Diet formulated with high cost animal proteins may increase the production cost of broiler chickens, and thereby can decrease the profit margin for farmers.

However, although the crude protein content of some animal protein sources such as blood meal and feather meal is high being 89.0 %, and 81.0 %, respectively (Hamilton, 2002), the low palatability, poor digestibility, and bioavailability of these animal proteins discourage the inclusion of higher amounts (>5 %) in poultry diets. In addition, the main reason for low nutritive value of these meals is amino acid imbalance (Summer *et al*., 1965; Moran *et al*., 1966). Moran and Summers (1968) reported that some animal protein sources such as feather meal reduce the growth rate of broilers.

Unlike other animal protein sources such as blood meal, feather meal has a poor amino acid balance with lysine (7.1 %) being relatively high and isoleucine being very low. McDonald *et al*. (1992) stated that blood meal can compensate the indispensable amino acids particularly lysine and methionine deficiencies in vegetable protein- based diets.

Giang *et al.* (2001) reported that fish meal contains a high level of protein (35- 60 %), but higher inclusion of this meal in broiler diet is limited due to problem of fishy smell in diet and poultry products (Singh and Panda, 1992), and also high cost along with an additional risk of feed-borne disease such as salmonella for poultry and human consumers (Veldman *et al*., 1995; Nesse *et al*., 2003).

Animal protein sources contain higher fat and protein, and diets formulated with this fat and protein-rich feedstuffs can negatively influence the quality of poultry products after ingestion by the birds. The ratio of lysine/arginine in animal proteins is higher than in vegetable proteins, which determines the effect on serum cholesterol level and the resulting degree of atherosclerosis (Kritchevsky, 1990). Broilers fed animal protein diets tend to accumulate higher abdominal fat content than those fed with plant protein diets (Mendonca *et al*., 1989; Hossain *et al*., 2011a). The fatty carcass produced from animal protein diets may deteriorate the meat quality, thus reducing the shelf life of the product, and ultimately reduce the consumer acceptance.

2.4. VEGETABLE PROTEIN SOURCES

2.4.1 Energy contents

Vegetable protein ingredients can also provide a substantial amount of energy, despite being rich sources of protein for broiler chickens**.** However, the energy values of vegetable proteins are comparatively lower than those of cereal grains. Soybean is a premier source of protein popularly used in poultry diet. Apart from containing higher level of protein (40-48 %), this ingredient also contributes a large amount of energy for poultry. The energy level of full-fat soybean is comparatively higher than that of the extracted meal.

Despite this, the energy content of this meal also has a greater level of variability in terms of feeding aspect to the birds (Douglas *et al.*, 2000). Hill and Totsuku (1964) reported a metabolizable energy (ME) value of 11.64 MJ/kg for commercial soybean meal, while Lautner and Zenisek (1965) obtained a value of 11.01 MJ/kg. Rojas and Scott (1969) reported the average ME was 11.60 MJ/kg for commercial solvent extracted soybean meal with CP 50 %.

In another study, they observed that treatment of soybean meal with phytase enzyme improved ME of soybean meal by 6.5 %, and 11 %, having 44 %, and 50 % protein, respectively. Renner and Hill (1960) showed that mild heat treatment (10 minutes at 107^{o}c) gave the highest ME value of soybean meal, or flakes for chickens. This was due to the inactivation of one or more trypsin inhibitors (Borchers *et al*., 1948), and the destruction of heat-labile soyin (Liener, 1953). The high ME values reported for commercial soybean meal is due to a unique combination of high available carbohydrate content, low crude fibre content and extensive denaturation of toxic compounds during processing.

The energy value of vegetable protein stuffs may be influenced by the amount of deleterious substances contained in each ingredient, and the processing strategy. Several researchers have observed a variable amount of energy in different processing systems. Phytic acid may also be a factor that can depress the energy value of cotton seed meal (Rojas and Sctott, 1969; Miles and Nelson, 1974). Phytase enzyme treatment apparently improved the ME value by complete hydrolysis of phytin, releasing some proteins from protein-phytate complexes and reduction in gossypol content of graded cottonseed meals. Leeson and Summers (1997) reported that ME values of sunflower meal, peas, and lupins were found 9.23, 10.68, and 12.56 MJ/kg, respectively, in broiler diets. The ME value of rape seed meal has also shown similar variation.

The ME value of rape seed meal was 4.62 MJ/kg reported by Lodhi *et al.* (1969), whereas March and Biely (1971) obtained a range of 4.7 to 7.24 MJ/kg. March *et al*. (1973) showed ME values of 6.32 and 6.13 MJ/kg for rape seed meal fed to broiler and white leghorn chicks, respectively. Clandinin (1973) referred average ME of 9.74 MJ/kg for three varieties of low hull rapeseed meal containing 7.76 to 10.30 % fibre, and 7.24 MJ/kg for regular rape seed (canola) meal containing 15 to 17 % fibre. The variation in energy values between oil seeds may depend on the quality, type, processing methods, anti-nutritive factors, amount of fat contents and so on.

2.4.2 Protein and amino acid contents

The protein content of vegetable sources (ingredients) is comparatively lower than those of animal sources of proteins. The protein quality, digestibility and availability of all indispensable amino acids of plant proteins are also inferior to those of animal proteins. The main function of protein is to supply all amino acids, as amino acids are the building blocks of protein in the body.

Although vegetable proteins do not contain all the indispensable amino acids in proper proportions as required by the birds, two or more vegetable proteins together can supply amino acids for the optimum growth and development of the birds. Besides energy, protein (amino acids) is a primary component of chicken diets. Lysine, methionine, and tryptophan are very important for poultry, as these amino acids are limiting in plant protein sources (McDonald *et al.*, 2002).

Many plant or vegetable protein sources such as soybean, canola, sunflower, cotton seed, rapeseed meal, lupins, peas, corn gluten meal etc., are used predominantly for poultry production. The nutritive values of these vegetable proteins with respect to protein and amino acid contents are also different.

Soybean meal is a well-established and relatively inexpensive excellent protein source for poultry diets. This meal contains high quality protein (40-48 %), but the carbohydrates (oligosaccharides, pectins, hemicelluloses, and cellulose) found in this meal are poorly digestible (Bach Knudsen, 1997). Soybean meal contains all essential amino acids, but the content of sulphur containing amino acids (methionine, cystine) is low, and similar to those of canola meal. Lysine digestibility in soybean meal is approximately 10 % units higher than in canola meal (Sauer *et al.,* 1982; NRC, 1994). The protein quality of soybean meal is about equivalent to fishmeal if it is supplemented with synthetic amino acid (DL-methionine). Full-fat soybean can be incorporated into the growing poultry diets at the rate of 15 to 35 % without any detrimental effect on performance. Lung and Man (1999) reported that fishmeal can be substituted by soybean meal up to 30 % in poultry diets for economic production.

The introduction of low-glucosinolate rape seed has enabled canola meal to become a good alternative to soybean meal in poultry diets. If lysine is supplemented in diet, canola meal can substitute up to 100 % of soybean meal and show no major detrimental impact on growth performance of birds, or nutrient (energy, mineral) utilization (Leeson *et al.*, 1987).

Rape seed contains CP (41 to 44 %) with a fair level of amino acid contents, in contrast to other plant sources (Newkirk *et al*., 1997). Canola is a variety of rape seed, which contains 20-22 % CP and 40-42 % fat, and lower levels of anti-nutritive factors (glucosynolates and erucic acid) (Swick and Tan, 1997). The protein content of this meal differs on the basis of type of cultivars, harvesting time, and processing methods. The CP content of this meal ranges from 37 to 38 %, if this meal is produced from a mixture of both *Brassica napus and Brassica Campestris* (Clandinin *et al*., 1981).

The deficiency of limiting amino acid (lysine) and the presence of toxic factor (gossypol) in cotton seed meal (CSM) make it unpopular for use in poultry diets. Selected CSM and canola meals (CM) up to 200 or 300 g/kg in starter diets, and up to 300 g/kg in finisher diets of broilers can be incorporated easily without affecting the performance of birds (Perez-Maldonado *et al*., 2001).

The nutritive value of lupin as poultry feed is dependent on the concentration of anti-nutritive factors and dietary fibre contents in the seed. Lupin seed meal contains crude protein ranges from 28 % to 47 % (Smith, 2005). Lysine in lupin is a highly available for poultry (Batterham, 1992).

Sunflower meal can be an alternative source of protein for poultry bird through extraction of oil, and proper processing. Villamide and San Juan (1998) reported that properly processed seed of high-oil sunflower varieties can become valuable protein sources for poultry, with a same amino acid profile as those found in soybean meal. The only demerit of this meal is lower level of lysine with a higher content of dietary fibre. High-oil sunflower meal contains 32.30 % CP, 18.8 % fat, and 11.54 % fibre, respectively (Senkoylu and Dale, 2006).

The protein and amino acid contents of different vegetable protein sources may vary depending on the species, cultivar, climate, topography of soil, toxic factors, dietary fibre, and processing strategy. Proper processing techniques may enrich the content of protein and amino acids of oil seed meals, and thereby nutritive value of these protein sources.

2.4.3 Lipids

Lipid, animal or vegetable fat or oil are used interchangeably and are used for both human and animal nutrition. Many vegetable oil or fat which are commercially available in the market, and these are mostly extracted from the different oilseeds, or vegetable protein stuffs namely soybean, canola, corn, mustard, sunflower, palm, linseed oils etc., are being used popularly in the poultry diets to supplement the energy requirement of the birds. These vegetable oil and or fat can be incorporated up to 10 % for fortifying energy in the practical diets of poultry diet (Singh and Panda, 1992).

Inclusion of fat or oil helps to enrich the diets with more energy, normally by two times higher than those of carbohydrate or protein. Apart from this, it makes diet palatable, improves pelleting, enhances fat-soluble vitamins i.e A, D, E, K, better absorbed by the birds, reduces pulverization, removes dustiness of the diets, and increases the efficiency of the ingested calorie. Moura (2003) reported that broiler chickens showed better performance when fed on diet supplemented with oil than those on diets with no oil, even though all diets were of similar nutritive values. Carcass composition and meat quality can be affected by the type of dietary fat or oils used in the poultry diet formulation. Level and profile of fatty acids of broiler meat or muscles can also be altered by feeding diets with different fat sources as reported by Scaife *et al.* (1994). The ratio of n-6 to n-3 fatty acids in broiler carcasses and abdominal fat pads to 5:1 is good for human health and can be increased by adding canola oil in broiler diets (Coetzee and Hoffman, 2002). It was further revealed that saturated fatty acids in the broiler carcass and fat can be decreased drastically by increasing the level of n-3 fatty acids in the diets.

2.4.4 Non-starch polysaccharides (NSPs)

Indigestible carbohydrates are considered as the most important components that affect the nutritive values of all-vegetable feeds. Vegetable feed proteins; particularly soybean meal, canola meal, and sunflower meal are rich in galacto-oligosaccharides and pectic polysaccharides (Kocher, 2002). These are generally regarded as anti-nutritive factors, which slow down the digestion of plant feedstuffs as well as bird's performance. The presence of anti-nutritive factors and indigestible NSPs limit the nutritive values of vegetable feedstuffs, for example, SBM, CM, peas, and so on (Castell *et al*., 1996; Dale, 1996). The effective utilization of these feedstuffs is often influenced by their high content of NSPs (17.9 % in CM vs 14.5 % in SBM) (Meng *et al*., 2005). Cellulose and pectic polysaccharides are the major NSP in these feedstuffs (Bach Knudsen, 1997). Other NSPs include rhamnogalacturonan, raffinose, and stachyose with associated side chains consisting of arabinose, galactose, and xylose residues (Bacic *et al*., 1988; Leeson and Summers, 2001).

Apart from these, other polysaccharides include cellulose, xylans, arabinoxylans, and xyloglucans, which are mainly present in the hull fraction of soybean, canola and peas. The total NSP content of vegetable protein sources ranges from 180 g/kg DM in peas and canola meal to over 350 g/kg DM in some lupin species (Kocher, 2001). However, these indigestible carbohydrates, particularly oligosaccharides and NSPs require microbial fermentation in the large intestine and ceaca, because the pancreatic enzymes of chicken are unable to hydrolyze these carbohydrates in the small intestine. It is reported that increased level of soluble NSP will increase fermentation in the upper gut which is detrimental for the overall performance as well as to bird health (Choct *et al*., 1996).

2.5 CONSTRAINTS TO USE OF VEGETABLE PROTEIN SOURCES

2.5.1 Formulation issues

Vegetable feed protein ingredients are comparatively cheaper in cost, and can be harvested more times in a year around the tropical areas, being important feed sources of many oil mills and feed industries (Vieira *et al*., 1992). Affordability, accessibility, and ease of processing for poultry industry make these proteins more attractive for feed formulation. Conversely, the vegetable protein sources contain numerous anti-nutritive factors, amino acids imbalance and deficiency, have lower protein, and deteriorate during storage. The quality of diets may be inadequate and affect bird performance adversely when diets are based solely on vegetable protein ingredients. The exclusion of animal by-products from diet formulation not only decreases the nutritive value of the formulated diets but also reduces the ability of the formulations to meet the required nutrients for the animals (Hossain *et al*., 2011a). Occasionally, feed costs may go up when birds fed on diets formulated exclusively with all-vegetable ingredients excluding animal proteins (Ristic and Damme, 2001; Vieira *et al.,* 2005; Bellaver *et al.,* 2005). These constraints of vegetable protein stuffs can be considered as formulation issues or barriers which discourage the producers from incorporating more vegetable ingredients in animal diets.

2.5.2 Anti-nutritive factors (ANFs)

Many feedstuffs, especially oilseeds and legumes that are fed to non-ruminant animals contain numerous detrimental factors that hamper proper nutrient utilization. These factors interfere with the utilization of the ingested feed materials in a variety of ways, including reducing protein digestibility, binding to various nutrients or damaging the gut wall and thereby reducing digestive efficiency.

These factors, which cause reduction in growth rate and feed conversion ratio and/or affect health status of the animals, can be termed as anti-nutritive factors (ANFs) (Liener, 1989; Huisman and Tolman, 1992).

However, extensive studies reported that cereal grains and other vegetable feedstuffs have got numerous anti-nutritional and toxic components that have detrimental effect on the nutritional value of vegetable and plant proteins (Lewis and Fenwick, 1987; Liener, 1989; Akande et *al*., 2010). Fasuyi and Aletor (2005) reported that these deleterious compounds prevent the birds from getting full nutritional benefits if these feeds are used in their diets without proper processing.

Among vegetable proteins soybean is used predominantly, but this bean is rich in protease (trypsin and chymotrypsin) inhibitors and lectin (phytohaemagglutinins), which interfere with the utilization of protein and mineral in the digestive tract (Siddhuraju *et al*., 2002), thereby reducing growth of the animals.

Rapeseed meals contain anti-nutritional substances importantly glucosinolate (commonly referred to as goitrogens), sinapine, tannins, erucic acid, and phytates (Kermanshahi and Abbasi Pour, 2006; Thanaseelaan *et al*., 2007). Feed palatability, growth or production of animals is found to be reduced by the detrimental effect of glucosinolates found in rapeseed meal (Peron and Partridge, 2010). The main anti-nutritional factors in lupins are alkaloids, together with phytates, protease inhibitors, and lectins (Mikić *et al*., 2009). Peas and beans contain ANFs importantly resistant starches, refractory proteins, tannins, and trypsin inhibitors, lectins (Crèpon, 2007). Main ANFs of pea are trypsin inhibitors and lectins (Mihailović *et al*., 2005b). Besides, among the toxic matters that make common vetch unsuitable for non-ruminant animals and humans, the most important are γ-glutamy l-β-cyanoalanine, that has a bad influence on metabolism of sulphur amino acids, and vicine and convicine, responsible for a disease called favism (Mihailović *et al.*, 2005a).

Apart from the above, many other ANFs are also remarkably present in vegetable protein sources. These include anti-vitamin factors, phytic acid, toxic amino acids, hydrocyanic acid (HCN) etc. Amongst these, phytic acid is a potent and common ANF found in all oilseed meals and plant feeds. Its presence in the diets help to form protein and mineral-phytate bond which results in unavailability of nutrients (e.g. protein and minerals) (Leeson and Summers, 2001; Al-Kaiesy *et al*., 2003). Vegetable proteins particularly canola meal contains relatively high phytate levels average 76.4 % of total P (Selle *et al*., 2003). The lack of endogenous phytases makes phytate phosphorus unavailable to non-ruminant animals, consequently inorganic phosphorus has to be added to poultry diets.

2.6 RESPONSE OF BIRDS TO VEGETABLE PROTEIN DIETS

2.6.1 Feed intake

Applying accuracy and a degree of precision in diet formulation requires an intimate knowledge of the bird, its daily nutrient requirements, and a more comprehensive understanding of the ability of the selected feeds to provide the most desirable nutrient status. The ingestion of the optimal level of dietary nutrients, whether for birds involved in egg or broiler meat production, is very much dependent on the level of feed intake. The complexities of the factors which determine nutrient intake and causative reasons and hypotheses for under- or over-consumption, have been reviewed extensively by others (Forbes, 1995; Van der Heide *et al.*, 1999; Forbes, 2006). Birds have precise requirements for nutrients, both macro and micro, and energy-yielding components. Therefore, knowledge of their feed-intake capacity is essential if dietary concentrations are to be appropriate. A bird's daily consumption of feed ultimately governs its health, development and potential for reproduction.

Many researchers have assessed feed consumption of broilers fed on both all-vegetable and traditional diets. Rechant (1995) obtained an increase in feed consumption of 6 % on plant protein diets enriched with enzymes and essential amino acids. No difference was observed in broiler chickens by feeding vegetable–based diets and the regular diets (Vieira *et al.*, 2005). Similar findings of feed intake of broilers was reported by Vieira *et al.* (2006) in a study on broilers fed all-vegetable diets containing acidulated soybean soapstock.

Protein originating from plant or animal sources might have a significant effect on the feed intake of broiler chickens. Ojewola and Ewa (2005) reported that broilers fed on diet containing cotton seed meal had the highest daily intake, and significantly different from the birds fed with the other diets having soybean meal, pigeon pea seed meal, groundnut cake and cashew nut meal. Rehman *et al*. (2002) reported that 100 % substitution of soybean meal with sunflower meal in broiler diets resulted in high feed consumption with poorer performances. This might be attributed to comparatively low nutritional values and mycotoxin susceptibility of sunflower meal.

Feed intake of broilers may be significantly improved in animal protein diets as compared to the birds fed pure vegetable diets (Alali *et al*., 2011; Hossain *et al*., 2011a, 2012a,b; Bhuiyan *et al*., 2012a,b). Animal protein ingredients such as fish meal are regarded as excellent sources of protein, and contain all indispensable amino acids in adequate quantities, particularly lysine and methionine as required for poultry (Singh and Panda, 1990). If fish meal is included in the diets it tends to stimulate the feed consumption and improve feed efficiency of the broiler chickens

(Solangi *et al.*, 2002). The higher feed intake may be affected by nature of protein, amino acid balance, micro-nutrient contents, particularly minerals (Na, Cl, Se), vitamin-E, choline, vitamin B_{12}, anti-nutritive factors, sensorial characters (smell, flavour, taste or palatability), and unidentified growth factors in the feeds. Feed intake of broilers on diets having soybean meal as sole protein source is reduced as compared to those fed animal protein or mixed plant proteins as reported by Irish *et al.* (1993).

2.6.2 Preference or feed selection by broiler chickens

The ancestors of modern meat chickens lived on self-selecting feeding systems. They thrived better under such feeding systems, because they were able to select or balance their own feed-mixture from a wide range of scavenging areas in the wild or range state. But this feeding system is not always available to modern meat chickens, because they are normally offered a single feeding system in confinement. They do not have any choice or opportunity to select their own feed by this system of rearing.

However, if the modern meat chickens are reared under preference-feeding system, they can show their innate ability to sort out an adequate diet mixture from a choice of variety of feeds that are individually insufficient (Sinurat and Balnave, 1986). In preference feeding system, individuality is the basic biological factor. The individual needs may be influenced by weight, production, age, and environmental conditions, enabling them to compose their own ration as per their actual body requirements (Emmans, 1977; Cumming and Mastika, 1987; Karunjeenwa, 1978). This may cause for their instinctive ability to choose proper food constituents, which is impossible when supplying single conventional feeds are provided the birds. When birds are given a choice, they have the ability to choose the diet of the appropriate content of CP and energy to meet their requirements for protein and energy independently (Forbes and Shariatmadari, 1994; Hruby *et al.*, 1995; Yo *et al.*, 1997). It was reported that fast-growing birds are more reactive to variation in indispensable amino acid concentrations than laying birds (Noble *et al*., 1993; Picard *et al*., 1993). It is generally considered that domestic fowls show a reduced responsiveness to the nutritional consequences of their feed intake when compared with their progenitors, the jungle fowl, particularly in terms of protein and energy regulation (Hughes, 1984). However, evidence from several species of poultry indicates that, birds are capable of sorting the required amount of protein when given a choice between high and low-protein diets. Many factors, including genotype, chronological age, physiological state, and prior experiences etc., can influence the dietary selection by the chickens (Rose and Kyriazakis, 1991; Forbes and Shariatmadari, 1994; Forbes, 1995).

In addition, the preference of broiler chickens for vegetable protein diet may be influenced by several factors, for example, nutrient requirements of individual birds, feed composition, and nutritive value, stage of production, environmental condition, genetics and so forth. Apart from these factors, organoleptic traits of diets such as colour, smell, odour, flavour, taste and texture might also influence the feed intake and feed regulation of broiler chickens (Cruze *et al.*, 2005). The choice of birds for foods is directed more towards the nutrition of food. Sensory characteristics of diet such as flavour and taste can initially influence intake and preference of birds, but this effect soon wanes as the birds perceive that there is no nutritional implication in the different sensory properties of food (Balog and Millard, 1989). Palatability of diets, metabolic and nutritional requirements might play a profound role in dietary self selection of the birds. So the ability of birds to self-select a complete feed may be affected by palatability, former experiences and social factors (Hughes, 1984; Forbes, 1995).

Dietary composition and sources of protein use in practical diets may also affect the feed intake and preference of broiler chickens. Bhuiyan *et al.* (2012b) reported that broilers preferred animal protein diet to vegetable protein diets when given a choice. This similar trend was also observed by Hossain *et al*. (2012a,b) in a several studies. Variation in different characteristics of individual protein sources may affect the feed preference of the birds, as no two single protein sources available in the nature are similar in characteristics reported by Singh and Panda (1992).

2.6.3 Body growth

Response of body growth in fast-growing chickens has been considered as an initial parameter for estimating the bioavailability of essential nutrients of feeds, because meat chickens are an ideal assay subject with a limited nutrient storage, high nutrient demand, and rapid growth rate (Ammerman, 1995**).** The growth response of broilers may be influenced adversely by many factors including type of feeds, level of nutrients, protein or amino acid requirements, dietary fibre level, and anti-nutritive factors and so on. Broiler chickens gained adequate body weight fed on all-vegetable based diets reported by Omenka and Anyasor (2010). Cancherini *et al.* (2004) also reported that corn and soybean meal-based diets promoted better growth response as compared to regular diets. The performance of birds fed vegetable protein was better than those of birds fed diets containing animal protein (Bellaver *et al.*, 2005). This result is supported that of Vieira *et al*. (2006), who revealed that body weight gain throughout the cycle was improved in broilers fed on all-vegetable diet supplemented with acidulated soybean soapstock (ASS), compared to gain on a diet supplemented with degummed soybean oil (DSO). However, body weight of birds fed veg-ASS was not different from that of birds fed the animal diet. Similar body weight gain was reported by Vieira *et al.* (2005), who conducted a study with broiler chickens fed an all-vegetable diet or a regular diet having a mix of animal by-products (pork and poultry by-products plus feather meal). They found no significant differences in the body weight gain of broilers by feeding these two feeds. Similar weight gain was found in broiler chickens (Hubbard) fed corn and soybean meal diets in the form of pellet and mash (Park *et al*., 1983). Results tend to suggest that similar growth responses can be obtained if the diet is formulated with similar nutrient composition and free from any detrimental factors exist in the feedstuffs.

2.6.4 Feed conversion ratio (FCR)

Feed conversion ratio or feed efficiency is also considered as one of the most important criteria used in determining the performance of broiler or layer chickens by the poultry industry. Whatever the system used to determine the FCR or feed efficiency of modern chickens, the idea gives a an estimation of how efficiently feed is utilized by the fast-growing chickens, and the quantity of meat or egg that can be recovered from these birds as a finished product.

Dietary protein sources might affect the FCR of meat chickens when used in practical diets of poultry. Different researchers have reported both positive and negative results of vegetable and animal proteins on the feed efficiency of broiler chickens. Higher rate of muscle protein and body weight gain was found in broilers fed the vegetable–based diets, compared to animal–based feeds (Omenka and Anyasor, 2010). Cancherini *et al.* (2004) observed better FCR when corn and soybean meal-based diets were fed to broilers. Colibar *et al.* (2001) reported higher rate of feed efficiency (14.16-13.80 %) of broilers that received vegetable proteins compared with other feeds. The FCR of broiler chickens was found by to be similar on all-vegetable or animal protein diets (Vieira *et al.,* 2005). In another experiment same responses were also observed for FCR when broiler chickens were on all-vegetable feeds supplemented with ASS as compared to the diet supplemented with degummed soybean oil (DSO) (Vieira *et al*., 2006).

Feeding of broilers with vegetable diets containing corn, barley, soybean, and cotton seed meal improved the feed efficiency and increased relative weight of breast muscle (Al-Ostwani *et al.*, 2000). Conversely, improved FCR was observed in the broilers when the birds offered animal proteins (fishmeal, poultry meat, and bone meal, poultry visceral meal) diets, compared to those fed the vegetable protein diets (Sadagopan *et al*., 1993; Cruz *et al*., 2009; Alali *et al*., 2011; Hossain *et al*., 2011a, 2012a, b). The variation in FCR between VP and AP diets may be due to complementary effects of well-balanced amino acids, better digestibility of protein or other nutrients.

2.6.5 Meat quality, carcass yield and visceral organ development

Poultry meat has little or no social, religious or cultural bias (Mounteney, 1975), therefore it is very popular and relished by many people all over the world. The meat from broiler chickens, whether male or female, is tender with soft, pliable and smooth texture and flexible breast bone cartilage (Singh and Panda, 1992). According to Ikeme (1990), poultry meat varies in acceptability to consumers according to the tenderness, juiciness, and flavour of the meat when cooked.

Feed sources originated of plant and animal might greatly affect the feed utilization and carcass quality of broiler chickens. The amount of deposition of fat in the carcass defines the meat quality of broiler chickens. Extra fat accumulation in broiler carcass is generally considered as an unfavourable characteristic in the poultry industry (Remignon and Le Bihan-Duval, 2003). Adoption of suitable feeding strategy and introduction of lean broiler lines by genetic selection appear to be the most effective way of reducing or limiting carcass fat content, and for improving the carcass quality. Less abdominal fat is accumulated by broilers fed on diets containing all-vegetable feeds than those fed on diets containing animal protein (Pawlak *et al*., 2005; Hossain *et *al*., 2011a). Broiler chickens supply premium quality meat that is rich in proteins with less fat (4-12 %) than other livestock (Kralik *et al.*, 2002).

Lipid content is the most variable components of meat and there are variations in amount of lipid deposited at different parts of poultry, being higher in high muscles than drumstick and lowest for breast (Olomu, 1995; Aduku and Olukosi, 2000). However, numerous studies have been performed on the meat quality of broiler chickens fed on all-vegetable or animal feed ingredients. Low fat content and higher protein meat was obtained by Omenka and Anyasor (2010), when broilers were fed with plant-based diets. The authors also reported a significantly lower serum total cholesterol and mean fat composition in heart, gizzard and muscles in the birds fed all-vegetable diets. This is also supported by other researchers (Anderson *et al*., 1995; Sirtori *et al.*, 1995) who stated that consumption of vegetable protein (soybean) diets can play an effective role in reducing the levels of serum triglycerides and cholesterol in humans and animals.

The sources of proteins used in the feed formulation for poultry not only affect the quality of the formulated diets but also influence their optimum growth, carcass yield parts, and other internal organ development of the broilers. Different researchers have been reported both positive and negative effects regarding body growth and the internal organs development of broilers fed on either vegetable protein or traditional diets. Feeding of broilers with vegetable protein diets had no effects on carcass characteristics and different digestive organ (crop, proventriculus, gizzard, duodenum, jejunum, ileum, caeca, colon, pancreas, and liver) development in comparison with a diet containing animal protein (Al-Masri, 2003 and 2006).

In contrast, Al-Ostwani *et al*. (2000) observed improved relative weight of breast muscle in broilers fed diets containing corn, barley, soybean, and cotton seed meal. Yousri *et al*. (1991) observed similar responses in the relative weight of stomach, gizzard, heart, lungs, liver, spleen, and kidney by feeding broilers with animal protein diets formulated with blood meal, fishmeal or meat-bone meal.

Morphometric measures of digestive organs (small intestine weight, oesophagus, crop, gizzard, liver and pancreas) were not influenced by level of feather, blood meal, poultry viscera, and bone meal in diets fed to broiler chickens (Xavier *et al.*, 2011 and 2012). Caires *et al*. (2010) observed no significant differences in different carcasses yield (carcass yield (%), breast bone, deboned breast, thigh, and drumstick) of broiler chickens fed diets containing meat and bone meal, blood meal, feather meal, and poultry offal meal. The similar organ development of the birds may be a result of the uniform growth of the broilers fed either type of diets (animal or vegetable protein based).

2.6.6 Skeletal development and quality

Modern meat chickens are very susceptible to leg problems, compared to other species of poultry. Birds with increased number of leg problems are culled from the flock or downgraded at slaughter (Kestin *et al.*, 1999). Weak bones result in breaking during processing and lower meat grade. Also weak legs often result in reduced feed intake thus affecting weight gain as well as product quality (Orban *et al*., 1999). The prevalence of leg problems of broiler chickens may be affected by numerous factors, including feed sources, nutrient density, dietary protein levels, deficiency of specific nutrients, especially vitamins and minerals in broiler diets (Bilgili *et al.*, 2006; Brickett *et al*., 2007). However, the incidence and severity of leg problems that is faced by the fast growing meat chickens is of great concern to the poultry enterprise, in terms of product quality and animal well-being viewpoint. Vegetable or plant feed sources contain high levels of anti-nutritive factors, including phytate. As previously mentioned, phytate reduces the availability of nutrients through formation of indigestible complexes with cations (Ca, Mg, Fe) and proteins (Reddy *et al*., 1982).

2.6.7 Litter quality

Litter or bedding material is used in the poultry house to provide comfortable environmental condition. Good litter condition results in better production. High litter moisture content, excreta moisture, drinking water etc., may deteriorate the quality of the litter materials. Apart from these, ammonia level emanating from excreta and pH may also worsen the environmental conditions and affect bird health and productivity. Ammonia can cause abundant pollution in broiler houses, and it may have a great impact on poultry welfare (Kristensen and Wathes, 2000). Ammonia is produced during the decomposition of uric acid and the efficiency of this conversion is directly related to the level of litter moisture. Growth depression and increase feed conversion in broilers may take place when ammonia levels exceeded 25 ppm in the broiler house.

Many dietary factors such as dietary protein level, electrolytic balance, ionophores, cereal contents, fibrous ingredients, legumes, and non-starch polysaccharides (NSPs) are associated with excreta/litter moisture and quality (Fleet and Saylor, 1983; Smith *et al*., 2000; Murakami *et al*., 2001; Francesch and Brufau, 2003). Excreta moisture of broilers may be affected by the frequency of water consumption of the birds, which in turn, may be influenced by any alteration in dietary feedstuffs. The NSP contents of vegetable diets arising from soybean and canola meals can affect digestion of other nutrients by attracting water and contributing higher litter moisture (Francessch and Brufau, 2004). Furthermore, vegetable ingredients, particularly soybean meal, can contain more potassium than the animal by-products (NRC, 1994; Viera and Lima, 2005). Potassium is known to increase water consumption which leads to improved moisture level in the excreta of broilers (Murakami *et al*., 2001), and thus result in sticky litter. Smith *et al.* (2000) showed that 0.1 % increase in dietary potassium level led to increase in excreta moisture content by 1.2 % in laying hens.

Environmental conditions may be worsened by the excessive moisture defecated from the birds, and it leads to predisposing challenge of microbial effect to the birds. In addition, the carbohydrates available in the vegetable protein soybean are poorly digested (Parsons *et al*, 1980; Pierson *et al*., 1980), and these can stimulate for increase bacterial growth in the litter. Broilers fed diets with only vegetable sources contributed a higher amount of moisture and ammonia in their excreta as reported by several researchers (Vieira and Lima, 2005; Eichner *et al*., 2007). This excess moisture affects the litter quality adversely and leads to an increased risk of development of footpad dermatitis and other diseases.

2.6.8 Tissue protein growth and digestive enzyme activities

Broiler chickens are non-ruminant, and therefore rely on enzymes from their own organ for nutrient digestion. Only a small amount of digestion takes place in ceaca by the microbial activity. Apart from this, the poultry industry relies on many microbial enzymes to enhance digestive function and improve bird productivity. Amongst multiple factors, the diet can play an important role in initiation of enzyme secretion and activities. Dietary modifications can alter both the synthesis and levels of pancreatic amylase, trypsin, and lipase, and can also induce their action due to presence of their respective substrates in gastrointestinal lumen (Branon, 1990). The activity of pancreatic amylase, and alkaline phosphatase was influenced by dietary energy level and bird age, but these did not affect the trypsin activities (Pinchasov *et al.*, 1990; Routman *et al*., 2003). The amount and type of fat (triglycerides) in the diets regulate pancreatic lipase activity, but the mechanism is still unclear (Ricketts and Brannon, 1994). Chicks fed with high energy content showed higher lipase activity than those treated with low energy level (Maiorka *et al*., 2004). Type of feeds or proteins used in the diet formulation of poultry might affect digestive enzyme activities. Broiler diets prepared exclusively with all-vegetable ingredients may increase dietary fibre considerably in association with phytic acid. More than 5.0 % crude fibre in the practical diet of broiler is not desirable at all, because chicken is a non-ruminant animal and so high fibrous feed materials depress their digestibility and thus reduce their performance.

2.6.9 Energy utilization

Feed is the most expensive recurrent cost of production and energy represents a large proportion of such feed. So the energy content of the diets represents the core theme in diet formulation for poultry. Cereal grains provide the major energy to poultry and may accounts for 60 to 70 % of the diets. Apart from this, animal fat, vegetable oil, legumes, oilseed meals, animal by-products etc., also contribute substantial amount of energy along with providing other essential nutrients such as protein, vitamins and minerals for commercial chickens. Protein sources may also supply considerable amount of energy and their interaction with the main energy sources has a bearing on overall energy supply and feed utilization. So, it is important to determine precisely energy values of diets containing vegetable sources, either for least–cost formulation purposes or for adapting feed supply to energy requirements of animals (Noblet, 2006).

The relative speed of broilers' body growth compared to other poultry species is mainly dependent on the efficient utilization of nutrients to produce body tissues and deposit fat and protein in the carcass (Leeson *et al.*, 1996). The trend of protein and fat deposition in the body is influenced by many factors including nutrition, genotype, sex, environmental condition, and body weight or degree of maturity of birds (Havenstein *et al*., 1994; Wiseman and Lewis, 1998; Decuypere *et al.,* 2003). However, although all-vegetable ingredients are inexpensive and potential sources of plant nutrients, their exclusive use is limited because many poultry nutritionists are still concerned about the quality issues and energy values of the diets as well as their pattern of utilization. There are numerous constraints in vegetable feeds which reduce their optimal utilization, thus limiting the benefits of these feeds to broiler chickens. This results in reduced digestibility and poor performance of the birds. In the diets of most non-ruminant animals, for example, pigs and poultry, the digestibility coefficient of feed energy varies in a range between 70 and 90 %, but this range usually increases (10 to 100 %) for individual feed ingredient (Sauvant *et al*., 2004), whether it is derived from plant or animal origin.

The ME intake of birds may be reduced due to a high fibre contents and presence of anti-nutritive factors contained in the vegetable protein diets (Barteczko *et al*., 2008; Warenham *et al*., 1994). In addition, phytic acid and other ANFs can adversely affect energy utilization and the availability of other nutrients in poultry diets (Ravindran *et al*., 2000 and 2005). Apart from this, increased activity of gut microflora on dietary components can also suppress energy utilization (Choct *et al.*, 1996), availability, and digestibility of other nutrients (Steenfeldt *et al.*, 1995; Smits *et al*., 1997). It is reported that most of the variation of digestibility of feed energy is closely associated with the presence of dietary fibre (Le Goff and Noblet, 2001). Variation in gross chemical composition between grains may affect the digestibility of feed energy to a great extend as well (Black, 2001).

However, the capacity of cereal grains to contribute energy to birds varies widely between and within grain species. This variation in energy value may arise from chemical or physical properties and botanical origin of individual feed components.

2.7 IMPROVING THE QUALITY OF VEGETABLE PROTEIN DIETS

2.7.1 Crop Breeding

Crop yields can be increased, production costs can be reduced, and new products can be generated through using new improved technologies, like crop breeding and genetic engineering, which allow improvements in crops beyond levels possible with any other approaches. Genetic manipulation of many cereal grains such as maize, sorghum, barley, wheat, soybean etc., can enrich their yield as well as nutritive quality many folds over the usual cultivation strategy.

The principal goal of this breeding strategy is to enhance the nutritive values of the crops as well as feedstuffs, by promoting the nutrient status (e.g. protein, indispensable amino acids, minerals, or fatty acids), or reducing the level of detrimental effects such as phytate in the seeds (Swiatkiewicz and Arczewska-wlosek, 2011). As a result of advances in crop breeding research, many crops are now available in the market for use in poultry diet formulation.

Excessive phosphorus excretion by intensive livestock operations is a growing concern worldwide. Application of high available phosphorus maize (HAP) in poultry diet resulted in increased availability of P compared to conventional maize, and better production indices in broilers through drastically reducing phosphorus excretion in the excreta (Waldroup *et al*., 2000; Yan *et al*., 2000). Identification of non-lethal, low phytic acid mutations in corn, barley, and more recently soybean have resulted in a substantial reduction in phytic acid content, with a corresponding increase in available phosphorus. Yuan *et al*. (2009) reported that two new mutants of soybean seed had no adverse effects on seed quality and nutritional traits. The authors also assumed that these two mutant genes could be valuable genetic resources for breeding soybean varieties with reduced levels of phytic acid seed, which may have the potential to promote the nutritive status of soybean meal, and lower environmental pollution due to phosphorus for a wide range of animal farming. An increase level of energy and digestible amino acids was observed in low-phytate soybean meal, compared to traditional soybean in trials on duck (Adeola, 2005). However, although genetically modified (GM) crops have demonstrated an improvement in nutritive value and thereby improved productive performance of animals, their safe use in feeding and the impact on consumer heath are not completely known.

2.7.2 Feed processing

Anti-nutrients pose a major constraint to the use of plant protein sources in livestock feeds when these are used in practical diet formulation without adequate and effective processing. The levels or concentrations of these anti-nutrients in plant protein sources vary with the species of plant, cultivars, and post–harvesting treatments (Akande *et al*., 2010). However, many food processing techniques have been highlighted as possible means of reducing or totally eliminating the anti-nutrient levels in plant feed sources to innocuous levels that can be tolerated by animals, particularly non-ruminants (Fasuyi and Aletor, 2005).

Examples of feed processing techniques are drying (sun, oven), chopping, grinding, mealing, pelleting, freeze drying, heat (steam) treatment, ammonia treatment, autoclaving, boiling, cooking, and refrigeration. Sterilization, blanching, ensiling, chemical treatment, extracting, irradiation, salting, etc., are other methods. These process can effectively eliminate the toxicity of these feedstuff (Singh and Panda,1992; Leeson and Summers, 2001). Several other processing methods including extrusion, roasting, toasting, and jet-sploding have been reported to enhance the nutritive value of whole oilseeds (Arieli, 1998; Chen *et al*., 2008). In fact, protease inhibitors and lectins (phytohaemagglutinin) are widely believed to be the most important of the anti-nutrients present in soybeans, and can be easily eliminated by heat processing techniques (Krogdahl *et al.*, 1994; Leeson and Summers, 2001). Ethanol extraction process is suitable for reducing the magnitude of the negative impact of NSP (oligosaccharides) (Coon *et al*., 1990; Leske *et al*., 1993).

Microbial breakdown of glucosinolates and their degradation products appears to be an economical strategy for improving the nutritional value of rape seed meal. Vig and Walia (2001) demonstrated that solid-state fermentation of rapeseed meal significantly reduced the level of glucosinolates in the ingredient. The benefits were greater with longer fermentation times (>2 days). Similar results were achieved with mustard meal, with complete hydrolysis of glucosinolates after 60-96 hours (Rakariyatham and Sakorn, 2002). Gamma irradiation can be an effective and safe process to enhance the nutritional value and inactivation or removal of certain anti-nutritive factors in feedstuffs (Siddhuraju *et al*., 2002; Farkas, 2006). This method can also be very effective to enhance the nutritional quality of oilseed such as canola meal for broiler chickens (Gharaghani *et al*., 2008).

Recently, treatment of soybean meal and canola meal with gamma irradiation was successful in reducing degradation of protein by rumen microorganisms and increasing intestinal digestibility of protein (Shawrang *et al.*, 2008). Other investigators reported that anti-nutritional factors, such as protease inhibitors (Siddhuraju, 2002; De Toledo *et al*., 2007), phytic acid (Al-Kaiesy *et al.*, 2003; Bhat *et al.*, 2007) were significantly inactivated by gamma irradiation. Overall, choosing and using the right processing technique can improve the nutritive values of plant feedstuffs many folds by eliminating anti-nutritive properties, and these processed ingredients can be used safely in the practical diets of poultry.

2.7.3 Nutrient supplementation

Diet formulation with only vegetable ingredients can increase the crude fibre level in the practical diet, thereby reducing the digestibility of broiler chickens. Different supplemental feeds such as exogenous enzymes, fat or oils, synthetic amino acids, vitamin and mineral premixes, in-feed antibiotics, etc., can be incorporated into broiler chicken diets safely in order to enhance the nutritional quality of such feeds for the optimum performance of poultry.

Exogenous enzymes are regarded as "pro-nutrient" and have been found to be beneficial for improving the nutritive value of feedstuffs, and thereby increased performance of poultry to an extent (Rosen, 2006). Examining three substrates (canola meal, soybean meal, and peas), Meng *et al*. (2005) observed significant NSP degradation ranging from 8.8 to 10.2 % by a pectinase enzyme. The most pronounced degradation of NSP was achieved when a combination of enzymes containing pectinase was used by another researcher (Slominski *et al*., 2006) on the substrate of full-fat flaxseed.

In addition, it has been recently demonstrated that the efficacy of enzymes (amylases, proteases, enzyme admixture) is inextricably linked to the digestibility of the diets to which they are added (Cowieson and Bedford, 2009; Cowieson, 2010). Using enzymes in diets, oligosaccharides (raffinose, stachyose) of soybean meal can be degraded by 55-70 %, and thereby growth performances of birds can be enhanced through efficient nutrient and energy utilization (Graham *et al*., 2002; Ghazi *et al*., 2003; Cowieson *et al*., 2003; Meng and Slominski, 2005).

Ristic and Damme (2001) reported significantly better performance in poultry fed on vegetable proteins admixture with a phytogenic digestion promoter in comparison with animal proteins.

Dephytinization is an interesting approach to overcoming the anti-nutritive properties of phyate using enzyme. Ravindran *et al*. (2001) reported that increasing phytase inclusion levels improved both average ileal amino acid digestibility and dietary available ME values in an essentially linear manner. Newkirk and Classen (2001) reported dephytinization of canola meal with a purified phytase resulted in increase in amino acid digestibility of between 39.8 % (proline) and 0.2 % (methionine). Micro-nutrient digestibility, particularly key amino acids, increased significantly when broiler chickens fed diets based on all-vegetable ingredients supplemented with microbial enzymes (Hossain *et al.*, 2013a).

Direct supplementation of synthetic amino acids to diets may decrease crude protein (3 to 4 %) levels in broiler diets without affecting performance, if free amino acids are included in the diet equivalent to the amino acid levels in a regular diet (Payne, 2007). Colibar (2000) reported that supplementation of synthetic amino acid (DL-methionine) or probiotic addition improved the productive performance of broilers by 12.17 to 26.28 %. Micronutrients, in form of premix can be supplemented in poultry diets to remove the gaps in vitamins and minerals. Supplementation of some minerals, amino acids, and vitamins could help reduce or neutralise the negative impact of anti-nutritional factors in plant protein sources for poultry and other farm animals.

2.8 IMPORTANCE OF THE CURRENT STUDY

There has been limited research on the use of diets entirely based on vegetable ingredients for broiler chickens. Due to complexities in the direct assessment of the nutrient availability of feeds and having numerous limitations in vegetable feedstuffs, the current research has been designed to examine the potential and full nutritional benefits of these feed ingredients by broiler chickens. As previously highlighted these ingredients are not only safer, they are inexpensive and would be preferred by many medium or large–scale producers.

Poultry industry, nutritionists, feed formulators, and the producers of specialized fowl, including organic, would also benefit from the findings. Besides, the digestion, absorption, excretion and efficacy of broiler chickens feeding exclusively on vegetable–based diets are not still well explicit, and so further study is recommended to obtain potential beneficial effects of using these feedstuffs in poultry diets, for optimum and economic meat chicken production.

Producing poultry products (meat and eggs) at economical rate may be feasible using vegetable protein sources in practical diets, as these are cheaper and safer than animal protein sources. Despite cutting feed cost considerably, this ingredient can serve as excellent sources of nutrient for poultry when processed properly and supplemented with other pro-nutrients (Cruz *et al*., 2009). Besides, information on available data of average macro and micro-nutrient contents of these locally available vegetable feedstuffs may help to ease the formulation strategy of many poultry raisers across the world.

Extra fat accumulation of broiler carcass is generally considered as the unfavourable trait in the broiler industry (Remignon and Le Bihan-Duval, 2003). Adoption of suitable feeding strategy may help to reduce extra-fat content of broiler carcasses, and thereby improve the meat quality. As it is reported that broilers contained less abdominal fat fed diets containing all-vegetable ingredients than those fed on diets containing animal protein (Jensen *et al.,*1980; Pawlak *et al*., 2005; Hossain *et al*., 2011a). The merit of this concept can be used in the formulation of diets for poultry and other livestock. When lean meat is desired, animals may be fed vegetable–based diets, which will result in lower fat and higher protein deposition in their carcasses (Singh and Panda, 1992). So this strategy of producing meat chickens excluding animal by-products could therefore bring about nutritional and economical benefits to consumers and producers alike.

Meat chicken productions entirely based on vegetable ingredients may be an emerging trend to producers, and have great demand to consumers as well. Because, this meat is being popularly accepted in some world market, especially in the European Union and Middle East countries (Mendes, 2003). Moreover, chicken meat fed on all-vegetable diets may contribute less fat with better profile of fatty acids in their carcasses, which may enhance shelf life or preservation quality of meat as well as consumer's preference.

2.9 CONCLUSION

From the review it is axiomatic that, considerable further research study is required concerning the constraints to the use of all-vegetable protein diets and how these diets can be used for livestock and poultry feeding. Despite numerous limitations of vegetable feedstuffs, appropriate strategies can be adopted to improve quality of all-vegetable diets, eliminating their intrinsic problems for economic poultry production. Further research on all-vegetable diets will go a long ways to improve such diets for profitable poultry production.

CHAPTER 3 COMPARATIVE PERFORMANCE OF BROILER CHICKENS FED ON VEGETABLE OR ANIMAL PROTEIN DIETS

ABSTRACT

This study was carried out to compare the performance of broilers fed vegetable protein (VP) diets with that of birds fed animal protein (AP) as regular diets. Cobb 500 day-old male broiler chicks (n=256) were randomly divided into four experimental groups and raised on AP diets containing fishmeal and soybean (SBF); fishmeal with canola (CMF) or VP diets containing predominantly soybean (SBM) or canola (CM) meals, with no animal products. All diets were iso-energetic and iso-nitrogenous, and were pelleted but amino acid levels were formulated on a total and not digestible basis. Feed intake up to 21 d was highest (P<0.001) on the AP diets, while the SBM group was the lowest. Birds in the AP diet groups were heavier at 21 (P<0.001) and 35 days than those on VP diets. Birds on AP diets to 35 d had superior feed conversion ratio while the CM group was the most inferior. Excreta moisture level was significantly higher in birds fed the VP diets than those on AP diets. Excreta pH and ammonia concentration were similar between treatments. Protein digestibility was higher on the AP diets than on the VP diets. Birds raised on VP diets had significantly lower abdominal fat content than birds on AP diets. Other meat characteristics measured in this experiment did not differ significantly. Bone development, in terms of breaking strength and latency-to-sit time, was significantly better on the AP diets than the birds on VP diets. The birds on the CMF diet had longest tibia bones while birds on SBM diet were the shortest. Total tibia ash (TBA) content on the CMF diet was significantly (P<0.001) increased along with these mineral (Fe, Cu) contents which were also significantly higher in birds on the same diet than others. The responses of birds generally indicated that the AP diets were superior to the VP diets.

3.1 INTRODUCTION

Animal by-products and processed animal proteins are commonly used in poultry diets as conventional feed resources to meet the essential nutrient requirements of broiler chickens. In some countries these feedstuffs are being excluded from poultry diets in order to prevent cross-contamination of diets for ruminant animals in situations where feed mills are shared. The exclusion of these feed ingredients from formulations not only reduces the nutritive value of the diets but also limits the ability of the formulations to meet the essential nutrient requirements for poultry (Hossain *et al*., 2011a).

The protein requirements of poultry are most commonly met by both vegetable and animal proteins. Animal by-products (meat meal, meat and bone meal, fish meal) are the most commonly used animal proteins in poultry diets and are considered excellent protein sources for chickens (Parsons *et al*., 1997; Giang *et al*., 2001). Despite being satisfactory sources of quality proteins, there are many constraints to the extensive use of animal by-products in diet formulation for poultry, the key one being the risk of zoonotic diseases. Furthermore, a critical cost appraisal of poultry feed formulate shows that protein of animal origin is more expensive than vegetable protein (VP) sources (Oluyemi and Roberts, 2000).

In view of the foregoing, there is the need to investigate the possibility of developing alternative VP diets for poultry. Broilers grown exclusively on VP diets are preferred in the European Union and Middle East (Mendes, 2003). This tendency is growing and intensifying the pressure on feed formulators and nutritionists to supply organic, safe and hygienic poultry products to consumers, through provision of quality diets to poultry that exclude animal by-products or any growth promoters. Incorporation of vegetable ingredients, notably soybean, canola, sunflower, mustard, etc., in diets, instead of animal meals as protein sources, can lead to satisfactory broiler performance as long as the diets are properly balanced with necessary nutrients. These ingredients are a good source of nutrients, comparatively inexpensive, easily available, easy to process, and pose less risk of disease contamination.

However, vegetable sources contain numerous anti-nutritive factors (ANF), are lower in protein quality, have lower digestibility, and lower biological value than animal protein sources (Liener, 1980; Tacon, 1992; Vieira *et al*., 2003). There is the possibility that diets formulated with vegetable proteins will be nutritionally inferior to diets with AP and broilers fed solely on the former will respond negatively. This study was undertaken to ascertain the growth responses, excreta quality, nutrient digestibility, leg bone development and meat yield characteristics of broiler chickens fed on VP or AP diets.

3.2 MATERIALS AND METHODS

3.2.1 Experimental design and bird management

The experiment was conducted at the Animal House of the University of New England (UNE), and approved by the Animal Ethics Committee of same University (Approval No**:** AEC10/076). A total of 256 day-old Cobb 500 male broiler chicks (46.04±0.88 g) were collected from a local commercial hatchery (Baiada Poultry Pty. Ltd., Tamworth, Australia). The chicks were immediately distributed in a completely randomized design into four dietary treatment groups (SBM, CM, SMF and CMF) having 64 chicks in each group. Each treatment had eight replicates consisting of 8 birds per replicate (details of these diets are provided below). The birds were reared from 1 to 35 days of age in a cage rearing system. The chicks were reared in brooder cages (42 × 75 × 25 cm) in the first 3 weeks and then larger metabolic cages (60 × 45 × 38 cm).

Each pen was furnished with a feeder and drinker. Feeders were cleaned before supplying diets, and drinkers were washed weekly to maintain hygienic conditions. Excreta trays were cleaned after three weeks, or earlier if they were filled with excreta material.

For the first two days the birds were provided with a temperature of 33 ^{o}C. The temperature was then gradually reduced by 1 or 2 ^{o}C every 1 or 2 days until the chicks were 19 days old at which point the temperature was maintained at 24 ^{o}C for the rest of the trial. Sixteen hours of lighting per day were provided throughout the trial period. Feed was provided *ad libitum* in pelleted form and birds had free access to water. In the first 3 weeks, the birds were given starter diets followed by the finisher diets for the last 2 weeks.

Mortality was recorded as it occurred, while body weight and feed intake were recorded weekly for the calculation of body weight gain, and FCR was corrected for mortality. On d14, excreta samples were collected to determine ammonia concentration, pH and moisture content for assessing litter quality. Four birds from each pen were randomly selected, weighed and killed by cervical dislocation to collect digesta samples from the ileum for the assessment of ileal nutrient (GE, protein and starch) digestibility on d 21. Latency-to-sit (LTS) and gait-scoring tests were conducted to enable assessment of leg bone development of birds on day 28. Two birds per pen were also selected randomly, weighed and killed humanely at day 35 to obtain the right tibia bone samples for measurements of bone strength and mineral concentration. Meat yield was also assessed at this stage.

3.2.2 Dietary treatments

Four experimental diets coded as SBM, CM, SMF and CMF were formulated with maize, wheat, and vegetable oil as the main energy sources, along with soybean meal, canola meal and fishmeal as protein sources and pelleted (Tables 3.1 and 3.2). Diets SBM and CM were formulated exclusively with ingredients of plant origin to meet or exceed NRC (1994) recommendations. These diets (SBM, CM) were formulated with soybean meal and canola meal at a ratio of 3:1 to one another along with the other feedstuffs.

In contrast, diets SMF and CMF were animal protein (AP) or regular diets formulated with ingredients of both animal and plant origin. These two diets (SMF and CMF) contained soybean and canola meals at a ratio of 2:1, and fishmeal was also included as a complementary source of protein in both diets. The fishmeal was incorporated at the rates of 7.74 % and 8.39 % in the SMF and CMF starter diets, and at the rates of 7.18 % and 7.0 % in finisher diets, respectively. All experimental diets were iso-caloric and iso-nitrogenous and supplemented with exogenous enzymes (Avizyme 0.5g/kg and Phyzyme, 0.1 g/kg diet) as well as Zinc Bacitracin (0.5 g/kg). Titanium dioxide (TiO_2) was incorporated into each diet at a rate of 5.0 g/kg as an indigestible marker to enable assessment of nutrient digestibility.

3.2.3 Ileal digestibility

The ileal digesta samples were collected on day 21, pooled and frozen at -20 ^{o}C until further processing. The samples were freeze-dried, and ground for analysis of concentrations of protein, gross energy and starch in feeds, and freeze-dried ileal digesta were analyzed along with the indigestible marker, and these data were used to calculate the digestibility coefficient. The apparent digestibility coefficient for nutrients was calculated using the following equation:

$$\text{Digestibility coefficient} = 1 - \frac{digesta\ nutrient\ (g/kg) / digesta\ TiO_2\ (g/kg)}{diet\ nutrient\ (g/kg) / diet\ TiO_2\ (g/kg)}$$

3.2.4 Meat yield parameters

At the end of the trial (d 35), two birds per replicate were selected randomly, weighed and killed humanely to collect right tibia bones and different meat parts and offal (feather, neck, thigh, breast, drumstick, giblet, and abdominal fat) for evaluation of meat yield characteristics of individual birds, and later bone characteristics including weight, width, length, breaking strength, total bone ash and mineral (Ca, P, Mg, Mn, Zn, Cu, Fe) concentration.

3.2.5 Leg bone (tibia) strength

The right tibia bones, collected on day 35 were boiled for 10 minutes in deionized water, to remove all the soft tissues and defatted. Length and head width were then measured using digital callipers (Mitutoyo, Japan) and the weight was recorded. The bones were then placed horizontally between brackets 10 mm apart and the breaking strength was measured by positioning a 10 mm diameter compression rod against the bones and applying pressure (Lloyd, Hampshire, UK). After that, the entire bone was ashed at 550 ^{o}C for 4 h to measure the ash content. The ash was further analysed on inductively coupled plasma (ICP) spectrophotometry machine to determine the relative concentrations of the individual minerals, particularly those associated with skeletal development, namely, calcium, phosphorus, magnesium, manganese, iron, copper and zinc.

Table 3.1 Composition of starter diets fed during the trial period (d1-21)

	Diets			
	SBM	**CM**	**SMF**	**CMF**
Ingredient composition (g/kg)				
Maize	405.9	377.5	414.0	617.0
Wheat	210.0	187.5	203.8	0.0
Vegetable oil	0.0	20.0	0.0	0.0
Soybean meal	246.9	93.8	154.9	84.0
Canola meal	82.3	281.2	77.4	167.9
Fishmeal	0.0	0.0	77.5	84.0
Limestone	20.1	13.5	25.7	12.0
Dicalcium phosphate	18.4	10.9	30.0	14
DL-Methionine	2.0	1.4	2.2	1.5
Lysine	1.7	1.2	2.1	1.9
Sodium chloride	3.5	4.0	3.2	3.7
Vitamin-mineral premix[1]	2.5	2.25	2.5	2.5
Choline chloride	0.6	0.6	0.6	0.6
Avizyme 1502	0.5	0.5	0.5	0.5
Phyzyme XP	0.1	0.1	0.1	0.1
Zinc Bacitracin	0.5	0.5	0.5	0.5
Marker (TiO_2)	5.0	5.0	5.0	5.0
Nutrient composition (g/kg)				
ME (MJ/kg)	12.4	12.3	12.3	12.3
Crude protein	211.0	211.1	211.1	211.2
Crude fibre	31.0	37.0	28.0	34.0
Ether extract	24.0	28.4	29.3	32.2
Calcium	12.0	12.2	12.5	12.4
Available P	6.2	6.3	6.4	6.2
Sodium	2.0	2.0	2.2	2.1
Chlorine	2.5	2.7	2.7	2.5
Lysine	13.0	13.1	13.3	13.4
Methionine + cystine	8.2	8.2	8.4	8.3
Threonine	8.2	8.4	8.4	8.5
Arginine	14.2	14.1	14.0	14.1

[1]Provided per kg of diet (mg): vitamin A (as all-trans retinol), 3.6 mg; cholecalciferol, 0.09 mg; vitamin E (as d-α-tocopherol), 44.7 mg; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; vitamin B_{12}, 0.2 mg; biotin, 0.1 mg; niacin, 50 mg; D- calcium pantothenate, 12 mg ; folic acid, 2 mg; Mn, 80mg; Fe, 60 mg; Cu, 8 mg; I, 1 mg; Co, 0.3 mg and Mo, 1 mg.

Table 3.2 Composition of finisher diets used for the trial period (22-35 days)

	Diets			
	SBM	**CM**	**SMF**	**CMF**
Ingredient composition (g/kg)				
Maize	412.7	393.4	430.0	413.0
Wheat	200.0	200.0	210.0	230.0
Vegetable oil	0.0	21.2	0.0	0.0
Soybean meal	225.0	81.0	143.7	70.0
Canola meal	75.0	243.0	71.8	140.0
Fishmeal	0.0	0.0	71.8	70.0
Limestone	35.2	22.0	24.5	23.6
Dicalcium phosphate	34.5	21.1	30.4	35.7
DL-Methionine	1.4	1.5	1.7	1.6
L-Lysine	2.0	2.6	1.8	1.8
Sodium chloride	5.0	5.0	5.0	5.0
Vitamin-mineral premix[1]	2.5	2.5	2.5	2.5
Choline chloride	0.6	0.6	0.6	0.6
Avizyme 1502	0.5	0.3	0.3	0.3
Phyzyme XP	0.1	0.3	0.3	0.3
Zinc Bacitracin	0.5	0.5	0.5	0.5
Marker (TiO_2)	5.0	5.0	5.0	5.0
Nutrient composition (g/kg)				
ME (MJ/kg)	12.4	12.4	12.4	12.4
Crude protein	191.5	191.4	191.5	191.3
Crude fibre	28.0	36.5	28.0	33.0
Ether extract	24.0	28.0	26.1	28.0
Calcium	14.8	14.2	14.6	14.7
Available P	6.2	6.3	6.2	6.3
Sodium	2.3	2.4	2.7	2.7
Chloride	3.3	3.3	3.7	3.7
Lysine	12.3	12.0	12.4	12.4
Methionine+cystine	8.2	8.1	8.2	8.2
Threonine	8.0	8.0	8.1	8.0
Arginine	13.1	12.0	13.0	12.0

[1]Composition as in Table 3.1

Latency-to-sit (LTS) and gait-scoring tests

On day 28, leg bone development was assessed through LTS and gait-scoring tests. The LTS tests were performed according to the method of Berg and Sanotra (2003), with some modifications. Birds were tested in pairs and in luke-warm water. The test is based on the fact that bodily contact with water is an aversive experience for broiler chickens. Standing time is therefore positively correlated to the strength of leg bone.

Two birds per replicate were used to carry out this test. Four plastic tubs containing warm water (32 ^{o}C) at a depth of 3 cm were used (Plate 3.1). The water temperature was checked every 10 minutes with a thermometer to ensure the correct temperature was maintained, and it was checked again before another batch of birds was tested. Hot or cold water was added to raise or lower the water temperature if needed. Two birds from each replicate were selected randomly from the cage and put into the water tubs. The tubs were covered with mesh lids to prevent birds from flying out. The birds were observed and left to stand in the tubs of water for up to 15 minutes. The time elapsed before the birds first attempted to sit down in the water was recorded in seconds with the aid of a stopwatch. If any bird was still standing after 15 minutes, the test was terminated.

Plate 3.1: Latency- to- sit test

The gait-scoring test conducted followed the 3-point scoring system developed by Kestin *et al*. (1992) and Webster *et al.* (2008). To conduct the test two birds were randomly selected each replicate group, and allowed to walk freely on the floor. The birds were then scored by visual observation against a number of criteria as described in Table 3.3.

Table 3.3: 3-point scoring system

Gait scores	**Degree of impairment**	**Criterion**
1	None	Bird can walk at least 1.5 meter with a balanced gait. Bird may appear ungainly but with little effect on function.
2	Obvious impairment	Bird can walk at least 1.5 meter but with a clear limp or decidedly awkward gait.
3	Severe impairment	Bird will not walk 1.5 meter. May shuffle on shanks or hocks with assistance of wings.

3.2.6 Chemical analyses

Dry matter (DM) content

The dry matter content was determined gravimetrically following drying at 105 ^{o}C in a forced air convection oven (Qualtex Universal Series 2000, Watson Victor Ltd, Perth, Australia) for 24 hours for diets and bone, or drying at 80 ^{o}C for 48 hours for excreta or freeze drying (Martinn Christ freeze dryer, Germany) at -50 ^{o}C for 72 hours for digesta.

Gross energy, crude protein and ash contents

Gross energy contents of diets and ileal digesta were determined using an IKA bomb calorimeter (IKA-WERKE, C7000, and Staufen, Germany). The nitrogen contents of digesta and diets were determined according to the Dumas combustion technique as described by Sweeney (1989) using a LECO FP-2000 automatic nitrogen analyzer (Leco Corp., St. Joseph, MI, USA).

For ash contents, tibia bone, diets and ileal digesta were weighed accurately and placed into crucibles, then ashed at 550 ^{o}C for 4 h in a Carbolite CWF 1200 chamber furnace (Carbolite, Sheffield, UK). Furnace temperature was set at 200 ^{o}C initially and then raised to the target temperature after one hour. The residue was weighed and crude ash content was calculated.

Mineral analysis

Based on different digestion systems, nitric acid, hydrochloric acid and perchloric acid were added to analyse for mineral contents in diets, excreta, digesta, and tibia bone ash. Diluted samples and the standard solution were separately put into a set of fresh tubes for spectroscopy (ICP) (Vista MPX, Melbourne, Australia). Copper, Fe, Mn, Zn, Ca, P and Mg were measured at 327, 238, 257, 213, 616, 231, 279 nm wavelength, respectively.

For bone ash, approximately 0.2 g was placed into a Schott bottle in a scrubbed fume cupboard. Two millilitres of a mixture of $HClO_4$ (70 %) perchloric acid and H_2O_2 (30 %) were added to each tube. Each tube was loosely covered with a lid and set overnight. Then one ml of H_2O_2 was added and tubes were tightly sealed and placed in an oven set at 80 ^{o}C for 30 minutes. The bottles were allowed to cool slightly and a further 1 mL H_2O_2 was added before they were capped tightly and digested for 1 hour at 80 ^{o}C. The solution was made to a volume of 25 mL by adding distilled water and filtered through Whatman No.1 filter paper for ICP analysis (Anderson and Henderson, 1986).

Titanium dioxide (TiO_2) analysis

The TiO_2 content of the ileal digesta and diet samples was measured as per the method of Short *et al.* (1996). Around 0.1g of the freeze-dried digesta or feed samples was first ashed in a porcelain crucible for 13 hours at 580 ^{o}C and then dissolved in 5 mL of 7.4 M sulphuric acid upon cooling. The samples were then boiled on a heating plate for 20 minutes at 200 ^{o} C and then for another 20 minutes at 250 ^{o}C until completely dissolved. After cooling, the solution was transferred quantitatively into a 50 mL volumetric flask through a filter paper (Whatman 541, hardened ashless, 90 nm ø Cat No. 1541 090, Whatman International Ltd Maidstone, England). After that, 10 mL of hydrogen peroxide (30 %, v/v) were added to each flask and the contents diluted up to 50 mL with Mili-Q water and mixed properly by inversion to eliminate bubbles. The solutions in the flasks turned a typical orange colour the intensity of which was proportional to the concentration of $TiO_{2.}$ An aliquot of the solution was obtained and, along with a similarly prepared standard solution was analyzed using a Hitachi 150-20 UV spectrophotometer (Hitachi Science System Ltd., Ibaraki, Japan) by measuring the absorbance at 410 nm. The TiO_2 content was determined from the standard curve and converted to mg/g of the sample.

Total starch assay

The starch contents of the ileal digesta and feed samples were determined using a Megazyme total starch kit (AMG/AA 05/2006) based on the method developed by McCleary *et al*. (1994). Finely ground samples (0.5 mm), approximately 100 mg, were weighed accurately into screw-capped reaction tubes (30 mL) and soaked with 0.2 mL 80 % ethanol and 2 mL DMSO. A further 3 mL of thermostable α-amylase (3000 U/mL; 45 U/mg at pH 6.0, Megazyme) in MOPS buffer (50 mM, pH 7) were added, followed by 0.1 mL amylo-glucosidase (3300 U/mL on soluble starch at pH 4.5, Megazyme) and incubated at 50 ^{o}C for 1 hour. Glucose released was determined calorimetrically after incubating an aliquot (0.05 mL) with 2.2 mL of GOPOD reagent (Megazyme) at 50 ^{o}C for 20 minutes and reading the absorbance at 510 nm against a reagent blank.

Determination of excreta moisture, pH and ammonia concentration

The excreta samples collected on d14 of the experiment were used to assess litter quality. Excreta samples were collected from each replicate group of birds into an aluminium foil set on the excreta trays. Collected excreta samples were stored at -20 ^{o}C prior to analyses.

Excreta moisture content was determined according to the method described in AOAC (1994). The pH of excreta samples was measured using a pH meter (Model LS, Sargent-Welch Co., Springfield, N. J). Five grams of each excreta sample was placed into 50 mL plastic tubes, 40 mL Mili-Q water were added and then the samples were homogenised using a homogeniser for two minutes (11000 rpm). After that, the samples were filtered through Whatmann-1 and diluted. The filtrate was used for pH and ammonia measurements.

Excreta ammonia was measured as per the technique of an ammonia assay kit (Catalogue Number AA0100), (Sigma-Aldrich, 3050 Spruce Street, Saint Louis, Missouri 63103, USA). Around 0.1 to 0.2 mL diluted excreta sample was placed into cuvettes and mixed thoroughly with 1 to 2 mL ammonia assay reagent, and then incubated for 5 minutes at 18-35 ^{o}C. After that, absorbance was read at 340 nm against blank samples similarly prepared with 0.1 mL water mixed with 1.0 mL ammonia assay reagent. After this reading, samples in each cuvette were mixed with 0.01 mL of L-Glutamate dehydrogenase solution (Catalogue Number-G2294) and incubated, as before, at 18-35 ^{o}C for 5 minutes. The absorbance of each solution was measured again at 340 nm. The concentration of ammonia (mg/mL) was calculated from the following equations:

Calculation:

ΔA_{340} for the reagent blank, Test and standard. For each: $\Delta A_{340=} A_{initial} - A_{final}$

Δ (ΔA_{340}) Test or standard

= ΔA_{340} (Test or standard) - ΔA_{340} (Blank)

=NH_3 (mg/ml) of original sample

$$= \frac{(A)(TV)(17)(F)X0.00273}{(SV)}$$

where, A=Δ (ΔA_{340}) Test or standard; TV=Total assay volume in mL; V=sample volume in mL; MW (Molecular weight) of Ammonia=17g/mole or equivalently 17 μg/μmole; F=dilution factor from sample preparation; SV=Millimolar extinction co-efficient for NADPH at 340 nm=1/cm; D=Light path (cm)=1 cm.

3.2.7 Statistical analysis

Statistical analyses were performed using Minitab software (Minitab version 14, 2000). The data were analyzed using a one-way ANOVA with diet as independent factor. The significance of difference between means was determined by Fisher's least significant difference at $P \leq 0.05$.

3.3 RESULTS

3.3.1 Gross response and mortality

Results shown in Table 3.4 indicate that birds on the SMF diet consumed a greater ($P<0.001$) quantity of feed (1344.2 g/b) than birds fed the VP diets during the period of1-21d. Feed intake was similar between the groups during the first and last week. Live weight gain was greater for the birds fed on the AP diets (SMF; CMF) than on the VP (CM; SBM) diets during d1-7 ($P<0.001$), d1-21 ($P<0.001$), and for the overall period ($P<0.05$). Feed conversion ratio (FCR) up to 35 days of age was superior on the CMF diet while the birds in the CM diet group were the least efficient. Birds of CMF diet group had similar FCR values to the birds of SMF diet over 35 days, and this differed significantly ($P<0.05$) from FCR on the VP diets. The FCR on the SBM, CM, SMF and CMF diets were 1.80, 1.84, 1.73 and 1.72, respectively, over 1-35d. Mortality during the entire experimental period was similar with no significant ($P=0.16$) differences between treatments. During d1-21, there was a mortality rate of 0 % in the CM diet group, 6.2 % in the SBM, and 4.6 % in the SMF and CMF groups. Over 1-35d, mortality was 10.9, 6.2, 10.9 and 12.5 % in the SBM, SMF, CM and CMF groups, respectively.

3.3.2 Excreta moisture, pH and ammonia contents

The excreta from birds on the VP diets had significantly ($P<0.05$) higher excreta moisture levels (78.3; 77.6) than excreta from the birds on the AP diets (Figure 3.2). Although the excreta pH values and the concentration of ammonia in the broilers fed the VP diets were higher than those of the birds on the AP diets, these differences were not significant ($P>0.05$).

3.3.3 Ileal digestibility of nutrients

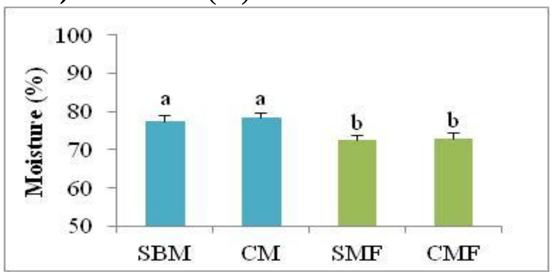
Protein digestibility was significantly (P<0.05) higher on the AP diets than the VP diets (Figure 3.3). Broilers fed SMF and CMF diets showed a similar trend in protein digestibility, whereas the digestibility of VP (SBM; CM) diets was also similar. Gross energy (GE) and starch digestibility was not significantly (P>0.05) affected by the dietary treatments.

Table 3.4 Growth responses of broiler chickens fed on different diets from d1 to 35 days

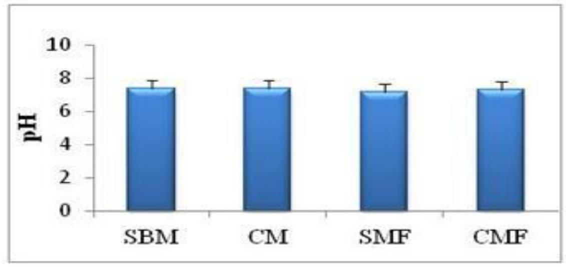
	Age (days)	**Treatments**				**SEM**
		SBM	**CM**	**SBF**	**CMF**	
Feed intake (g/b)	1-7	135.4	136.8	149.4	145.1	2.07
	1-21	1154.7^{c}	1252.7^{b}	1344.2^{a}	1290.4^{ab}	13.36***
	1-35	3677.7	3879.4	3856.6	3840.6	48.86
Live weight gain (g/b)	1-7	113.1^{b}	104.0^{b}	132.9^{a}	123.0^{a}	2.03***
	1-21	762.9^{b}	784.0^{b}	900.6^{a}	909.6^{a}	12.28***
	1-35	2037.4^{b}	2110.5^{b}	2219.1^{a}	2228.3^{a}	24.56*
FCR	1-7	1.20^{b}	1.31^{a}	1.12^{c}	1.18^{bc}	0.017*
	1-21	1.51^{b}	1.59^{a}	1.50^{b}	1.42^{c}	0.020*
	1-35	1.80^{a}	1.84^{a}	1.73^{b}	1.72^{b}	0.017*
Mortality (%)	1-7	1.5	0.0	1.5	0.0	0.55
	1-21	6.2	0.0	4.6	4.6	1.00
	1-35	10.9	6.2	10.9	12.5	1.00

Data represent mean values of eight replicate groups consisting of eight birds per replicate during 1-21 days, and four birds per replicate during the period 21 to 35 days of age; [a,b,c] Means within a row bearing unlike superscripts are significantly different at *P<0.05 and *** P<0.001; SEM= Pooled standard error of means.

a) Moisture (%)



b) pH



c) Ammonia (mg/L)

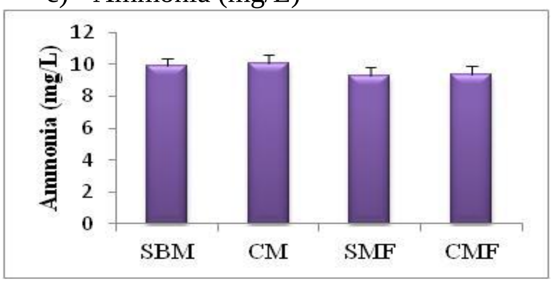
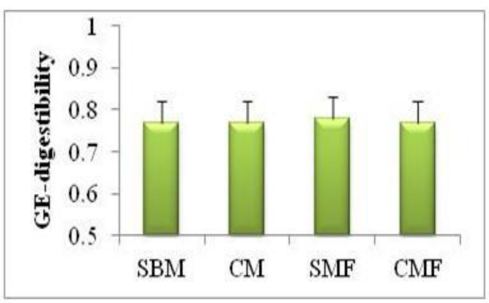
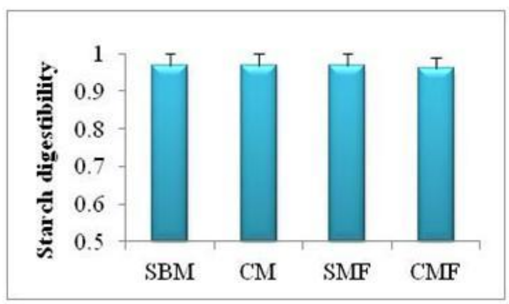


Figure 3.2: Moisture, pH and ammonia contents of excreta material of broilers fed on test diets to 14 days of age; Bars bearing different letters are significantly different (*P<0.05).

a) Gross energy (GE)

b) Starch





c) Protein

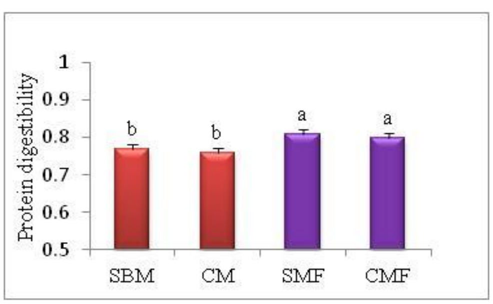


Figure 3.3: Ileal digestibility of gross energy (GE), starch and protein by chicks on test diets at 21 days; Bars not having similar superscripts are significantly different (*P<0.05).

3.3.4 Leg bone strength and gait score

The results of latency-to-sit and gait-scoring tests are presented in Figure 3.4. Birds fed on the AP (SMF and CMF) diets spent the longest (P<0.05) time (14.58 and 13.26 minutes, respectively) standing in the water, while birds on the SBM diet spent the shortest time (8.84 minutes). Statistically similar standing times (14.58 and 13.26 minutes, respectively) were observed in the two AP diet groups. There were no significant (P>0.05) differences in the gait scores of the different dietary groups. However, comparatively higher scores (1.56, 1.56) were recorded on the VP (SBM and CM) diets than the value (1.31) recorded for both AP diets (SMF and CMF).

a) Gait score b) Latency-to-sit (LTS)

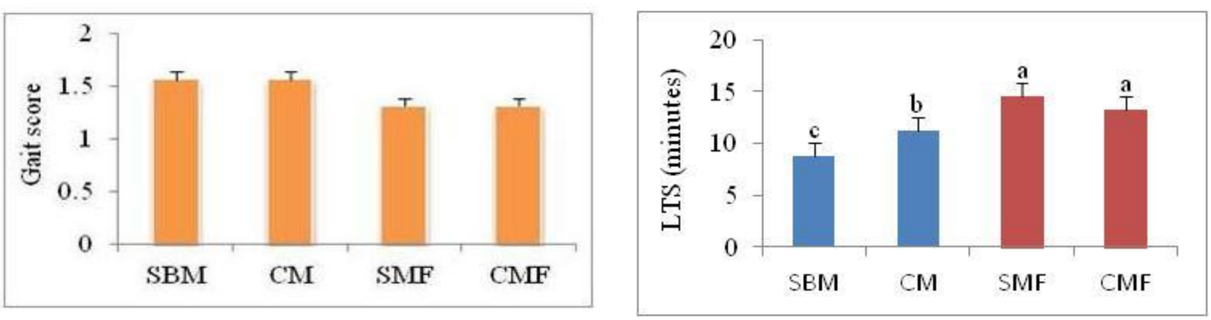


Figure 3.4: Latency-to-sit (minutes) and gait-score for different test diets; Bars bearing unlike superscripts are significantly different (*P<0.05).

3.3.5 Meat yield

Results of meat yield parameters shown in Table 3.5 demonstrate that only the abdominal fat content was significantly (P<0.05) affected by the dietary treatment. The highest abdominal fat content (0.92 g) was found in birds on the CMF diet and the lowest abdominal fat content (0.72 g) was on the SBM diet. Broilers fed on animal protein (SMF; CMF) diets accumulated similar fat content in their carcasses. Other meat characteristics such as, dressing percentage, and weights of giblet, breast, drumstick, thigh, shank, neck and feather were not significantly different between treatments.

3.3.6 Leg bone characteristics and bone mineral concentrations

The results of bone characteristics (length, weight, width, breaking strength), and total tibia bone ash and bone mineral concentrations are presented in Table 3.6 and Table 3.7. Birds on the CMF diet had the longest ($P<0.05$) tibia bone (67.1 mm) while SBM were the shortest (63.0 mm). Lengths for the other dietary groups were 66.0 and 65.0 mm in the SMF and CM groups, respectively. Similarly, birds on SMF had significantly ($P<0.05$) highest bone breaking strength (31.3 kg). The lowest bone strength (21.5 kg) was recorded in birds fed on the SBM diet (Table 3.6). Statistically similar bone strengths of 31.3, 30.0 kg and 28.2 kg were found in the birds fed on the SMF, CMF and CM diets, respectively. Bone ash content on CMF diet group were significantly ($P<0.001$) the highest (1.69 g/b) while the birds on SBM diet group had the lowest (1.51 g/b) bone ash. Macro-mineral (Ca, P, and Mg) contents on different treatments were identical. Of the micro-mineral contents, only Fe and Cu were significantly ($P<0.05$) different between the diet groups, with the highest amount of Fe (2.86 μg) and Cu (0.048 μg) in the CMF diet group, while the CM and SBM diet groups contained the least amounts 2.29 and 0.030 μg, respectively. Statistically similar Cu contents of 0.03, 0.03 and 0.03 μg were found in the bones of birds fed on SBM, CM and SMF diets, respectively. The Fe content of the CM diet group of birds was also similar to the birds of SMF diet group.

Table 3.5 Carcass traits and meat yield (g/100g body weight) of broilers

	Treatments				**SEM**
	SBM	**CM**	**SMF**	**CMF**	
Feather	4.4	4.0	5.4	5.1	0.29
Dressing percentage	74.6	74.5	74.0	73.9	0.38
Breast	25.0	22.5	23.4	23.0	0.35
Drumstick	9.0	8.7	8.6	8.8	0.14
Thigh	10.6	10.2	10.0	10.1	0.17
Abdominal fat	0.7^{c}	0.8^{b}	0.9^{a}	1.1^{a}	0.04
Giblet	5.7	5.2	5.4	5.3	0.09
Shank	2.6	2.3	2.4	2.5	0.05
Neck	2.8	2.7	2.8	2.8	0.04

Data represent mean values of two birds of eight replicate groups taken at the end of 35 days trial period ; $^{a, b, c}$ Means within a row bearing unlike superscripts are significantly different (* P<0.05).

Table 3.6 Bone (right tibia) traits and quality of broilers fed on test diets

	Treatments				SEM
	SBM	**CM**	**SMF**	**CMF**	
Length of bone (mm)	63.0^{c}	65.0^{ab}	66.0^{a}	67.1^{a}	0.37
Weight of bone (g/kg body weight)	3.5	3.4	3.6	3.7	0.07
Width of bone (mm)	11.5	11.3	12.0	11.6	0.12
Breaking strength (kg/bone/bird)	21.5^{c}	28.2^{ab}	31.3^{a}	30.0^{a}	1.05

Length and width are measured in millimetre per bone per bird basis; Data represent mean values of two birds obtained by slaughtering at 35days; $^{a, b, c}$ Means bearing uncommon superscripts in a row are significantly different (*P<0.05).

Table 3.7 Mineral concentration of bone ash (μg/g bone ash) of broilers fed on test diets

	Treatments				**SEM**
	SBM	**CM**	**SMF**	**CMF**	
[1]TTA	1.51^{c}	1.57^{b}	1.64^{a}	1.69^{a}	0.009***
Ca (g/kg bone ash)	236.3	243.6	246.6	246.5	3.50
P (g/kg bone ash)	111.0	112.3	113.2	114.3	1.52
Mg (μg)	47.6	46.8	45.9	47.0	0.62
Mn (μg)	0.06	0.06	0.06	0.05	0.002
Zn (μg)	3.4	2.7	2.8	2.8	0.13
Fe (μg)	2.8^{b}	2.3^{c}	2.4^{c}	2.9^{a}	0.07*
Cu (μg)	0.03^{b}	0.03^{b}	0.03^{b}	0.05^{a}	0.001*

[1]**TTA**-Total tibia ash (mg/g bone weight); Data represent mean values of two birds obtained by slaughtering at the end of the trial (35days); $^{a, b, c}$ Means bearing uncommon superscripts in a row are significantly different at *P<0.05, and ***P<0.001.

3.4 DISCUSSION

3.4.1 Gross response of broiler chickens

From the current study it is clear that the gross response of broilers fed on fishmeal supplemented diets (CMF; SMF) were better than those fed on the sole VP diets. Birds fed on AP diets attained significantly higher body weight gain, and better feed efficiency throughout the trial period. The results suggest that the positive response to AP diets was mainly due to increase in feed intake at mid growing stage (21d). A similar increase in feed intake by broiler chickens fed on AP diets was reported by Bhuiyan *et al.* (2012 a, b) without any improvement in growth. The difference in growth response between the studies might be due to variation in feed composition and formulation. Furthermore, uses of different protein sources in the formulated diets might be other reasons for affecting growth response of the birds.

However, the improved growth responses might be due to increase feed intake of broilers fed on AP diets compared to VP diets. Apart from this, other factors, for example, increased protein or amino acid digestibility, better amino acid balance, lower anti-nutritive effects, combination of different protein sources, the level of inclusion, and the amino acid profile of the protein mixtures used in the practical diets might be responsible for this growth response of birds. The results agree with the findings of previous researchers (Radhakrishnan *et al.*, 2001; Karimi, 2006; Alali *et al*., 2011; Hossain *et al*., 2012a,b) who found similar performance with inclusion of various sort of animal proteins in vegetable–based diets.

The results of the current study further indicate that the beneficial effect of AP diets on broiler performance becomes more obvious at higher levels during the early phase of the growth period, with the greatest benefit manifesting between 1 and 21d on weight gain, mainly via stimulation of higher feed intake during this period. Several researchers have concluded that fishmeal contains unidentified growth factor. This factor improves the palatability of fishmeal containing diets which, when fed to broilers, results in an improvement in growth (Pierson *et al*., 1979; Barlow and Windsor, 1984; EI Boushy and van der Poel, 1994). It might be possible that the higher growth response to the AP diets may be due to the synergistic effect of the balance of animal and vegetable proteins, and dietary amino acids resulting from the addition of fishmeal to the AP/regular diets. This supposition is supported by several researchers, including Woodham and Deans (1977) and Pike (1979). Dublecz (2003) reported that fishmeal is basically devoid of anti-nutritive factors, is balanced in amino acids, and has higher biological value than vegetable proteins. These traits of fishmeal might influence broilers to grow more rapidly through efficient utilization of feed than on diets without fishmeal. Conversely, performance of birds fed on VP diets was poorer than AP diets. The reasons behind this might be the presence of anti-nutritive factors, amino acid imbalance, lower biological value and or poor digestibility of the nutrients.

The FCR was improved on the AP diets compared to the VP dietary groups in this study. The improved FCR of birds might be due to better protein digestibility of the AP diets as well as increased efficiency in utilization of energy, as recently reported by Hossain *et al.* (2012a,b) in a trial on similar diets. Our findings are in agreement with Solangi *et al*. (2002) and Alali *et al*. (2011), who reported that fishmeal and or other animal protein meal increase feed intake and also enhance the feed efficiency of the broilers. Singh and Panda (1990) reported that fishmeal contains all indispensible amino acids in adequate amounts, particularly lysine and methionine needed for poultry, and is considered as a standard feed ingredient in pig and poultry diets (NRC, 1994; Pike, 1999).

3.4.2 Excreta moisture, pH and ammonia

The excreta of broilers fed the VP diets contained significantly higher moisture than that of the AP diets. The moisture levels of the excreta of broilers in the VP groups were 5.21 % higher than levels in the AP diet groups. Many dietary factors such as protein level, electrolytic balance, ionophores, cereal contents, fibre content, legumes and NSPs are associated with regulation of excreta/litter moisture and quality (Fleet and Saylor, 1983; Smith *et al*., 2000; Murakami *et al*., 2001and Francesch and Brufau, 2004).

Any dietary change that increases water intake of birds will lead to an increase in excreta moisture level. The NSP contents of VP diets arising from soybean and canola meals can affect digestion of other nutrients by attracting water and contributing to higher litter moisture (Francessch and Brufau, 2004). Furthermore, vegetable ingredients, particularly soybean meal, can have up to four times more potassium than the animal by-products used in poultry feeds (NRC, 1994; Viera and Lima, 2005). Potassium is known to increase water intake and increase moisture of excreta (Murakami *et al*., 2001), and thus result in sticky litter. In addition, the carbohydrate fraction of soybean meal is poorly digested (Parsons *et al*, 1980; Pierson *et al*., 1980) and may serve as a substrate for increased bacterial activity in the litter. The current findings supported the results of Vieira and Lima (2005), Takahashi *et al*. (2004) as well as Eichner *et al*. (2007), who reported that broilers fed diets formulated with all-vegetable ingredients produce a higher amount of moisture and ammonia in their excreta. This excess moisture affects the litter quality adversely and leads to an increased risk of development of footpad dermatitis and other diseases. Conversely, the lower moisture content in the excreta of birds fed on the AP or regular diets containing fish meal may be attributed to better osmotic regulation. Fishmeal contains some specific nutrients, e.g. taurine (Comb, 1998) that can regulate the osmotic pressure of birds (Park and Choi, 1997).

3.4.3 Ileal digestibility

Ileal protein digestibility on the VP diets was lower than on the AP diets. This finding supports the report of Vieira and Lima (2005). This may partly be responsible for the better growth of broiler chickens on the AP diets observed in this study. The poor protein digestibility of VP diets may be due to various factors, including the presence of ANF, the nature of the protein and amino acid balance as well as a higher proportion of crude fibre of vegetable feeds (Hossain *et al.*, 2011b). No two protein sources are similar in properties such as mode of digestibility, biological value, protein quality, physical and chemical properties, etc. (Singh and Panda, 1992). These characteristics of individual protein sources might affect the protein digestibility and performance of birds when used in feed formulation. However, several researchers (Gatel, 1994; Smits and Annison, 1996) reported that the major factor limiting the use of vegetable proteins in practical diets is the existence of naturally occurring deleterious factors, including NSP, tannins and trypsin inhibitors, which have adverse effects on nutrient digestibility and absorption. Although properly processed oilseed meals may have little or no residual ANF, the complex structure, physical and chemical properties of all-vegetable diets might prevent the birds from getting full nutritional benefits by suppressing their nutrient digestibility and energy utilization.

Vegetable protein ingredients are particularly high in trypsin and chymotrypsin inhibitors, which interfere with the digestibility of dietary protein. Besides, these sources have a higher level of lectins which have the ability to bind to the epithelial cells lining the small intestine and thus interfere with nutrient absorption (Liener, 1989). Canola contains tannins, which form complexes with dietary protein and carbohydrates as well as inhibit the activity of various digestive enzymes and, to a lesser extent, glucosinolates, a precursor of toxic breakdown products produced by myrosinase activity (Warenham *et al.,* 1994). In this study, ileal energy digestibility of broilers of all dietary groups was similar. A possible reason for this may be due to the fact that similar cereals (maize and wheat) constituted the major proportions of the basal diets.

3.4.4 Leg bone development and strength

The results of the LTS test more clearly confirmed better leg bone development in birds on the AP diets. Berg and Sanotra (2003) have found dissimilar results between the LTS and gait scoring, the latter being more subjective than the former. For the LTS results, it may be assumed that birds fed on AP diets had better leg development and health than birds fed on VP diets. Poor leg health of broilers on the VP diets might have resulted from the phytate content of the plant feeds, although these diets were supplemented with microbial phytase. The anti-nutritive properties of phytate can not be eliminated completely by phytase supplementation, because degradation of phytate is incomplete (Selle and Ravindran, 2007). The lack of endogenous phytases makes phytate phosphorus unavailable to monogastric animals. The benefit of such enzyme supplementation are further examined in Chapter 5.

3.4.5 Meat yield

Most of the meat yield traits were similar and the differences between the treatment groups were non-significant. These findings support the report of Al-Masri (2003), who observed no significant differences in the various carcass traits of broiler chickens fed with VP diets and those fed a conventional diet with meat and bone meal as animal protein. The difference in the abdominal fat content observed in this study was similar to the findings of previous researchers who evaluated diets containing animal proteins (Takahashi *et al*., 1984; Janocha and Milczarek, 2006). The probable reason for higher abdominal fat accumulation in the AP dietary groups may be more rapid growth, leading to precocious maturation of birds.

3.4.6 Bone characteristics and mineral concentration

One of the common traits of bone breaking is the bending moment, which is a measure of the amount of force the bone can withstand (Crenshaw *et al*., 1981). This is usually related to bone mineralization, although there were no significant differences in the macro-mineral concentration from different diet groups. However, total bone ash contents of the birds on AP diets were found to be significantly increased compared to values in vegetable protein diets in this study. The increased length, width and weight of bones of the individual birds of each treatment group might contribute to higher bone ash accumulation in the broilers fed the AP/regular diets. Apart from this, despite non-significant differences of bone macro-mineral concentration of broilers between treatments, the concentrations of major minerals, namely Ca and P, which are closely associated with bone or skeletal development, were found in comparatively higher amounts in the bones of broilers fed on AP compared to those fed on the VP diets. Conversely, the concentrations of Mg and Mn were found to be slightly higher in birds on the VP diets than those on the AP diets. However, it should be pointed out the two minerals are only required in trace amounts and make less contribution to bone development than Ca and P. The concentrations of Cu and Fe contents of the animal protein (CMF) diet were also significantly higher than in the other groups.

The increased mineral contents might be a result of higher tibia ash content of AP diets as compared to the VP groups. Trace mineral (e.g. Cu, Fe) deficiency inhibits bone growth and decreases bone strength even with adequacy of macro-minerals (Ca and P) in the practical diet (Medeiros *et al.,* 1997). So these increased micro-mineral (Fe and Cu) contents of CMF animal protein diet might stimulate bone growth and slightly enhance the bone strength.

3.5 CONCLUSION

The present study showed that broiler chickens fed on fishmeal or AP diets had comparatively better performance in terms of live weight gain, feed intake and FCR than those on VP diets. In addition, the ileal digestibility of proteins and bone development were better on the former diets than on the latter. Chicks on the regular (AP) diets also maintained good litter quality but were fattier than those on VP diets. It may be inferred from the results of this experiment that broiler chickens fed on VP diets supplemented with fishmeal in different combinations might, for a variety of production criteria, be more beneficial than rearing them on VP diets only. Further studies are required to define the energy utilization of birds on the two sets of diets, and possibly improve the nutritional value of the VP diets.

CHAPTER 4 ENERGY UTILIZATION AND PERFORMANCE OF BROILER CHICKENS ON VEGETABLE PROTEIN DIETS

ABSTRACT

This study was undertaken to assess the gross response and energy utilization of broiler chickens fed on vegetable protein (VP) and animal protein (AP) diets. To investigate this, a total of 204 day-old Cobb-500 male broiler chicks were randomly assigned to four experimental groups and raised on AP diets (SMF; CMF) containing fish meal or VP diets (SBM; CM) with main source of vegetable proteins (soybean or canola meals). Results showed that feed intake was highest (P<0.001) on the AP diets, while the vegetable protein (SBM) diet was the lowest. Birds in the AP diets were heavier (P<0.001) than the VP diets. Birds on AP diets achieved superior FCR, while birds on CM vegetable protein diet were the poorest. There were no significant effect (P>0.05) of treatments on the mortality of birds. The dietary apparent metabolizable energy (AME) contents were similar, but ME intake on the AP diets was significantly (P<0.001) higher than in the VP. Fat intake was highest (P<0.001) on CMF animal protein diet while protein intake was unaffected (P>0.05) between treatment. Heat production (HP) was identical, but net energy of production (NEp) was significantly (P<0.05) improved in the birds on AP diets. Whole body energy as well as fat (P<0.05) and protein contents were significantly increased (P<0.01) in the AP diet groups as well. Birds on AP diet groups retained significantly (P<0.05) higher energy as fat (RE_f), while energy retention as protein (REp) was also highest (P<0.01) on the same diets (SMF; CMF). The efficiencies of utilization of ME for energy (k_{RE}), protein (k_{REp}) and fat (k_{REf}) retentions were unaffected. The results demonstrated that birds on the AP diets utilized energy better, and as such grew faster than the birds on VP diets.

4.1 INTRODUCTION

Feed costs represent as major part of costs (about 70 % of variable costs) in poultry production, with dietary energy sources occupying the greatest portion (70 to 75 %) of the diets (Vander Klis *et al.*, 2010). Birds tend to eat feeds mainly to satisfy their energy requirements and once this is met, they will not consume any more feeds, even if the requirements of other nutrients like protein, vitamins or minerals have not been met (Singh and Panda, 1992). For this reason, the energy content of the diets represents the core item in diet formulation for poultry. Most of the dietary energy comes from plant sources in the form of carbohydrates, from cereal grains. These cereal grains provide the major energy which accounts for 60 to 70 % of the nutrient requirement for poultry. Protein is supplied by plant and animal sources. Plant sources are cheaper and safer than animal sources but the former often lack nutrient balance. This may affect the utilization of nutrients and growth of birds on such diets. Protein sources may also supply a substantial amount of energy and their interaction with the main energy sources has a bearing on the overall energy supply and utilization. So, it is important to determine the energy value of diets containing vegetable protein sources. The performance of birds is closely associated with feed nutrients and energy utilization, which is primarily related to availability of more nutrients and energy from the feed ingredients (Olukosi *et al*., 2008).

In poultry research studies, metabolizable energy (ME) and net energy (NE) are usually used to measure energy availability to and utilization by the birds. The ME can be accurately determined from the difference between the gross energy of the feed and the gross energy of excreta derived from such feeds (NRC, 1994). Metabolizable energy has been commonly accepted and extensively used to compare energy values of feedstuffs, and diets for poultry, and energy requirements are commonly expressed in this form. Net energy is a more accurate measure of energy utilization, as it measures the amount of gross energy that is used for productive purposes. It can be measured in a number of ways, including direct and indirect calorimeter which are expensive.

There are a number of constraints in VP sources, which reduce their optimal utilization, thus limiting the benefits of these feeds to broiler chickens. This results in reduced digestibility and poor performance of the birds. From the results obtained in Chapter 3, it was shown that the nutrient digestibility and overall performance of the broiler chickens fed on VP diets were comparatively poorer than those of birds fed on AP/conventional diets.

The current study was designed to provide some mechanisms for these differences. Apart from direct measurements of energy intake and retention, the deposition of fat and protein were assessed. This information is required to understand the partitioning of retained energy in the body between fat and protein, and the utilization of ingested ME by broilers for this purpose (Lopez and Leeson, 2005). The objectives of the present study were to determine the energy intake and utilization from VP diets; evaluate protein and fat retention, and assess the efficiency with which fat and protein are used for depositing energy.

4.2 MATERIALS AND METHODS

4.2.1 Experimental design and bird management

A total of 204 day-old Cobb 500 broiler male chicks (46.65±0.21g) were used in this study. At one day old, 192 chicks were allocated in a completely randomized design into four dietary treatment groups, with 48 chicks in each group. Each treatment had six replicates, with 8 birds per replicate cage. The remaining 12 birds were used for the initial slaughter group, to provide basal compositional data. The second group of 192 birds was slaughtered at 21 days, to enable measurement of nutrient retention.

A total of 24 brooder cages were set up on the floor in two climate-controlled rooms (12 cages in each room); and after 17 days, four birds from every pen were transferred to metabolic cages in order to collect excreta samples during the later part of the trial. Each pen was equipped with two troughs, one for feed and the other for water. Wood shaving was placed to a depth of 2 c.m as litter. The birds were brooded at 33 ^{o}C for the first two days, and then the temperature was reduced gradually to 24 ^{o}C at 19 days of age, and maintained at this level to the end of the trial. Feed, in pellet form, and water were provided *ad libitum.* The birds were on a starter diet for the duration of the trial.

Body weight and feed intake data of the birds were recorded weekly. Mortality was recorded as it occurred. Feed conversion ratio (FCR) was calculated weekly, and corrected for mortality. On d19, 20 and 21, excreta samples were collected from each cage and pooled. The excreta were immediately frozen at -20 ^{o}C before being dried (80^{o}C for 8 hours) in an oven to a constant weight. Excreta were pooled within each pen and ground prior to analyses. At the end of the trial, two birds per replicate cage were killed by cervical dislocation and processed as described below. All animal care, handling and management procedures of this experiment were approved by the UNE Animal Ethics Committee (Approval No: AEC10/076).

4.2.2 Dietary treatments

Four experimental diets (starter), coded as SBM, CM, SMF and CMF were formulated as shown in the previous Chapter 3 (Section 3.2.2), and these diets were used for the birds of this trial from hatch to 21 days.

4.2.3 Growth responses and chemical analyses

Feed intake (FI), live weight (LW), FCR and survivability were measured as described above in section 4.2.1. The starter diets were analysed for nutrient composition. Excreta and diet samples were analysed for gross energy in order to determine the ME. Samples were dried at 105^{o}C in a drying oven (Precision Scientific Co., Chicago, IL, USA) for 24 h for DM determination. Gross energy was determined in a bomb calorimeter (Parr 1261; Parr Instruments Co., Moline, IL, USA) using benzoic acid as a calibration standard. Titanium dioxide concentrations in the diets and excreta samples were measured according to the method of Short *et al*. (1996), as described in previous chapter (Section 3.2.6). At 21 days, two birds per replicate were killed and the whole intact carcasses were frozen immediately and later processed. Both chicks from the same cage were processed together. After chopping and coarse-grinding individual chickens, they were thoroughly mixed and two subsamples (approximately 200 g each, wet weight) were taken, finely ground and freeze-dried as described by Olukosi *et al*. (2008). The two subsamples were mixed together after drying and ground again, from which a smaller sub-sample was taken for chemical analysis. The ground carcass samples were analysed for gross energy (GE), diethyl ether extractable fat (EE) and nitrogen (N).

Nitrogen content of the diets and meat samples was determined according to the Dumas combustion technique as described by Sweeney (1989) using a LECO® FP-2000 automatic nitrogen analyser (Leco FP analyser model 602600; Leco Corp., St Joseph, Michigan, USA) with EDTA as a calibration standard. Crude protein (CP) equivalent of the ingredients was calculated as N (%) × 6.25. The EE was determined indirectly by the Soxhlet method for fat extraction. Around 6-8 g of finely ground sample was weighed into pre-weighed filter paper (N^{o}1 Whatman 185 mm) and extracted for 48 to 50 hours with chloroform, using a Soxhlet apparatus. After that, the samples were allowed to drain and dry at 80^{o} C for 72 hours. The EE was calculated as loss in weight and expressed as a proportion of dried sample weight.

To calculate the nutrient retention and other variables the following formulae were used:

$$ME\ (MJ/kg) = GE_i - [GE_o \times (T_i/T_o)]$$

where, GE_i is gross energy (MJ/kg) in feed; GE_o is the gross energy (MJ/kg) in excreta, T_i is the concentration of titanium dioxide in the diets; and T_o is the concentration of titanium dioxide in the excreta.

Net energy of production (NE_p) was calculated as follows:

Initial GE of carcase (kj) = carcase GE (kj/g) × body weight of bird (g) --- (1)

Final GE content of carcase (kj) = carcase GE (kj/g) × body weight of bird (g) --- (2)

NE_p (kj) = (2) - (1)

Heat production (HP), which consists of the heat increment of feeding and fasting HP, was calculated as the difference between NEp and ME intake:

$$HP\ (kj) = MEI - NE_p$$

where, ME intake (MEI) was calculated using the following formula:

$$MEI\ (kj) = ME\ (kj/g) \times \text{feed intake (g)}$$

Energy retained as fat (RE_f) and as protein (RE_p) were calculated as follows:

$$RE_f\ (kj) = \text{Carcase fat (g)} \times 38{\cdot}2\ kj/g$$

$$RE_p\ (kj) = \text{Carcase crude protein content (g)} \times 23{\cdot}6\ kj/g.$$

The values 38·2 and 23·6 kj/g are energy values per gram of fat and protein, respectively, as derived by Larbier and Leclercq (1992):

$$\text{Efficiency of ME use for energy retention } (k_{RE)\ =}\ NE_p/MEI$$

$$\text{Efficiency of ME use for lipid retention } (k_{REf}) = RE_f/MEI$$

$$\text{Efficiency of ME use for protein retention } (k_{REp}) = RE_p/MEI.$$

4.2.4 Statistical analysis

Statistical analyses were performed using Minitab software (Minitab version 16, 2000). The data were analyzed using one-way ANOVA with diet as factor. The significance of difference between means was determined by Fisher's least significant difference at $P \leq 0.05$.

4.3 RESULTS

4.3.1 Gross performances

The gross performance of birds fed on the different diets is shown in Table 4.3. Feed intake up to 14 and 21d was highest ($P<0.001$) on the AP (CMF and SMF) diets, respectively, with the SBM diet being the lowest in feed consumption. Birds fed the AP diets were significantly heavier ($P<0.001$) at 7, 14 and 21d than the birds on the VP diets, of which CM diet group of birds weighed the least. Over the entire test period, birds on CM diet were the poorest ($P<0.001$) in FCR, while the FCR on the AP diets were the best. The birds on the SBM diet were also better in FCR than the birds on CM diet throughout the trial. The FCR of the SMF diet group was similar to the FCR of the CMF dietary group during 1-21d. The mortality of broiler chicks in different diet groups was similar during the entire experimental period (d1-21), with no significant ($P=0.28$) differences between the treatments.

4.3.2 Energy utilization of broilers fed on test diets

The ME contents of the experimental diets were similar, with a range between 12.1 and 12.3 MJ/kg, but gross energy intake (GEI), ME intake (MEI) and fat intake were significantly higher ($P<0.001$) in the AP diets than in the other diet groups (Table 4.4). There was no significant difference in the protein intake of the birds.

Birds on AP diets had significantly higher ($P<0.05$) NEp (327.9; 326.2 kj/d, for SBF and CMF, respectively), while birds on the VP diet groups had the least NEp (263.2; 266.3 kj/d, for SBM and CM, respectively) (Figure 4.1). Heat production ranged from 304.7 to 358.7 kj/d, and was not affected by treatment.

Energy and fat deposition was increased ($P<0.05$) on the AP diets, and protein deposition was also elevated ($P<0.01$) on these two diet groups compared to VP diets (Table 4.5). Birds on the AP diets deposited energy ($P<0.05$), fat ($P<0.05$) and protein ($P<0.01$) at a higher rate than those on the VP diets. Birds of the VP diet groups had similar rates of energy, fat and protein deposition. The rates of nutrient (energy, fat and protein) deposition were also similar in the AP diet groups.

Table 4.3 Gross response of broilers fed vegetable and animal protein diets at different phases of growth

	Age (days)	**Treatments**				Pooled SEM
		SBM	CM	SMF	CMF	
Feed intake (g/b)	1-7	146.4^{b}	160.0^{a}	152.0^{ab}	146.0^{b}	1.80*
	1-14	498.5^{c}	603.0^{a}	588.9^{ab}	606.5^{a}	6.23***
	1-21	1085.3^{c}	1139.6^{b}	1294.0^{a}	1291.5^{a}	9.02***
Live weight (g /b)	1-7	144.8^{b}	130.3^{c}	178.4^{a}	165.7^{a}	1.55***
	1-14	372.4^{b}	317.4^{c}	460.5^{a}	453.4^{a}	3.85***
	1-21	668.3^{bc}	602.9^{c}	921.9^{a}	904.8^{a}	14.55***
FCR	1-7	1.48^{b}	1.92^{a}	1.15^{c}	1.24^{c}	0.023***
	1-14	1.53^{b}	2.23^{a}	1.42^{b}	1.49^{b}	0.021***
	1-21	1.80^{b}	2.00^{a}	1.50^{c}	1.50^{c}	0.036
Mortality (%)	1-7	0.0	0.0	0.0	4.1	0.66
	1-14	2.0	0.0	1.0	4.1	0.78
	1-21	2.7	0.0	4.1	4.1	0.83

Data represent mean values of eight replicate groups consisting of eight birds per replicate during d1-18 days, and four birds during 19 to 21 days; $^{a, b, c}$Means bearing uncommon superscripts within a row are significantly different at *P<0.05 and ***P<0.001; SEM= Pooled standard error of means.

Table 4.4 Metabolizable energy (ME) of diets, intake of gross energy (GEI), metabolizable energy (MEI), protein and fat of different diets fed to broilers

	Treatments				Pooled SEM
	SBM	CM	SMF	CMF	
ME (MJ/kg)	12.3	12.1	12.3	12.3	0.05
GEI (MJ/bird)	15.8[c]	17.5[b]	19.3[a]	18.8[a]	0.13
MEI (kj/d/bird)	567.9[b]	592.0[b]	686.6[a]	672.2[a]	4.79
Protein intake (g/b)	48.0	44.7	49.9	49.1	1.30
Fat intake (g/b**)**	50.6[c]	63.7[b]	65.2[b]	71.7[a]	0.47

Data (calculated on DM basis) denote mean values of six replicate cages with four broilers per replicate cage at 21 days; [a, b, c]Means bearing uncommon superscripts within a row are significantly different (P<0.001).

a) NEp (kj/d) b) HP (kj/d)

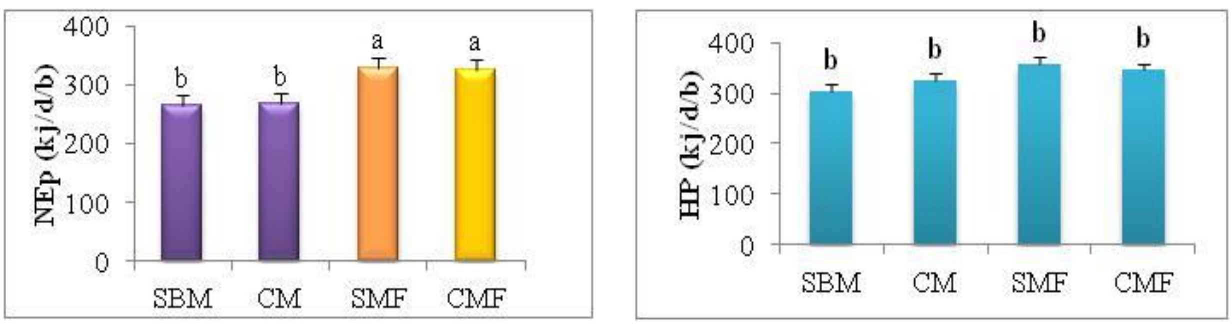


Figure 4.1: (a-b) Net energy for production (NEp), and Heat production (HP) of the birds fed test diets; Bars with dissimilar letters are significantly different at P<0.05.

Energy retained as fat (RE_f) (P<0.05) and as protein (REp) (P<0.01) was significantly different between the treatment groups; these being higher in the AP diets than in the VP diet groups (Table 4.6). The efficiencies in utilization of ME, protein and fat for energy retention were 0.45 - 0.49, 0.22-0.26 and 0.21-0.24, respectively, and were generally not affected by dietary treatment.

Table 4.5 Whole body energy, fat and protein contents at 21 days of age

	Treatments				Pooled SEM
	SBM	CM	SMF	CMF	
Total deposition (g/bird)					
Energy (kj/bird)	5833.8^{b}	5898.9^{b}	7192.1^{a}	7156.2^{a}	182.91*
Fat	72.5^{b}	67.6^{bc}	88.7^{a}	87.9^{a}	2.24*
Protein	126.8^{b}	115.8^{bc}	157.5^{a}	150.8^{a}	3.57**
Rate of deposition (g/day)					
Energy (kj/day)	277.8^{b}	280.9^{b}	342.5^{a}	340.8^{a}	8.70*
Fat	3.5^{b}	3.2^{b}	4.2^{a}	4.2^{a}	0.10*
Protein	6.0^{b}	5.5^{b}	7.5^{a}	7.2^{a}	0.17**

Data denote mean values of six replicate cages with four broilers per replicate cage at 21 days; [a,b,c]Means bearing uncommon superscripts within a row are significantly different (*P<0.05; **P<0.01).

Table 4.6 Energy retention (kj/d) as fat (RE_f) and as protein (REp), and efficiencies of ME, fat and protein use towards energy retention

	Treatments				Pooled SEM
Energy retention	SBM	CM	SMF	CMF	
RE_f (kj/d)	131.8^{b}	123.0^{b}	161.3^{a}	160.0^{a}	4.09*
REp (kj/d)	142.5^{b}	130.1^{b}	176.9^{a}	169.5^{a}	4.01**
Efficiencies of energy utilization					
K_{RE}	0.46	0.45	0.48	0.49	0.013
K_{REp}	0.25	0.22	0.26	0.25	0.006
K_{REf}	0.23	0.21	0.23	0.24	0.006

Data denote mean values of six replicate cages with four broilers per replicate cage at 21 days; [a,b,c]Means bearing uncommon superscripts within a row are significantly different (*P<0.05; **P<0.01).

4.4 DISCUSSION

4.4.1 Gross performances of birds

The gross response of broiler chickens has been regarded as the primary criterion for determining the feed nutrient requirements because the broiler chick is an ideal experimental subject with a limited nutrient store, high nutrient demand and rapid growth rate (Ammerman, 1995). In this study overall gross performance of broiler chickens was reduced by feeding VP diets, confirming the results obtained in Chapter 3 and also reported by Hossain *et al*. (2011a, 2013b).

Birds consumed significantly greater quantity of animal protein diets than the VP diets in this study. The result is in agreement with the report of Bhuiyan *et al*. (2012a,b). The reduced feed consumption of broilers on VP diets may be due to deficiency of essential amino acids, poor quality, or indigestibility of nutrients, anti-nutritive effects, and lower palatability of the diets. Jackson *et al*. (1982) reported that essential amino acid imbalances in diets reduced the biological value of the diets and decreased the feed intake. Lower palatability of the diets might be another reason for reduced feed consumption by the broiler chickens (Mahmoudnia *et al*., 2011). Furthermore, vegetable ingredients, particularly canola meal, contain anti-nutrients, predominantly glucosinolates, which impart bitter taste and thus affect the feed consumption of birds (Peron and Partridge, 2010). Other deleterious substances which may be present in the diets are protease inhibitors, lectins, polyphenolic compounds, saponins and non-starch polysaccharides (Hughes and Choct, 1999; Ravindran *et al*., 2005), can also affect the feed consumption. Apart from these, the probable causes of depressed performance of broilers on VP diets have also been highlighted in the previous Chapter 3.

4.4.2 Energy utilization by birds

The results of this study provide an indication of how efficiently broiler chickens utilize energy and other nutrients from VP and animal protein diets. In the current study, the ME contents of the experimental diets were similar, but ME intake was reduced in groups on the VP diets. This reduction in ME intake may be due to a high fibre content and presence of anti-nutritive factors in these diets, as observed by some researchers (Warenham *et al*.,1994; Barteczko *et al*., 2008). Phytic acid, in particular can adversely affect energy utilization and the availability of other nutrients in poultry diets (Ravindran *et al*., 2005), although these diets were supplemented with phytase and mechanically processed. The anti-nutritive effect of phytate and other deleterious factors of plant proteins can not be eliminated completely by enzyme supplementation (Selle and Ravindran, 2007) and processing technique. Apart from this, increased activity of gut microflora on the dietary factors can lead to energy wastage (Choct *et al*, 1996), and availability and digestibility of other nutrients (Smits *et al*., 1997). Le Goff and Noblet (2001) also reported that most of the variation in digestibility of feed energy is related to the presence of dietary fibre. Variation in gross chemical composition between grains may greatly affect digestibility of feed energy (Black, 2001).

Metabolizable energy intake was high in birds on the AP diets, possibly as a result of higher feed intake and increased nutrient digestibility of these diets (Barteczko *et al*., 2008), which was also observed in Chapter 3. Fishmeal supplementation to VP diets might enhance the digestibility of amino acids of the feeds as a result of synergistic action or combination of vegetable and animal proteins in the regular diets. Fishmeal contains all the essential amino acids as required by poultry, which can ensure better utilization of energy and other nutrients in traditional diets.

In this study, broilers on the AP diets used energy better, as shown by higher NEp with no significant differences in HP. This group of birds also attained heavier body weight than the VP groups. Olukosi *et al*. (2008) reported that an improvement in energy utilization may be due to improvement in nutrient and energy availability.

The heat production (HP) of birds did not differ significantly between treatments, although birds in the AP had a numerically higher level of HP than the VP diet groups. This increase in HP on the AP diets may be due to higher feed intake, in particular protein intake (Johnson, 2007). The AP diets also resulted in increased whole body energy, fat and protein contents, a trend supported by Boekholt *et al.* (1994) who observed that when protein is not limiting in the diets of broilers, extra energy available in the diet is used for both fat and protein accretion. On the other hand, the rate of deposition of energy, fat and protein was reduced in birds on the VP diets. This could be caused by higher fibre content, as previously reported by Uberoi *et al.* (1992).

In general, more energy is retained as protein than as fat, especially on the AP diets. This also represents a more efficient use of energy and the carcass would be less fatty as a result. Leeson and Summers (1997) have reported that abdominal fat of birds increases with age whereas protein retention decreases. This is simply related to the maturity of the birds and is found commonly in most strains (Leeson, 1995). In the present study, protein retention was found to be higher than fat retention as birds in the tested age group (0-21d) were still in the actively growing phase of production (Bregendhl *et al*., 2002). The efficiency of utilization of metabolizable energy for energy, protein and fat retention was unaffected by dietary treatment, the ME being more efficiently used for energy deposition and less for protein and fat deposition. It is uncertain what the implications of this trend means, but it may explain the increase in fat deposition as birds become older.

4.5 CONCLUSION

The present study showed that AP diets supported higher body growth and better FCR not only through higher feed intake but also through a more efficient utilization of dietary energy. The latter is supported by higher ME intake and higher NEp. There is a need to assess the utilization of proteins in these diets in order to make a complete recommendation as to their quality.

CHAPTER 5 ASSESSING THE PROTEIN AND MINERAL DIGESTIBILITY OF VEGETABLE PROTEIN DIETS FED TO BROILER CHICKENS WITH OR WITHOUT MICROBIAL ENZYMES

ABSTRACT

This study was conducted to assess the effects of two protein sources (soybean or canola) and microbial enzyme supplements (carbohydrase/protease and phytase) of broiler chickens. Day-old male Ross-308 chicks (n=256) were assigned to four treatments, each replicated eight times, eight chicks per replicate, in a 2 × 2 factorial arrangement. Two basal diets (soybean meal or canola) were fed as such or supplemented with microbial enzymes (Avizyme 1502; Phyzyme-XP) ad libitum from 1 to 35 days. Results showed that feed intake of birds to 21 and 35 days on the canola (CM) diet was higher ($P<0.001$) than that of birds on the soybean (SBM) diet. Live weight was also higher on the CM diet than on the SBM diet at 7 ($P<0.01$) and 21 ($P<0.05$) days. Feed conversion ratio (FCR) was better ($P<0.01$) on the SBM diets than on the CM diets at 21 days. Enzyme supplementation generally improved ($P<0.001$) feed intake, and live weight. Feed conversion ratio was also improved ($P<0.001$) by enzyme supplements up to 21 days of age but over the entire feeding period, this was significant ($P<0.01$) only on the SBM diets. The digestibility of histidine was significantly ($P<0.05$) better on CM diet, but not lysine, which was higher ($P<0.01$) on SBM diet at 21 days. The digestibility of threonine and lysine at 35 days was higher ($P<0.01$) on the SBM diet than on the CM diet. A similar trend was observed for valine, isoleucine and leucine but these were significantly different at $P<0.05$ on day 35. Threonine ($P<0.001$) and lysine($P<0.01$)as well as other amino acids such as histidine, valine, iso-leucine and phenylalanine digestibility measured at day 21; and the digestibility of histidine, threonine, lysine and isoleucine assessed on day 35 were significantly ($P<0.05$) improved in supplemental diets compared to control diets. At 21 day, diets had no impact on mineral digestibility, but enzyme supplementation increased ($P<0.05$) the digestibility of K, Mn, and Cu, compared to the non-supplemented diets. Enzymes had no significant ($P>0.05$) effect on mineral digestibility at 35 days. The digestibility of Cu, Zn and Mg was higher ($P<0.05$) on the CM diet, whereas Ca digestibility was higher on the SBM diet. Tissue protein content and endogenous enzyme activities (except for maltase) were unaffected by protein source and microbial enzyme supplementation. The interactions between the two factors were not significant. The results suggest that canola meal supported growth better than soybean and this could be attributed to higher feed intake on the canola diet rather than nutrient digestibility.

5.1 INTRODUCTION

Protein is an essential nutrient required by broiler chickens for their optimum growth and development. The key element of feather, muscle tissue or cell is protein, which plays an important role in many processes of life. Proteins of animal origin in the feed, such as fish meal, meat meal or milk product, as a class, are superior to the proteins of plant origin in the feed. In terms of protein quality, eggs are best, followed closely by milk. Muscle meats as well as fish, and meat from glandular organs rank a little lower, but are better than most of the plant proteins (Singh and Panda, 1992). This fact was confirmed by results in the preceding chapters of this thesis. The type of proteins used in feed formulation not only affects the quality of the formulated diets but also influences the performance of broiler chickens and the quality of meat.

Diets containing animal proteins can fulfil the protein and amino acid requirements of the birds with a minimum intake. If the dietary proteins are deficient in one or more essential amino acids, they will not be able to provide proper protein nutrition even with excessive intake (Sing and Panda, 1992). However, globally the poultry industry is seeking alternative feed proteins due to rising cost, hazard of zoonoses in animal protein meal (e.g. meat and bone meal), and great demand of intensive poultry production across the world (CEC, 2000; FAO, 2004). So it might be beneficial to use vegetable ingredients instead of animal protein meals to forestall the cross-contamination as well as to reduce feed cost of poultry production all over the world.

In this regard, proteins in diets containing only vegetable ingredients, such as, soybean meal, canola meal etc., may have potential for providing safe, quality diet formulation for poultry. Soybean is the premier vegetable source of protein, while canola seed is also becoming popular, and these are currently the largest protein meals produced worldwide (USDA, 2010; Hossain *et* *al*., 2012a). These meals contain a higher proportion of protein (37-48 %), and in a typical diet of broiler chickens these meals can be used to provide up to 60 % of the crude protein (Newkirk and Classen, 2002).

However, the effective utilization of these feedstuffs is often influenced by their high content of NSP (17.9 % in CM vs 14.5 % in SBM) (Meng *et al*., 2005). Cellulose and pectic polysaccharides are the major NSP in these feedstuffs (Bach Knudsen, 1997). Apart from these, such ingredients contain many other anti-nutritive factors, particularly phytic acid, which are resistant to animal enzymes and tend to suppress the nutritive quality of diet as well. Furthermore, certain proteins of plant origin are harmful to chickens, and interfere with enzyme activity in the intestine by reducing digestibility and absorption of nutrients thus depress growth (Singh and Panda, 1992). Some of these problems can be overcome by use of microbial enzymes in addition to other supplements in the practical diets of broiler chickens. Response to such enzymes depends partly on the nature of the diet and many other factors. Feed formulated entirely with vegetable ingredients may increase dietary fibre level considerably, and young birds may encounter a lower feed digestibility, resulting in poor performance. Young chicks lack certain types and amounts of enzymes, which are necessary to utilize a high carbohydrate and vegetable protein in the diet at an early stage, thus affecting performance (Classen and Bedford, 1999). It is reported that, inclusion of enzymes to diets enables the birds to degrade the anti-nutrient feed components along with promoting the breakdown of starch, cell walls, and storage proteins (Troche *et al*., 2007).

In previous studies (Chapter 3 and 4), growth performance, nutrient (protein) digestibility and energy utilization of broilers fed on all-vegetable diets was poorer than response on animal protein diets. Therefore, the current study was undertaken with the aim of improving growth performance of the birds through supplementation with microbial enzymes and how these affect nutrient digestibility, tissue protein growth and endogenous enzyme activities.

5.2 MATERIALS AND METHODS

5.2.1 Animal husbandry and bird management

The experiment was conducted at the Animal House of the UNE, and approved by the Animal Ethics Committee of the UNE (Approval No**:** AEC11/067). A (2 × 2) factorial experiment, having two diet types (soybean or canola-based) and two enzyme levels (with or without), was conducted to examine the response of broiler chicks in terms of feed intake, amino acid and mineral digestibility, and the activities of intestinal and pancreatic enzymes as well as tissue protein growth. A total of 256 day-old Ross male broiler chicks (46.34±0.27g) were obtained from a local commercial hatchery and tested from hatch to 35 days. The chicks were weighed and distributed randomly into four dietary treatments (details are shown in section 5.2.2), each treatment replicated 8 times, 8 birds per replicate. The birds were reared in brooder cages for the first 3 weeks, and then transferred to large metabolic cages for the last 2 weeks of the trial. The management and care of birds was as described in previous chapters (3 and 4).

5.2.2 Dietary treatments

Two basal diets were formulated with maize, wheat, and vegetable oil as the main energy sources, along with soybean (SBM) and canola (CM) meals as sole protein sources, and pelleted, as shown in the previous Chapter 3 (Section 3.2.2). The diets were fed as such (SBM- or CM-) or supplemented with exogenous enzymes (Avizyme 1502 and Phyzyme XP), SBM+ or CM+ (Tables 5.1 and 5.2). The exogenous enzymes were included at a rate of 0.5g/kg of Avizyme 1502 (containing amylase 800, xylanase 1200, protease 8000 U/g) and 0.1 g/kg of Phyzyme XP (1000 FTU). Both of these enzymes were supplied by Danisco Animal Nutrition, UK. All diets were iso-caloric and iso-nitrogenous, and were supplemented with Zinc Bacitracin (0.5 g/kg diet). The birds were fed starter diets for the first three weeks, and finisher diets were used in the last two weeks of the trial period. Titanium dioxide (TiO_2) was incorporated into each diet at a rate of 5 g/kg as an indigestible marker to enable assessment of nutrient digestibility.

5.2.3 Gross responses and sample collection

Three birds from each pen on day 21, and two birds on day 35, respectively, were randomly selected, weighed and killed by cervical dislocation to collect digesta samples from the ileum for the assessment of amino acid and mineral digestibility. In addition, pancreatic and jejunal tissue samples were also collected on d 21 only to assess the tissue protein contents and activities of endogenous enzymes (chymotrypsin amidase, alkaline phosphatase, sucrase and maltase). The ileal digesta from Meckel's diverticulum to the caeca junction were collected and pooled by pen, frozen immediately after collection and subsequently freeze-dried. Dried ileal digesta samples were ground to pass through a 0.5 mm sieve and stored in airtight containers at -20 ^{o}C for chemical analyses. Gross responses in terms of live weight (LW), feed intake (FI), feed conversion ratio (FCR) were measured as described in section 4.2.3 (Chapter 4).

Table 5.1 Ingredient and nutrient composition of the starter diets (0-21 days)

	Diets			
	SBM-	**SBM+**	**CM-**	**CM+**
Ingredient composition (g/kg)				
Maize	406.6	406.6	363.6	363.6
Wheat	211.0	211.0	181.7	181.7
Vegetable oil	0.0	0.0	21.7	21.7
Soybean meal	246.9	246.9	96.4	96.4
Canola meal	82.3	82.3	290.0	290.0
Limestone	20.1	20.1	14.8	14.8
Dicalcium phosphate	17.0	17.0	21.0	21.0
DL-Methionine	2.0	2.0	1.7	1.7
Lysine	1.7	1.7	1.2	1.2
Sodium chloride	3.5	3.5	4.2	4.2
Vitamin-mineral premix1	2.5	2.2	2.5	2.0
Choline chloride	0.6	0.6	0.6	0.6
Sodiumbicarbonate	0.3	0.0	0.1	0.0
Avizyme1502	0.0	0.5	0.0	0.5
Phyzyme XP	0.0	0.1	0.0	0.1
Zinc Bacitracin	0.5	0.5	0.5	0.5
Marker	5.0	5.0	5.0	5.0
Nutrient composition (g/kg)				
ME (MJ/kg)	12.37	12.37	12.38	12.38
Crude protein	211.0	211.0	211.1	211.1
Crude fibre	31.0	31.0	36.2	36.2
Ether extract	24.0	24.0	28.1	28.1
Calcium	12.3	12.3	12.2	12.2
Available P	6.2	6.2	6.2	6.2
Sodium	2.0	2.0	2.0	2.0
Chlorine	2.5	2.5	2.7	2.7
Lysine	13.0	13.0	13.1	13.1
Methionine+cystine	8.3	8.3	8.3	8.3
Threonine	8.3	8.3	8.4	8.4
Arginine	14.1	14.1	14.2	14.2

[1]Provided per kg of diet (mg): vitamin A (as all-trans retinol), 3.6 mg; cholecalciferol, 0.09 mg; vitamin E (as d-α-tocopherol), 44.7 mg; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; vitamin B_{12}, 0.2 mg; biotin, 0.1 mg; niacin, 50 mg; D-Calcium pantothenate, 12 mg ; folic acid, 2 mg; Mn, 80mg; Fe, 60 mg; Cu, 8 mg; I, 1 mg; Co, 0.3 mg and Mo, 1 mg.

Table 5.2 Ingredient and nutrient composition of finisher diets

	Diets			
	SBM-	**SBM+**	**CM-**	**CM+**
Ingredient composition (g/kg)				
Maize	414.1	414.1	382.2	382.2
Wheat	211.0	211.0	190.0	190.0
Vegetable oil	0.0	0.0	18.3	18.3
Soybean meal	227.0	227.0	89.0	89.0
Canola meal	75.6	75.6	267.0	267.0
Limestone	24.0	24.0	21.0	21.0
Dicalcium phosphate	23.0	23.0	20.4	20.4
DL-Methionine	2.2	2.2	1.8	1.8
L-Lysine	2.3	2.3	1.6	1.6
Sodium chloride	5.0	5.0	5.0	5.0
Sodium bicarbonate	5.1	4.6	0.1	0.0
Vitamin-mineral premix[1]	4.5	4.5	2.5	2.0
Choline chloride	0.6	0.6	0.6	0.6
Avizyme 1502	0.0	0.5	0.0	0.5
Phyzyme XP	0.0	0.1	0.0	0.1
Zinc bacitracin	0.5	0.5	0.5	0.5
Marker (TiO_2)	5.0	5.0	5.0	5.0
Nutrient composition (g/kg)				
ME (MJ/kg)	12.4	12.4	12.4	12.4
Crude protein	191.4	191.4	191.2	191.2
Crude fibre	29.4	29.4	36.0	36.0
Ether extract	24.0	24.0	28.0	28.0
Calcium	14.3	14.3	14.2	14.2
Available P	6.3	6.3	6.2	6.2
Sodium	2.3	2.3	2.2	2.2
Chlorine	3.3	3.3	3.3	3.3
Lysine	12.4	12.4	12.3	12.3
Methionine + cystine	8.2	8.2	8.1	8.1
Threonine	8.0	8.0	8.1	8.1
Arginine	13.1	13.3	13.0	13.0

[1] **Composition as in Table 5.1**

5.2.4 Chemical analyses

Amino acid composition

The amino acid analysis was carried out by the Australian Proteome Analysis Facility (APAF), Macquarie University, NSW, Australia. Amino acid concentrations in the diet and ileal digesta samples were determined using pre-column derivatisation amino acid analysis with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) followed by separation of the derivatives and quantification by reversed phase high performance liquid chromatography (HPLC), according to Cohen and Michaud (1993), and Cohen (2001). Amino acids were detected by UV absorbance. Approximately 100 mg of sample was hydrolyzed in 20 % HCl for 24 h at 110 ^{o}C. An internal standard (α amino butyric acid; AABA) was added to each sample following hydrolysis. Ten µL of the solution were then derivatised using an AccQ Tag Ultra Derivatization Kit (Waters Corp. USA; 70 mL borate buffer +20 αL AccQ Tag solution, incubated for 10 min at 50 ^{o}C). The HPLC analysis was based on the method of Cohen (2001), but adapted for use with an ACQUITY Ultra Performance LC (UPLC; Waters Corp. State USA) system. The column employed was an ACQUITY UPLC BEHC18 1.7 µm column (water) with detection at 260 nm and a flow rate of 0.7 mL/min. Samples were analyzed in duplicates and results were expressed as mean.

Mineral concentrations and titanium dioxide of diets and digesta samples were determined by following the method as described in Chapter 3 (Section 3.2.6). The analyzed mineral and amino acid were later used to determine the apparent digestibility in a similar way as stated in the previous Chapter 3 (Section 3.2.3).

Tissue protein and digestive enzyme analysis

The jejunal tissue samples were processed according to the method developed by Shirazi-Beechey *et al.* (1991) and modified for poultry by Iji *et al.* (2001b). The pancreas was processed in a similar way to the jejunum tissue, except that Milli-Q water (Millipore Australia, North Ryde, Australia) was used instead of buffer, and the entire tissue was homogenized (Nitsan *et al*., 1974; Iji *et al*., 2001b).

The specific activities of jejunal and pancreatic enzymes were assessed by incubation with fixed substrate concentrations as standardized for poultry by Iji *et al*. (2001b). On the jejunal homogenates, the assays were conducted for mucosal protein content and activities of alkaline phosphatase (EC 3.1.3.1), maltase (EC 3.2.1.20) and sucrase (EC 3.2.1.10). For the pancreas, assays were conducted for chymotrypsin amidase (EC 3.4.21.1) and lipase (EC 3.1.1.3). The specific activities of enzymes were measured according to the methods previously described for other species. The concentration of protein in both the jejunal mucosa and pancreatic tissue was measured using the Comassie dye-binding procedure described by Bradford (1976). All the raw data for protein concentration were processed through the Lowry Software (McPherson, 1985) before statistical analysis.

5.2.5 Statistical analysis

Statistical analyses were performed using Minitab software (Minitab version 16, Minitab, 2000). The data were subjected to general linear model (GLM) and tested for significance between the dietary treatment means by Fisher's least significance difference at $P \leq 0.05$.

5.3 RESULTS

5.3.1 Gross responses

Diet type had no effect on FI up to 7d, but birds on the CM diet significantly ate more (P<0.001) than birds on the SBM diet at 21 and 35 days (Table 5.3). Feed intake (FI) was similar on the two enzyme supplemented diets (SBM+; CM+), but FI to 7 d was improved significantly (P<0.001) by enzyme supplementation. Except for 35d, LW on the CM diet was also higher than that on the SBM diet at 7 (P<0.01) and 21(P<0.05) days. At 35d, LW was identical between the two basal diets, but was significantly improved (P<0.001) by enzyme supplementation. Diets had no significant effect (P>0.05) on FCR of broilers to 7 and 35 days of ages; but FCR was significantly (P<0.01) better in the broilers on SBM diet when fed for 21 days. In general, FI and LW was improved significantly (P<0.001) in chickens as a result of enzyme supplementation to diets compared to the birds fed non-enzymes diets between hatch and 7, 21 and 35 days. Except for 7d, FCR was also improved on supplemental diets when fed for 21 (P<0.001) and 35 (P<0.01) days, respectively. There were no significant effects (P>0.05) of diet × enzyme interaction on the gross response of broiler chickens.

5.3.2 Amino acid digestibility

There was no significant (P>0.05) effect of diets on amino acid digestibility as measured at 21 days of age except for histidine and lysine (Table 5.4). The digestibility of histidine was the highest (P<0.05) in birds on diet CM, whereas the digestibility of lysine was higher (P<0.01) on the SBM diet. Enzyme supplementation increased the digestibility of all indispensable amino acid except for arginine, methionine and leucine.

Methionine digestibility tended to be significant (P=0.07) between the two test diets. Besides these, the digestibility of leucine was also improved marginally (P=0.09) on supplemental diets as compared to those fed basal diets. The digestibility of histidine, valine, iso-leucine and phenylalanine was similar in birds on the two enzyme-supplemented diets, but the digestibility of threonine (P<0.001), lysine (P<0.01), and the remaining amino acids (P<0.05) was all improved due to enzyme supplementation.

Table 5.3 Feed intake (g/b), live weight (g/b) and feed conversion ratio (FCR) of broiler chickens between hatch and 7, 21 and 35 days

Diet	**Enzyme**	**Feed intake**			**Live weight**			**Feed conversion ratio**		
		Day 7	Day 21	Day 35	Day 7	Day 21	Day 35	Day 7	Day 21	Day 35
SBM	-	110.6^{b}	1017.7^{c}	3577.6^{d}	119.8^{c}	678.2^{c}	1930.5^{b}	1.49	1.62^{b}	1.90^{a}
	+	150.2^{a}	1155.7^{b}	3681.8^{b}	152.4^{a}	811.8^{b}	2088.0^{a}	1.41	1.51^{c}	1.80^{b}
CM	-	132.3^{b}	1181.4^{b}	3633.1^{c}	134.7^{b}	714.4^{b}	1942.2^{b}	1.52	1.77^{a}	1.92^{a}
	+	148.6^{a}	1266.6^{a}	3796.4^{a}	156.4^{a}	838.6^{a}	2111.1^{a}	1.35	1.60^{b}	1.84^{b}
Pooled SEM		3.44	8.78	7.83	1.46	5.81	12.96	0.039	0.018	0.014
Significance										
	Diet (A)	0.156	0.001	0.001	0.003	0.011	0.506	0.828	0.002	0.287
	Enzyme (B)	0.001	0.001	0.001	0.001	0.001	0.001	0.124	0.001	0.004
	A × B	0.101	0.145	0.069	0.073	0.688	0.827	0.607	0.410	0.805

Data represent means of eight replicate groups consisting of eight birds per replicate during d1-21, and five birds during 22- 35 days; [a, b, c, d]Means bearing uncommon superscripts within a column are significantly different at the levels shown; SEM= Pooled standard error of means.

At 35d, the digestibility of threonine and lysine was significantly (P<0.01) higher on SBM diet than on CM diet (Table 5.5). Similarly the digestibility of valine, isoleucine and leucine was also higher (P<0.05) on SBM diet than in CM diet. The digestibility of histidine and arginine in SBM diet appeared to be somewhat better than on CM diet. Apart from this, addition of enzymes also significantly (P<0.05) increased the digestibility of histidine, threonine, lysine and isoleucine, but not the digestibility of other amino acids measured at 35d. Furthermore, the digestibility of leucine in birds on enzyme-supplemented diets also increased marginally (P=0.09) compared to the birds fed the non-supplemented diets. There was no significant (P>0.05) effect of diet × enzyme interaction on the digestibility of amino acids at 21 and 35 days.

Table 5.4 Ileal digestibility coefficients of amino acids in birds on diets with or without supplemental enzymes at 21 days

Diet	Enzyme	His	Arg	Thr	Lys	Met	Val	Ile	Leu	Phe
SBM	-	0.76^{c}	0.83	0.66^{b}	0.82^{b}	0.89	0.72^{c}	0.74^{b}	0.75	0.76^{b}
	+	0.79^{b}	0.84	0.71^{a}	0.85^{a}	0.90	0.74^{b}	0.75^{b}	0.77	0.77^{b}
CM	-	0.78^{b}	0.83	0.67^{b}	0.80^{c}	0.90	0.73^{c}	0.74^{b}	0.76	0.76^{b}
	+	0.81^{a}	0.85	0.70^{a}	0.83^{b}	0.91	0.76^{a}	0.77^{a}	0.78	0.80^{a}
Pooled SEM		0.004	0.004	0.004	0.003	0.003	0.005	0.005	0.006	0.006
Significance										
	Diet (A)	0.037	0.278	0.99	0.007	0.072	0.159	0.443	0.235	0.948
	Enzyme (B)	0.019	0.209	0.001	0.002	0.269	0.013	0.025	0.094	0.012
	A × B	0.947	0.983	0.70	0.963	0.614	0.795	0.918	0.825	0.908

Data represent means of three chickens from five replicate groups at 21 days of age; $^{a, b, c}$Means bearing uncommon superscripts within a column are significantly different at the levels shown.

Table 5.5 Ileal digestibility coefficients of amino acid in birds on test diets with or without supplemental enzymes at 35 days

Diet	Enzyme	His	Arg	Thr	Lys	Met	Val	Ile	Leu	Phe
SBM	-	0.81^{b}	0.85	0.73^{b}	0.85^{b}	0.92	0.77^{a}	0.78^{b}	0.80^{a}	0.80
	+	0.84^{a}	0.87	0.76^{a}	0.87^{a}	0.93	0.79^{a}	0.81^{a}	0.82^{a}	0.83
CM	-	0.80^{b}	0.84	0.69^{c}	0.83^{c}	0.92	0.74^{b}	0.76^{c}	0.78^{b}	0.77
	+	0.82^{b}	0.85	0.72^{b}	0.84^{b}	0.93	0.77^{a}	0.78^{b}	0.79^{a}	0.79
Pooled SEM		0.004	0.003	0.005	0.003	0.002	0.005	0.005	0.004	0.012
Significance	Diet (A)	0.075	0.068	0.002	0.003	0.367	0.023	0.014	0.041	0.123
	Enzyme (B)	0.019	0.124	0.018	0.036	0.233	0.279	0.050	0.092	0.927
	A × B	0.627	0.315	0.792	0.307	0.472	0.626	0.388	0.523	0.204

Data represent means of two chickens from five replicate groups at 35 days of age; $^{a, b, c}$Means bearing uncommon superscripts within a column are significantly different at the levels shown.

5.3.3 Mineral digestibility

The ileal digestibility of minerals of broilers fed the two VP diets to 21 and 35d are shown in Tables 5.6 and 5.7. There was no significant (P>0.05) effect of test diets on the mineral digestibility on day 21. However, the digestibility of Zn tended to be significantly (P=0.06) different between the two test diets at this stage (21d). The digestibility of Cu was significantly (P<0.01) increased by enzyme supplementation of both diets while enzyme supplementation improved (P<0.05) the digestibility of Mn and K (Table 5.6). Besides this, the digestibility of Mg on supplemented diets tended to be significant (P=0.08) compared to the birds fed diets with no enzymes.

Table 5.6 Ileal mineral digestibility coefficients of broilers fed on vegetable protein diets with or without supplemental enzymes at 21 days

Diet	**Enzyme**	**Mn**	**Cu**	**Zn**	**Ca**	**Mg**	**K**	**P**
	-	0.42^{b}	0.45^{b}	0.46	0.52	0.47	0.91^{b}	0.63
SBM	+	0.45^{b}	0.47^{b}	0.52	0.58	0.52	0.90^{b}	0.67
	-	0.43^{b}	0.44^{b}	0.44	0.52	0.48	0.90^{b}	0.60
CM	+	0.52^{a}	0.52^{a}	0.45	0.53	0.54	0.92^{a}	0.67
Pooled SEM		0.010	0.008	0.011	0.013	0.014	0.002	0.010
Significance	Diet (A)	0.100	0.952	0.06	0.424	0.679	0.172	0.634
	Enzyme (B)	0.028	0.008	0.161	0.207	0.088	0.012	0.056
	A × B	0.237	0.508	0.281	0.342	0.983	0.164	0.715

Data represent means of three chickens from five replicate groups at 21 days of age, [a, b]Means bearing uncommon superscripts within a column are significantly different at the levels shown.

The digestibility of K, Na, P, and Mn at 35d of age was identical between the two test diets (Table 5.7). The digestibility of Cu, Zn and Mg was significantly (P<0.05) higher on birds on the CM diet than in those on the SBM diet in this study. Calcium digestibility was, however, higher (P<0.05) on the SBM diet than on CM diet. Apart from this, the K digestibility of broiler chickens on SBM diet was also marginally (P=0.09) higher than that of birds fed the CM diet.

There was no significant ($P>0.05$) effect of enzymes on mineral digestibility at 35d. Diet × enzyme interaction on the digestibility of minerals at 21 and 35 days was also similar between treatments.

5.3.4 Tissue protein growth and activity of digestive enzymes

There was no significant effect ($P>0.05$) of diet or enzyme supplementation on pancreatic tissue protein content and chymotrypsin amidase activities (Table 5.8). However, tissue protein content in the pancreas tended to be higher ($P=0.06$) in birds on the enzyme-supplemented diets. Jejunal mucosal protein content was also not different between treatments. Sucrase, maltase and alkaline phosphatase activities in the jejunum were not influenced ($P>0.05$) by addition of enzymes to diets, but the activity of maltase was significantly ($P<0.05$) higher in birds on the CM diet than on the SBM diet. Apart from this, the activity of sucrase marginally increased in birds fed on diets with enzyme supplementation, but this effect was not significant ($P>0.05$). The activities of alkaline phosphatase in chicks on enzyme-supplemented diets declined, although the difference between the two test diets was insignificant ($P=0.53$). There was no significant ($P>0.05$) effect of diet × enzyme interaction on the tissue protein contents and enzyme activities.

Table 5.7 Ileal mineral digestibility coefficients of broilers fed on vegetable protein diets with or without supplemental enzymes at 35 days

Diet	**Enzyme**	**Mn**	**Cu**	**Zn**	**Ca**	**Mg**	**K**	**P**	**Na**
	-	0.46	0.44[b]	0.53[b]	0.65[a]	0.54[b]	0.88	0.63	0.39
SBM	+	0.49	0.47[a]	0.55[a]	0.66[a]	0.56[a]	0.89	0.63	0.42
	-	0.44	0.49[a]	0.57[a]	0.62[b]	0.57[a]	0.86	0.61	0.42
CM	+	0.46	0.49[a]	0.57[a]	0.65[a]	0.57[a]	0.86	0.63	0.47
Pooled SEM		0.006	0.009	0.006	0.004	0.006	0.006	0.004	0.018
Significance	Diet (A)	0.118	0.041	0.045	0.018	0.022	0.089	0.141	0.255
	Enzyme (B)	0.534	0.53	0.901	0.265	0.925	0.762	0.112	0.746
	A × B	0.103	0.759	0.945	0.477	0.748	0.528	0.190	0.333

Data represent means of two chickens from five replicate groups at 35 days of age, [a, b]Means bearing uncommon superscripts within a column are significantly different at the levels shown.

Table 5.8 Tissue protein content and enzyme activities of broiler chickens at day 21 with or without microbial enzyme supplementation

		Pancreas		**Jejunum**			
Diet	Enzyme	Protein (mg/g tissue)	CA[2]	Protein (mg /g tissue)	AP[3]	Maltase (ηmol/mg protein)	Sucrase (ηmol/mg protein)
SBM	-	55.1	3.8	64.5	5.8	0.48[b]	0.15
	+	58.8	4.9	66.7	5.6	0.52[a]	0.15
CM	-	57.3	4.8	57.2	7.1	0.58[a]	0.14
	+	61.8	5.6	62.5	6.0	0.64[a]	0.18
Pooled SEM		1.03	0.35	2.01	0.48	0.022	0.006
Level of significance							
	Diet **(A)**	0.213	0.258	0.169	0.352	0.023	0.295
	Enzyme **(B)**	0.06	0.193	0.362	0.539	0.224	0.09
	A × B	0.838	0.839	0.714	0.663	0.799	0.200

Data represent means of six replicates at 21 days of age; **CA[2]**, Chymotrypsin amidase (ηmol/mg protein); **AP[3]**, Alkaline phosphatase (µmol/min/mg protein); [a, b]Means bearing uncommon superscripts within a column are significantly different at the levels shown.

5.4 DISCUSSION

5.4.1 Gross responses

In this study, the results show that interactive action between diets and enzymes on the gross responses of broiler chickens fed vegetable protein diets was negligible. Rather, diets and enzymes individually had significant effects on the feed intake, body weight and FCR of broiler chickens. Birds on the CM diets consumed a significantly higher amount of feed compared to those fed the SBM diet. In addition, broilers in this diet group (CM) demonstrated a similar trend in feed consumption regardless of supplementation with enzymes. The results agree with the report of previous studies (Bhuiyan *et al*., 2010, 2012a; Hossain *et al*., 2011a, 2012a, b). The reason for greater feed intake of broilers on enzyme supplemented diets may be a result of birds growing more rapidly on these diets and therefore requiring more nutrients. Besides, the reason for greater feed intake of broilers on enzyme supplemented diets may be an outcome of increase fibre digestion, as the fibre may initially create gut fill, once such fibre is digested, chicks are able to increase FI to meet their nutrient requirement. The higher feed intake on CM diets could also be caused by faster growth of birds and the consequent higher nutritional requirement (Shrivastava *et al*., 1981). Moreover, supplementation of exogenous microbial enzymes to the diet might stimulate the broilers to consume more feed as a result of increased nutrient digestibility of the feeds. Trace minerals (Cu and Zn) in association with enzymes play an active role (Stefanidou *et al*., 2006), which might accelerate the metabolic process leading to improved digestion, absorption and assimilation of the diets inside the body. Feed intake and body growth rate of broilers may be impaired by the deficiency of these minerals in practical diets (Larbier, 1992).

However, although broilers from both diet groups (SBM; CM) with or without enzyme supplementation showed a similar trend in growth to 35 days, birds from the CM diet group grew faster than those from the SBM diet group during this period. This improved growth of broiler chickens fed on CM diet might be the result of higher feed consumption or utilization of the nutrients. The FCR was significantly improved on the chickens of SBM diets irrespective of enzyme supplementation. The improved feed efficiency of broilers on the SBM diets may be an outcome of better feed digestibility and nutrient availability of these diets. For example, the amino acid digestibility of this diet (SBM) was found to be significantly better than that on the CM diets. The improved FCR of broiler chickens fed on enzyme-supplemented diets is supported by the findings of previous researchers (Rassmussen and Petterson, 1997; Peng *et al*., 2003; Cowieson *et al*., 2006; Khajali *et al.*, 2007; Cowieson and Ravindran, 2008).

5.4.2 Amino acid digestibility

The pattern of amino acids digestibility was different between the two vegetable protein diets, particularly at the finisher period. The improved amino acid digestibility of SBM diet may be due to better protein quality of soybean meal compared to canola meal. Soybean meal also tends to have a lower fibre content and less anti-nutritive factors than CM. Le Goff and Noblet (2001) reported that most of the variation in digestibility of nutrients is related to the presence of dietary fibre. In the current study, enzyme addition to the two VP diets improved the digestibility of key amino acids both days 21 and 35 compared to the birds fed on the control diets. The results agree with the previous researchers (Ravindran *et al.*, 2000; Cowieson and Ravindran, 2008). Exogenous enzymes in VP diets might enable the broilers to breakdown the anti-nutritive factors, especially cell wall polysaccharides, to improve access to other nutrients. Moreover, microbial enzymes may decrease the digesta viscosity and facilitate improved contact between endogenous enzymes and nutrients, thereby improving digestibility (Lazaro *et al.*, 2004).

However, the digestibility of amino acid was not uniform, but variable. The response of threonine and lysine was more pronounced than for other amino acids assessed in this study especially during the starter period. Threonine is an important component of gastrointestinal mucin, and over half of the absorbed dietary threonine is used by the enterocytes. The improvement in threonine metabolizability with enzyme supplementation will provide more threonine for mucin synthesis (Pirgozliev *et al*., 2011).

The digestibility of arginine and methionine measured at 21 days, and the digestibility of valine and phenylalnine along with the former amino acids at 35 days, were not improved by supplementation with enzymes. This is almost similar to the report of Zanella *et al*. (1999), who demonstrated that lysine, arginine and methionine digestibility were not affected by supplemental enzyme (Avizyme). The effect of diets on amino acid digestibility of broiler chickens was more pronounced at 35 days of age than was observed at 21 days. But the impact of enzymes on digestibility was more pronounced at 21 days than at 35 days in this study. Digestibility of the majority of essential amino acids (histidine, threonine, lysine, valine, iso-leucine and phenylalanine) was increased by supplemental enzymes during the mid-growing period (21d), while the digestibility of most of the essential amino acids (arginine, methionine, valine, leucine and phenylalanine) was unaffected by addition of enzyme to vegetable protein diets at 35 days. This implies that enzymes exerted more action on amino acid digestibility during the early stage of growth than at the later stage. This difference between the two time periods may be due to differences in digestive function relative to age.

5.4.3 Mineral digestibility

The difference between mineral digestibilities of the two diets might be caused by differences in phytate contents or other anti-nutrient effects found in plant feed ingredients. The presence of such factors in many plant ingredients can affect nutrient utilization has been highlighted by many researchers (Thompson and Yoon, 1984; Wappnir, 1989; Sebastiana *et al*., 1997). However, the use of different diet formulations, the bird strain, feedstuffs, crude fibre level, anti-nutrient components etc., may also be responsible for these differences in the digestion of mineral nutrients (Pirgozliev *et al*., 2011).

The improvement in the digestibility of some minerals due to enzyme supplementation during the starter period of growth, partly agree with results of Selle *et al*. (2000) and Zyla *et al.* (2001), who showed a positive effect of dietary enzyme (phytase) on the utilization of minerals and energy, and nutrient digestibility in wheat-based diets. The addition of microbial phytase in the vegetable protein diets might help the birds to break down the mineral-phytate bond, thus making available the nutrients and increasing the mineral digestibility (Rama Rao *et al*., 2006; Narcy *et al*., 2009). The improvement in mineral digestibility due to enzyme supplementation was more in younger chicks while differences between two basal diets was more pronounced in the finisher phase. Nutrient digestibility in chicks has been shown to improve with the growth of digestive organs and the increase in enzyme activities (Noy and Sklan, 1995).

5.4.4 Tissue protein growth and digestive enzyme activities

In this study, maltase activity was higher on CM diet. This might stimulate the terminal digestion of starch and enable the birds to grow faster. Although there was a slight increase in pancreatic tissue protein content and activity of sucrase in enzyme-treated diets, the activity of alkaline phosphates declined somewhat in birds on the supplemental diets in this study. The activities of enzymes might exert a more stimulating effect on chyme passing through the digestive tract (Duke, 1986). Jiang *et al*. (2008) reported that the activities of lipase, pancreatic protease and trypsin were not influenced by addition of exogenous enzymes except for increased activity of trypsin in the intestinal lumen of the broiler chickens.

5.5 CONCLUSION

The results obtained in this study revealed that feed intake, mineral digestibility, and efficacy of enzyme (maltase) of broiler chickens on CM diet were significantly higher than those of birds fed on SBM diet. Birds of the SBM diet group had significantly better FCR, improved amino acid digestibility and lower feed intake, but similar body weight to the birds of the CM diet group. These results demonstrate better value of SBM than CM. However, both ingredients possessed their advantages and would be better combined, as is done in practical diets. The role of supplemental enzymes was pronounced but the exact mechanisms were not so obvious. Further studies are needed to define the preference of broiler chickens for the two oilseed meals.

CHAPTER 6 PREFERENCE FOR AND NUTRITIVE VALUE OF VEGETABLE PROTEIN DIETS IN RELATION TO PRODUCTION PHASE OF BROILER CHICKENS

ABSTRACT

This experiment consisted of two sub-trials, conducted to assess the impact of phase at which vegetable protein (VP) diets are introduced to broiler chicks, and preference of birds for diets based on soybean or canola meal. Two hundred and ten day-old Cobb 500 chicks were distributed into five dietary groups. One group was fed on animal protein (AP) diet all through to 21 days of age; two other groups were started on AP diet for 7 days and then switched to diets containing soybean meal (AP-SBM) or canola meal (AP-CM), while two other diets (SBM-AP and CM-AP) were started on one of the VP diets for 7 days and then switched to AP diet. A sub-trial on thirty birds raised on a commercial diet to 7 days was used in a feed selection test of the two main VP diets, containing SBM or CM. Chicks were reared under similar care and management conditions and the diets were iso-caloric and iso-nitrogenous. Results of the main trial showed that chicks on CM-AP diet ate more ($P<0.05$) than those on the other diets on day 7. Body weight gain was highest ($P<0.001$) on the AP-SBM diet while birds on the CM-AP diet weighed the least at 7d. Feed intake, body weight gain, FCR, mortality, bone growth, visceral organ development, and activities of digestive enzymes were similar between the groups from hatch to 21 days of age. Results of second sub-trial showed that chicks preferred the CM-based diets to the SBM-based diets at 8-14d ($P<0.001$) and 15-21d ($P<0.01$) when given a choice.

6.1 INTRODUCTION

In chicks, the development of the digestive system is much faster than the rest of the body immediately after hatch. The gastro-intestinal tract (GIT) is completely formed at the embryonic stage (Moran, 1985), although it develops to a full-fledged functionally within a few days after hatching (Nitsan *et al*., 1991; Iji *et al.*, 2001a, b). This post-hatch development is very important for future performance of efficient feed utilization and growth (Faria *et al*., 2005).

Provision of high quality nutrition to chicks in early life is necessary, to ensure the rapid development of the GIT and the rest of the body. Protein appears to be most essential component of such nutrition, as it drives the muscle development in later phases (Hargis and Creger, 1980). The quality and therefore the source of such protein may be important. This was demonstrated by differences in growth performance of the birds in the preceding experiments (Chapters 3 and 4), and also reported by Hossain *et al*. (2011a, 2012a, b).

However, it is generally assumed that no two protein sources are similar in characteristics. The pattern of digestibility, biological value, quality, physical or chemical structure or properties of protein sources vary widely between sources. These characteristics of individual protein ingredients might affect neonatal intestinal development and function, and thus performance of the broilers when used in practical diets. The interaction between dietary nutrients, intestinal growth and digestive function is crucial during the post-hatch period (Ullah *et al*., 2012). Nutrient processing by the GIT determines the amount of nutrients that is available to the intestinal tissues for metabolic activities of the birds. The efficiency of nutrient supply to endogenous tissues would be dependent on dietary factors, including dietary energy and protein contents (Swatson *et al*., 2003). Besides, feed management, feeding behaviour, diet preference or selection by the animal may also affect feed consumption and growth of the birds.

The ancestors of modern meat chickens lived on self-selecting feeding systems. They thrived better under such feeding systems, because they were able to select or balance their own feed mixture from a wide range of scavenging areas in the wild or range state. However, this feeding system is not always available to modern meat chickens, because they are normally offered a single feeding system in confinement. They do not have any choice or opportunity to select their own feed within this system of rearing. So there is need to explore alternative strategies in which meat chickens are able to select an appropriate mixture from a wide variety of available feedstuffs. Free-choice feeding system can be adopted as an alternative to regular system to meet the nutrient needs of broilers. This strategy may be economically viable because it can save feed costs. As feed is the most expensive of all the variable-cost inputs required for poultry production, a self-selection feeding regimen may offer a potential beneficial effect for achieving optimum production through reducing feed costs (Zulkifli *et al*., 2001). The objectives of this study was to assess the impact of stage at which vegetable or animal protein diets are introduced to young broiler chicks on subsequent growth and their preference for the two main vegetable protein sources tested in this project.

6.2 MATERIALS AND METHODS

6.2.1 Animal husbandry and bird management

Two sub-trials were conducted simultaneously with a total of 240 day-old male broiler chicks (Cobb 500; 45.0 ± 0.23 g) from hatch to 21 days. In the main trial, 210 chicks were assigned to five dietary groups i.e. AP, fed on animal protein diet from hatch to 21 days of age; AP-SBM and AP-CM, fed on AP for 7 days and then switched to diets with predominantly soybean meal or canola meal, respectively as protein source; SBM-AP or CM-AP, fed on a diet with soybean or canola as main protein source for 7 days and then switched to AP diet. Details of these treatments are shown in Table 6.1. Each diet group was replicated 6 times, 7 birds per replicate in a completely randomized design. Three basal diets (SBM; CM and SBF) were used alternately for the chicks of five dietary groups in this experiment. These diets were similar in ingredient and nutrient compositions to those described in Chapter 3 (Section 3.2.2). Diets (SBM; CM) were VP diets while diet SBF was treated here as AP diet, and these were allocated accordingly into five dietary treatment groups (Table 6.1).

The birds were raised on floor-cage system in a climate-controlled housing. Wood shaving was placed to a depth of 2 cm as litter material on the floor, which was replaced every week. The birds were brooded at 33 ^{o}C for the first two days, and then the temperature was reduced gradually to 24 ^{o}C at 19 days of age, and maintained at this level to the end of the trial. Feed, in pellet form and water were provided *ad libitum.* Feed intake, body weight gain, and feed conversion ratio were assessed weekly. Mortality was also recorded as it occurred.

Table 6.1 Feeding regimes followed for the main trial

Treatment groups	**Feed allocation by time (days)**		
	1-7	**8-14**	**15-21**
AP	AP	AP	AP
SBM-AP	SBM	AP	AP
CM-AP	CM	AP	AP
AP-SBM	AP	SBM	SBM
AP-CM	AP	CM	CM

Two birds from each replicate on day 21, were randomly selected, weighed, and killed humanely to measure relative visceral organ weights (small intestine, pancreas, liver, proventriculus, ventriculus, spleen, bursa of Fabricius). In addition, tissue samples from the pancreas and the proximal region of the jejunum were also collected on day 21 to assess the tissue protein content (pancreas and jejunum), and specific activities of digestive enzymes as described in Section 5.2.4. The right tibia bone was also collected at this time (21d) from two birds, killed in a similar way, to measure the bone characteristics (weight, length, width, tibia ash content, and bone strength) as per the methods described in Section 3.2.5. All collected samples were pooled by pen and stored at -20 ^{o}C until further processing for laboratory analyses.

6.2.2 Feed selection test

A sub-sample of 30 day-old male chicks of similar strain and initial weight as stated above were sub-divided into 6 cages (replicates), with five chicks per cage. All chicks were fed on a commercial diet until 7 days of age, then provided access to two VP (SBM and CM) diets in separate feeders from 8 to 21 days of age. Feed intake from each feeder was calculated daily until the end of the trial.

6.2.3 Animal ethics approval and statistical analysis

All animal care, handling and management of this experiment were approved by the Animal Ethics Committee of UNE (Approval No**:** AEC11/068), Australia. Statistical analyses were performed using Minitab software (Minitab Version 16, 2000). The data were subjected to one-way analyses of variance for completely randomized design and tested for significance between the dietary treatment means by Fisher's least significant difference at $P \leq 0.05$.

6.3 RESULTS

6.3.1 Growth performance of broiler chickens on main trial

Results of growth performance demonstrated that apart from the first 7 days, dietary regime had no ($P>0.05$) effect on feed intake (FI) when assessed from hatch to 14 or 21 days (Table 6.2). Chicks in the CM-AP and AP dietary groups had significantly ($P<0.05$) higher FI than other diet groups between hatch and 7 days. Up to 7 days, birds on SBM-AP diet group had the highest ($P<0.001$) body weight gain (BWG), while birds on CM-AP diet group gained the least. From hatch to 14 days, birds on the SBM-AP regime gained the highest ($P<0.001$) BWG, while birds on the CM-AP gained the least. Body weight gain in birds on the SBM-AP was similar to the birds fed the AP diet to 14 days, but was significantly different ($P<0.001$) from the other groups. Birds on the AP-CM regime had the best FCR, while birds in the CM-AP group had the poorest FCR at 7 days. The FCR on SBM-AP diet group was also better ($P<0.05$) than on the AP-CM regime, the latter being the worst in FCR to 14 days. There were no significant differences ($P>0.05$) in FI, BWG and FCR between the groups when assessed between hatch and 21 days.

6.3.2 Gastro-intestinal development

The relative weight of visceral organs of broiler chickens fed on the different plans to 21 days is shown in Table 6.3. The weight of all the organs (small intestine, proventriculus, ventriculus, pancreas, liver, spleen and bursa) of birds was identical (P>0.05) between treatments. The relative weight of the spleen tended to be significant (P<0.08) between treatments.

Table 6.2 Feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and mortality of broilers from d1 to 21 days of age

	Age (days)	Treatments					SEM
		AP	SBM- AP	CM-AP	AP-SBM	AP-CM	
	1-7	180.8^{ab}	171.7^{b}	196.5^{a}	177.3^{b}	171.5^{b}	2.64*
FI (g/b)	1-14	631.3	631.6	645.0	623.8	649.6	5.31
	1-21	1324.0	1338.7	1349.7	1317.6	1354.8	8.55
	1-7	161.8^{bc}	160.8^{c}	146.2^{d}	170.9^{a}	168.5^{ab}	1.10***
BWG (g/b)	1-14	523.3^{ab}	530.0^{a}	496.4^{c}	507.3^{bc}	498.0^{c}	2.60***
	1-21	916.7	960.7	902.5	893.2	896.3	8.66
	1-7	1.12^{b}	1.07^{bc}	1.34^{a}	1.04^{bc}	1.02^{c}	0.014***
FCR	1-14	1.20^{b}	1.19^{b}	1.30^{a}	1.23^{ab}	1.30^{a}	0.012*
	1-21	1.45	1.40	1.50	1.48	1.51	0.014
Mortality (%)	1-21	4.76	2.38	2.38	2.38	2.38	1.126

Each value represents the mean of 6 replicates consisting of 7 birds in each replicate cage from d1-21 days; $^{a, b, c}$ Means bearing uncommon superscripts within a row are significantly different at *P<0.05 and ***P<0.001; SEM= Pooled standard error of mean.

Table 6.3 Relative weight of visceral organs (g/100g of body weight) of broiler chickens

Visceral organs	Treatments					SEM
	AP	SBM-AP	CM-AP	AP-SBM	AP-CM	
Small intestine[2]	4.8	4.9	5.3	4.9	5.9	0.18
Proventriculus + Gizzard[2]	3.4	3.5	4.0	3.7	3.6	0.13
Pancreas	0.26	0.25	0.30	0.29	0.32	0.008
Liver	3.6	3.4	3.8	3.7	3.8	0.10
Spleen	0.09	0.09	0.07	0.07	0.11	0.005
Bursa of Fabricius	0.14	0.15	0.13	0.13	0.13	0.008

Each value represents the mean of 6 replicates of 7 chicken each replicate cage at 21 days; [2]Organs were weighed with contents.

6.3.3 Tissue protein growth and digestive enzyme activities

Results of pancreatic and jejunal tissue protein contents as well as activities of digestive enzymes (chymotrypsin amidase, maltase, sucrase, alkaline phosphatase and lipase) measured at 21 days of age are shown in Table 6.4. The results demonstrate that there was no significant (P>0.05) difference in tissue protein contents, or pancreatic and intestinal digestive enzymes activities between treatments.

Table 6.4 Tissue protein and enzyme activities of broilers on different feeding regimes

	Treatments					SEM
	AP	SBM- AP	CM-AP	AP-SBM	AP-CM	
Pancreatic protein (mg/g)	46.0	32.0	33.0	37.4	46.7	2.44
[2]Chymotrypsin amidase	3.4	5.8	4.4	4.2	4.0	0.28
Jejunal protein (mg/g)	30.0	33.0	36.6	35.2	36.0	1.52
Maltase (ήmol/mg)	0.62	0.58	0.48	0.47	0.52	0.027
Sucrase (ήmol/mg)	0.13	0.13	0.14	0.12	0.13	0.006
[3]Alkaline phosphatase	3.6	3.0	3.0	3.1	4.3	0.24
[3]Lipase	2.9	2.9	3.8	3.8	2.6	0.25

Data represent means of six replicates at 21 days of age; [2]Chymotrypsin amidase (ηmol/mg protein); [3]Alkaline phosphatase and pancreatic lipase (μmol/min/mg protein).

6.3.4 Bone (tibia) development

There were no significant (P>0.05) differences in the various bone characteristics of broilers between treatments (Table 6.5). However, birds on the SBM-AP regime had the highest tibia ash content (1.20 mg), while the birds in the AP-SBM diet group had the lowest tibia ash content (1.01 mg), but differences were not significant (P>0.05) between treatments.

Table 6.5 Bone (right tibia) characteristics of broiler chickens

	Treatments					SEM
	AP	SBM-AP	CM-AP	AP-SBM	AP-CM	
Length of bone (mm)	63.3	65.8	64.5	64.8	65.4	0.58
Weight of bone (g/kg body weight)	4.3	4.5	4.4	4.5	4.2	0.06
Width of bone (mm)	9.3	10.0	9.4	9.1	9.2	0.11
Breaking strength (kg/g bone)	4.4	4.1	4.2	3.8	3.7	0.10
Total tibia ash (mg/g bone)	1.0	1.2	1.0	1.0	1.0	0.02

Length and width are measured in millimetres per bone per bird basis; data represent means of two chickens from six replicate groups at 21 days of age.

6.3.5 Feed selection

In the choice-feeding trial, the birds ate more of the CM diet than the SBM diet from 8 to 14 days (P<0.001) and from 15 to 21 days (P<0.01) (Figure 6.1). From 8 to 14 days, the choice was 62.2 % in favour of CM while from 15 to 21 days; selection was 58.42 % for CM, and 41.58 % for SBM, respectively.

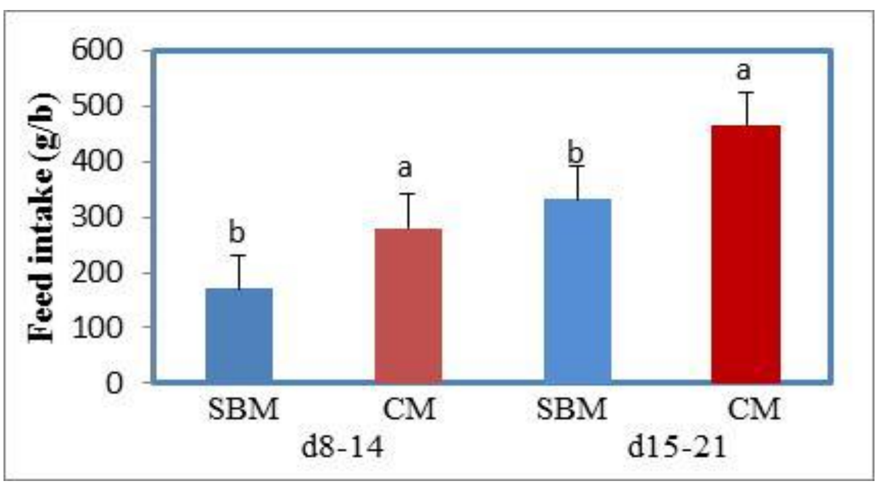


Figure 6.1: Weekly feed selection of broilers on two VP diets (SBM; CM) from d8-14, and d15-21 in choice-fed trial; [a, b]Means bearing uncommon superscript are significantly different at P<0.001 from d8-14, and at P<0.01 from d15-21 days.

6.4 Discussion

6.4.1 Gross responses of broiler chickens

The results demonstrated that birds on different diet groups achieved similar body weight gain with identical FCR at 21 days with the exception of the early stage of growing period (7 and 14d). This improved body weight gain of the birds fed on AP diets may be due to higher feed intake and improved feed efficiency of broilers in the younger stage of growth. Besides, variation in digestive organ development and enzymatic action of the birds may also affect the growth of birds at earlier period.

However, FI, BWG and FCR did not differ in spite of when the test diets were introduced at later stage. For FI, it appears that the birds have specific breed standards to meet and will achieve these targets in FI, if the nutrient composition is ideal. For BWG and FCR, it assumed that, the starting diets did not confer any advantage in the long term. Providing the VP and then AP diets enabled the birds to grow equally well, unlike what was observed in previous chapters when they were raised on VP diets from hatch to the end. Furthermore, similar growth responses of birds regardless of feeding them with VP or AP diets may be explained by the complementary effect of AP and VP diets for each treatment group. The broilers of all dietary groups consumed both AP and VP diets at the end of the trial period and this may have resulted in identical growth responses by the birds. More probably, broiler fed on a single diet may have lacked some nutrients which were then replaced by using another diet at a later stage of the growing period, and this fortification of the diets might have given rise to similar growth responses in the broiler chickens in this study.

6.4.2 Gastro-intestinal development of broiler chickens on test diets

Morphometric measures of digestive organs revealed no significant difference between the treatments. These results agree with those of Al-Masri (2006) and Xavier *et al*. (2012), who observed similar growth of digestive organs in broiler chicks on diets based on animal or vegetable protein ingredients. It is generally known that visceral organs are associated with digestive mechanism and develop most rapidly in the first 7-10 days of life (Nitsan *et al*., 1991; Iji *et al*., 2001a, b). However, how the nature of the starter diets influences this development has not been adequately studied.

In this study, the relative weight of spleen was marginally increased in the birds fed AP-CM; AP and SBM-AP diet groups compared to the birds of other diet groups. The thymus, spleen and bursa of Fabricius are the main immune organs of the chickens. Higher spleen weight would suggest better ability to protect from infection (unlike thymus and bursa). Higher protein intake may result in increased spleen mass and improved immunity of the birds. The increased relative weight of the spleen may alter the development and promotion of the immune function of the animals (Liu *et al.*, 2011); but this was not assessed in this current study. There were no significant differences in mortality between the groups.

6.4.3 Tissue protein growth and digestive enzyme activities

There was no significant difference between treatments in terms of digestive enzyme efficiency, or pancreatic and intestinal tissue protein contents of broiler chickens. There are no previous study on the effect of VP diets on intestinal structure and function. However, enzymes tend to respond to the presence of target substrates in the lumen of the intestine.

6.4.4 Bone development

In this study, broilers on the different feeding regimes developed similar bone characteristics. This may be an outcome of the identical body growth. The results are in contrast to the findings in the previous Chapter 3, when a single type of diet was used throughout the test cycle. Amongst bone characteristics measured, total bone ash was found to be slightly increased in the SBM-AP group compared to other groups. The higher concentration of bone ash in this group of chicken may be a result of the numerically longer, heavier and wider bones of the birds.

6.4.5 Feed selection

The results demonstrated that birds preferred the CM diet when they were given a choice between the two vegetable protein diets in this study. Birds consumed 20 % more CM diet than the SBM diet. A similar trend was also observed in previous experiments (Chapters 3 and 4), and also reported by Hossain *et al*. (2012 a,b; 2013b) in a trial on similar diets. It is not obvious what is the actual reason of higher feed intake by broilers on canola diet (CM), but the preference may be influenced by several factors, for example, nutrient requirements of individual birds, genotype, chronological age, prior experience, feed composition, nutritive value, stage of production, protein sources, vitamins and minerals, and so forth (Rose and Kyriazakis, 1991; Forbes and Shariatmadari, 1994). Apart from these factors, sensory characters of food materials such as colour, smell, flavour, taste or palatability might also play a key role in feed selection of broiler chickens (Cruze *et al*., 2005).

Certain minerals such as Na, Cl, and Zn might affect the feed consumption of birds by increasing the palatability of the given diets to the broiler chickens. Trace mineral (e.g. Zn and Cu) digestibility was found to be increased in the birds fed on CM diet compared to those fed on

SBM diets in previous study (Chapter 5), and also reported by Hossain *et al.* (2013a). The increased mineral digestibility of CM diet might affect slightly the feed consumption of broilers, because trace mineral in association with enzymes might play an active catalytic role which can help in metabolic processes for effective digestion, absorption and assimilation of the ingested feed materials.

As is evident from the formulation profile, the higher fibre content of CM diet perhaps induced slightly higher feed intake. Besides, higher feed intake may be stimulated by faster growth of individual bird and the consequent higher nutritional requirement (Shrivastava *et al*., 1981). In addition, variation in individual characters (e.g. mode of digestibility, biological value, and protein quality, physical and chemical properties) of protein sources used in the test diets might also affect greatly the feed preference of the broilers. Apart from these, birds can exhibit their innate ability instinctively to select a diet from a given choice of feed with the appropriate content of CP and energy in order to satisfy their nutrient requirements (Forbes and Shariatmadari, 1994; Hruby *et al.*, 1995).

6.5 CONCLUSION

Birds grew equally well regardless of feeding broiler chickens with starter diets at different phases. This may be due to complementary supply of nutrients at different stages of growth. The preference of chicks for the CM diet warrants further investigation as this diet did not appear to be nutritionally better than SBM in terms of growth rate and feed efficiency.

CHAPTER 7 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 INTRODUCTION

The modern meat chicken is a fast growing bird, and is an efficient converter of feed nutrients to meat. To obtain quality meat or protein from animals such as meat chickens, a high quality, well-balanced protein diet is required. As reviewed in Chapter 2, animal protein ingredients, for example, meat meal, meat and bone meal, fishmeal etc., are commonly used in poultry diets as sources of protein. These feedstuffs are known to have well-balanced amino acids with higher biological values than vegetable protein. Optimum performance can be attained from meat chickens when the poultry are raised on diets formulated with animal proteins. However, vegetable proteins have great potential in poultry diets because they are inexpensive, safer and the raw materials are easier to process than animal proteins.

However, the search for and the appropriate use of vegetable proteins demand more research to explore their potential use in poultry diets and the diets of other farm animals. The research on vegetable protein needs to focus on a number of areas including the variations in their chemical and physical nature, amino acid composition, and presence of deleterious materials, mode of digestion, absorption and assimilation, biological value, nutritive status, and the changes that take place during processing owing to the source, type, variety, breeding, harvesting technique, and storage methods.

Despite the advantages of vegetable proteins, there are some associated problems. These include productivity, utilization of their energy content, nutrient digestibility, and acceptability by chickens, intestinal morphologies, gut physiology and the immune response of birds fed these diets can be seriously affected by the nutritional quality of the vegetable protein diets. However, previous studies on the nutritional quality of vegetable diets with or without the addition of enzymes, other supplements, animal proteins and so forth have reported varying degree of success in terms of growth response, feed utilization, digestibility, feed efficiency and selection. There are a number of impediments to improving vegetable protein diets for broiler chickens.

Firstly, proper feed selection, post-harvest processing and storage techniques need to be devised to eliminate the inherent detrimental factors of vegetable diets, and to preserve their optimum quality. Secondly, there are inconclusive results on the effectiveness of enzyme supplementation, and problems associated with amino acid imbalance, poor digestibility, and the physical and chemical nature of the vegetable diets. Thirdly, research into the interrelationship between gut micro flora and microbial enzyme efficacy on feed utilization and performance of broiler chickens on these diets is either lacking or there are gaps.

Diets free of animal proteins (fish meal, meat and bone meal, blood meal etc.) have been examined by several workers, utilizing different combinations of vegetable proteins with or without supplementation of enzymes and synthetic amino acids (Baghel and Netke, 1987; Reddy and Eswaraiah, 1989, 1991). However, the inferences drawn from their studies were widely different as the results were dependent upon the type of vegetable protein utilized, the combination of different sources, the level of inclusion in diets and the amino acid profile of the protein mixtures used in the practical diets of broiler chickens (Sadagopan *et al*., 1993). It is in this context that an attempt has been made to develop VP diets by utilizing two popular and commercially available protein sources namely soybean and canola meals along with a combination of basal grains for economic poultry production.

The four experiments that were conducted in the provided data on productivity, meat and litter quality, leg bone development, energy utilization, nutrient digestibility and feed selection of broilers fed on vegetable protein diets with or without fishmeal and enzymes.

7.2 PRODUCTIVITY, LITTER QUALITY, MEAT YIELD TRAITS AND LEG BONE DEVELOPMENT OF BIRDS ON TEST DIETS

The aim of this project was to establish a degree of difference in the productive performance between chicks on all-vegetable diets and those on traditional diets. In Chapters 3 and 4, the results revealed that the response of broiler chickens with respect to feed intake, body weight and feed efficiency was better on regular or traditional diets than in exclusively vegetable diets. Liveability of broiler chickens was similar in both diet groups. The impaired growth of broilers fed sole vegetable diets might have been a result of lower feed intake, poor nutrient digestibility, poor energy utilization, and presence of anti-nutritive factors and other non-specific nutrient deficiencies of these diets.

The higher feed intake of the birds on AP diets as opposed to VP diets was also observed by Bhuiyan *et al.* (2012b). However, vegetable protein diets might have deficiencies of certain amino acids, particularly creatine along with other indispensable amino acids, which might affect the performance of the birds. Ringel *et al*. (2007) reported that creatine supplementation to VP diets improved the performance of broiler chickens significantly as compared to birds on sole VP diets.

The broiler chickens raised on the AP diets demonstrated significantly improved live weight, with a superior FCR to the VP treatment groups. This trend was maintained throughout the production cycle and became more pronounced towards the end of the experimental period. The results suggest that birds fed AP diets containing fishmeal supported better growth and better efficiency mainly through the stimulation of feed intake. Moreover, the better performance of broilers fed AP diets might be due to the combination of protein sources which would better amino acid balance. The results agree with the findings of previous researchers (Radhakrisnan *et al*, 2001; Alali *et al*., 2011; Hossain *et al.*, 2011a), who observed similar responses with diets containing animal proteins. The improvement in growth of the birds might also result from improved nutrient digestibility and presence of unidentified growth factors in fishmeal (Barlow and Windsor, 1984; EI Boushy and van der Poel, 1994) along with interactions of known nutrients such as vitamin B_{12}, selenium (Grastilleur, 2003), creatine (Ringel *et al*., 2007), carnitine and taurine (Comb, 1998). Conversely, the growth on VP diets might be impaired by the deficiency of some nutrients in these diets. Previous studies have reported improvement in growth performance of broilers when they were fed VP diets supplemented with carnitine and taurine (Rabie and Szilagyi, 1998).

In Chapter 3, excreta moisture level, pH and ammonia concentration were measured on day 21 to ascertain the litter quality of broiler chickens raised on vegetable or animal protein diets. The excreta material of birds on animal protein diets had lower moisture content than that of birds on VP diets. There was no significant difference in excreta pH and concentration of ammonia between the two diet groups (Chapter 3).

Higher moisture content in excreta material might cause deterioration in the litter quality, which, apart from causing inconvenience to the animal shed workers, might expose the birds to litter-borne diseases and affect their health status. The findings from this project supported the results of Vieira and Lima (2005), Takahashi *et al*. (2004) as well as those of Eichner *et al*. (2007), who reported that broilers fed diets formulated solely vegetable ingredients produce a higher amount of moisture and ammonia in their excreta. This excess moisture affects the litter quality adversely and leads to an increased risk of development of footpad dermatitis and other diseases. Conversely, lower moisture content in the excreta of birds fed on the AP or regular diets may be due to better osmotic regulation, a response that may be due to the presence of taurine in fish meal (Comb, 1998; Park and Choi, 1997).

Broilers grown on either vegetable or animal protein diets gave similar meat yield (dressing percentage, carcass weights, breast, drumstick, thigh, giblet, neck and shank weights) and internal visceral organ (small intestines, proventriculus, gizzard, pancreas, spleen, liver, bursa) development except for the abdominal fat content (Chapter 3 and 6). Birds fed on animal protein diets accumulated more abdominal fat content than the birds fed on VP diets. This implies that broilers grown on VP diets tended to produce less fat in their carcass. The advantage of this concept can be used in the formulation of diet for broiler chickens. When lean meat is desired, birds may be fed vegetable–based diets, which will result in lower fat and higher protein deposition in their carcasses (Singh and Panda, 1992). The substantial reduction in carcass fat, cholesterol and improvement of fatty acid make up of poultry meat could therefore bring about nutritional and economical benefits to consumers and producers alike. A resultant increase in concentration of unsaturated fatty acids can lower the cholesterol level of human consumers, reducing the risk of diseases.

Broilers raised on animal protein diets developed stronger leg bone than those on VP diets (Chapter 3). This was confirmed by higher bone breaking strength and longer latency-to sit times by the group of birds fed the regular (animal protein) diets. The poor leg health of broilers on the

VP diets might have resulted from the phytate content of the plant feeds, although these diets were supplemented with microbial enzymes. The anti-nutritive properties of phytate cannot be eliminated completely by phytase supplementation because degradation of phytate is incomplete (Selle and Ravindran, 2007).

The lack of endogenous phytases makes phytate phosphorus unavailable to monogastric animals. Research findings, as observed in Chapter 5, indicated that in the vegetable diets, macro-mineral (Ca, P, Mg) digestibility was unaffected by any dietary treatment or supplementation with enzymes, and also reported by Hossain *et al*. (2013a). Therefore, higher phytate content (76.4 % of total P) in plant feedstuffs particularly canola meal (Selle *et al.*, 2003) might affect the mineral availability leading to depressed leg bone development of the broilers fed VP diets. This idea is supported by Thorpe and Beal (2000) who reported that phytate is a common anti-nutritive factor of oilseed meals and plants, which has the potential to form indigestible complexes with cations (Ca, Mg, Fe) and bind with protein (Reddy *et al*., 1982) to reduce the availability of these nutrients (Leeson and Summers, 2001; Al-Kaiesy *et al.*, 2003) as well as increase environmental pollution.

Bone mineralization was very similar in both diet groups except for total tibia ash, Fe and Cu contents. The concentration of Fe and Cu was significantly higher in animal protein diet (CMF) which might also contribute to the better bone quality for this diet group of birds. It has been reported that the deficiency of trace minerals such as copper and iron can retard bone growth and reduce bone strength despite the adequacy of macro-minerals (Ca, P) in the diet (Medeiros *et al.*, 1997). The increased micro-nutrient (Fe, Cu) contents of AP diet might, to some degree, stimulate better bone growth and improved bone strength. However, other bone characteristics (weight, length, width, total tibia ash and breaking strength) of broiler chickens were not influenced by the dietary treatments up to day 21, as shown by the results presented in Chapter 6. This similarity in bone growth and development might be due to equal availability of nutrients in both AP and VP diets.

7.3 ENERGY UTILIZATION OF BIRDS ON TEST DIETS

Energy content plays a pivotal role in formulating diets for poultry. Most of the dietary energy comes from plant sources in the form of starch from cereal grains. Protein sources may also supply a substantial amount of energy and their interaction with the main energy sources has a bearing on the overall energy supply and utilization. The performance of birds is closely associated with feed nutrients and energy utilization, which is primarily related to availability of more nutrients and energy from the feed ingredients (Olukosi *et al*., 2008). Hence, modern poultry meat production is now highly competitive and a small difference in the efficiency of utilization of the supplied feedstuffs to the birds can be economically significant (Pirzgoliev and Rose, 1999).

In Chapter 4, the results showed that there was no difference between the dietary AME contents and protein intake of VP and AP (fishmeal) diets, but ME and fat intake on the fishmeal-supplemented diets were higher than in the birds on VP diets. The higher ME intake of birds may be a result of higher feed consumption of birds in this study. The higher feed intake may also imply higher consumption of fat. Conversely, the reduction in ME intake of broiler chickens on VP diets may be due to a high fibre content and presence of anti-nutritive factors in these diets, as has been observed by some researchers (Warenham *et al*., 1994; Jan *et al.*, 2008). Phytic acid, in particular can adversely affect energy utilization and the availability of other nutrients in poultry diets (Ravindran *et al*., 2005), although the diets in the present study were incorporated with exogenous microbial enzymes.

Broilers on AP diets utilized energy better as shown by higher NEp with no significant differences in HP as evident from the results shown in Chapter 4. This group of birds (AP) also attained heavier body weight than the VP diet groups. This improvement in NEp and performance of birds is evidence of more efficient utilization of energy on the animal protein diets. Olukosi *et al.* (2008) reported that an improvement in energy utilization may be due to improvement in nutrient and energy availability. Heat production of birds did not differ significantly between treatments, although birds in the regular or animal protein diet groups had a numerically higher level of HP than the VP diet groups. This increase in HP on the AP diets may be due to higher feed intake, in particular protein intake (Johnson, 2007).

In general, more energy is retained as protein than as fat, especially on the AP diets (Chapter 4). This also represents a more efficient use of energy utilization which might stimulate to give rise to less fatty carcass of the broiler chickens. Leeson and Summers (1997) have reported that abdominal fat of birds increases with age whereas protein accretion decreases. This is simply related to the maturity of birds and is found commonly in most strains (Leeson, 1995). Results shown in Chapter 4 revealed that the proportion of the retention of protein was found to be higher than that of fat as birds in the tested age group (0-21d) are still in the actively growing phase of production (Bregendahl *et al.*, 2002).

7.4 RESPONSE TO ENZYME SUPPLEMENTATION

Protein digestibility of broilers was higher with fishmeal supplemented diets than on VP diets only, but this did not significantly affect energy (gross energy) and starch digestibility (Chapter 3). The protein digestibility of fishmeal-supplemented diets (AP) was similar, and the digestibility of vegetable protein diets (SBM; CM) was also statistically similar in nature. The higher fibre content of VP diets, particularly canola meal might contribute to poorer digestibility of these VP diets than the AP diets. Apart from this, various factors, such as the presence of anti-nutritive factors, the nature of the protein and amino acid balance as well as a higher proportion of crude fibre might also be responsible for the poor digestibility of vegetable feedstuffs (Viera *et al*., 2005; Hossain *et al.*, 2011b). Furthermore, although properly processed oilseed meals (e.g. soybean, canola and cotton seed meals) might be devoid of deleterious agents, the complex structure, chemical or physical nature of VP diets might prevent the birds from getting the full nutritional benefits from the diets by suppressing their digestibility and efficient nutrient utilization.

However, micronutrient (amino acid and mineral) digestibility of VP diets increased significantly when they were supplemented with microbial enzymes as observed in Chapter 5. The main function of enzymes is to increase digestibility of feeds. Thus, supplementation of VP diets with microbial enzymes might help to increase the digestibility of such diets by degrading the anti-nutritive factors, particularly NSPs and phytate of the diets. Therefore, the addition of microbial enzymes to VP diets might help the birds to break down the mineral-phytate bond, thus making available the nutrients and increasing the mineral digestibility (Rama Rao *et al*., 2006; Narcy *et al*., 2009). Furthermore, it has been reported that carbohydrases help to increase the access of phytase to its substrate and facilitates the absorption of nutrients by reducing intestinal viscosity and releasing the encapsulated nutrients (Ravindran *et al.*, 1999; Selle *et al.*, 2003).

The gross response of broilers with respect to feed intake and live weight was significantly increased as a result of supplementation with microbial enzymes in VP diets while FCR was improved (Chapter 5). The beneficial effects of using these enzymes in plant–based diets has been reported by many previous researchers (Zanella *et al*., 1999; Ravindran *et al*., 2000; Cafe *et al*., 2002; Cowieson and Ranvindran, 2008; Bhuiyan *et al*., 2010, 2012a).

However, the tissue protein contents and intestinal and pancreatic enzyme activities (except maltase) were not influenced by supplemental enzymes to the VP or AP diets as seen from the results described in Chapters 5 and 6. The mechanism of internal enzymes activities was not obvious, but the response might likely be due to increased tissue protein synthesis at the intestinal and whole body levels.

7.5 FEED SELECTION AND/OR PREFERENCE OF TEST DIETS BY BIRDS

In Chapter 6, the results confirmed the improved performance of birds on animal protein diets throughout the production cycle compared to when they are started on the VP diets. However, switching the latter to AP diet ensured recovery in growth. Bird's response in terms of feed intake, BWG, FCR and mortality along with tissue protein growth, internal enzyme efficacy, digestive organs and bone development were unaffected, possibly as a result of adoption of new feeding mechanisms, nutrient metabolism or combination of these strategies, which would need to be investigated in future studies.

However, it was obvious that the growth of broilers was significantly better on AP diets than the birds fed VP diets in the earlier stage of growing period. The results are similar to our previous studies as shown in earlier Chapters (3 and 4), and reported by Hossain *et al*. (2011a and 2012a, b, 2013b). There tends to be a benefit of including AP in the diet or at least switching birds to such a diet at some point during the production cycle.

The results of the feed selection test in Chapter 6 indicated that birds preferred canola diet (CM) to soybean diet (SBM) when given a choice. A similar trend in feed consumption was also observed in the previous experiments (Chapters 3 and 4). The reason for the higher feed intake of broilers on CM diet is not clear, but the preference of birds may be influenced by several factors, for example, crude fibre content, physical and chemical properties of individual ingredient, etc. Apart from these factors, organoleptic properties of the food materials such as colour, smell, odour, flavour, taste or palatability might also play a key role in feed selection of broiler chickens (Cruze *et al*., 2005). Palatability of diets, metabolic and nutritional requirements might have a profound effect in dietary self-selection of the birds (Hughes, 1984; Forbes, 1995).

As is evident from the formulation profile, the higher fibre content of CM diet perhaps induced slightly higher feed intake. Besides, higher feed intake may be a result from faster growth of individual birds and the consequent higher nutritional requirement (Shrivastava *et al*., 1981). In a similar trial, broilers preferred AP diets to VP diets when they were given a choice between the two diets although performance was not different on the two diets (Bhuiyan *et al*., 2012b). The reason for this choice was not identified.

7.6 CONCLUSIONS AND RECOMMENDATIONS

An overview of the results obtained in this project indicates that broiler chickens fed on fishmeal-supplemented diets had comparatively better performance in terms of live weight gain, feed intake and FCR than birds fed exclusively with VP diets. In addition, ileal digestibility of proteins and bone development were better on the former diets than on the latter. Litter quality was also better with AP diets but carcasses were fattier on such diets. The latter may have some implications on consumer choice.

Enzyme supplementation of VP diets may be ideal as it significantly boosted the growth performance of the broiler chickens. Of the two main VP ingredients tested, birds tended to prefer the canola-containing diet to the soybean diets. The reason for this choice is unclear but it may be due to differences in physical and chemical properties of the two ingredients.

Generally there were only minor differences in performance between birds on VP and AP diets. It is likely that these differences can be closed through microbial enzyme supplementation. The results indicated that broilers can be raised satisfactorily on VP diets when duly supplemented with microbial enzymes and limiting amino acids to meet the requirements. There is a need for further research on the value of these supplementations. Another area that warrants investigation are changes in gut microbiology which may have a bearing on health.

There is a need to conduct detailed analyses of the anti-nutritional factors, including fibre and toxic substances, present in the VP ingredients. The protein quality of the two main VP ingredients, particularly amino acids balance should also be investigated. While the data on response to enzyme supplementation would be valuable towards improving these sources, it may need to be combined with other interventions such as feed processing, e.g. grinding technique (particle size) and pelleting. A cost benefit analysis will identify the economics of feeding AP against the VP diets. Further down the line is the possibility of using plant breeding to improve the nutrient balance of the ingredients.

REFERENCES

Adeola, O. (2005). Metabolizable energy and amino acids digestibility of high-oil maize, low-phytate maize and low-phytate soybean meal for White Pekin ducks. *British Poultry Science, 46*, 607-614.

Agbede, J. O., and Aletor,V. A. (2003). Comparative evaluation of weaning foods from Glycidia or Leucaena leaf protein concentrate and some commercial brands in Nigeria. *Journal of Science, Food and Agriculture, 84*, 21-30.

Aduku, A. O., and Olukosi, J. O. (2000). Animal Products Handling and in the Tropics, 2nd edn. GU Publications, Abuja, Nigeria, pp.1-67.

Ahmad, M. H., Miah, M. Y., Ali, M. A., and Hossain, M. A. (2006). Effect of different protein concentrates replacement of fish meal on the performance of broilers. *International Journal of Poultry Science, 5*, 952-956.

Akande, K. E., Doma, U. D., Agu, H. O., and Adamu, H. M. (2010). Major Anti-nutrients Found in Plant Protein Sources. *Pakistan Journal of Nutrition, 9,* 827-832.

Alali, W. Q., Hofacre, C. L., Mathis, G. F., and Batal, A. B. (2011). Effect of plant-based protein meal use in poultry feed on colonization and shedding of Salmonella Heidlberg in broiler birds. *Journal of Agriculture, Food and Analytical Bacteriology, 1*, 45-53.

Al-Kaiesy, M. T., Abdul-Kader, H. A., Mohammad, M. H., and Saeed, A. H. (2003). Effect of [gamma]-irradiation on anti-nutritional factors in broad bean. *Radiation Physics and Chemistry, 67*, 493-496.

Al-Masri, M. R. (2003). Effect of feeding broiler chicks a completely vegetable finishing ration on the apparent metabolizable energy, total protein efficiency and carcass marketing properties. Report on Scientific Laboratory Study, no.496/RSS. *Atomic Energy Commission*, Damascus, Syria, pp.1-50.

Al-Masri, M. R. (2006). Productive performance and digestive tract size of broiler chicks fed a conventional or all-vegetable ingredient diets. *Journal of Applied Animal Research*, *30*, 81-84.

Al-Ostwani, A. G., Hassan, E., Hashem,Y., Saadi, M. A., and Adawi, N. (2000). Using of barley and cotton seed meal in broiler diets in terms of reducing the cost. *Al-Thawra J. No.7695, p.11, Damascus, Syria*.

Ammerman, C. B. (Ed.). (1995). *Methods for estimation of mineral bioavailability. In Bioavailability of Nutrients for Animals: Amino Acids, Minerals, and vitamins, pp. 83-94 [C. B. Ammerman , Baker, D. H. and Lewis, A. J., editor], New York Academic Press.*

Anderson, D. L., and Henderson, L. J. (1986). Sealed chamber digestion for plant nutrient analysis. *Agronomy Journal*, *78*, 937-938.

Anderson, J. W., Johnstone, B. M., and Cook-Newwel, M. E. (1995). Meta-analysis of the effects of soy protein intake on serum lipids. *The New England Journal of Medicine*, *333*, 276–282.

AOAC. (1994). Association of Official Analytical Chemists. Official Method of Analysis (13th edition), Washington, DC, USA.

Arieli, A. (1998). Whole cottonseed in dairy cattle feeding: a review. *Journal of Dairy Science*, *72*, 97-100.

Bach Knudsen, K. E. (1997). Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology*, *67*, 319-338.

Baghel, R. P. S., and Netke, S. P. (1987). Economic broiler rations based on vegetable proteins. *Indian Journal of Animal Nutrition*, *4*, 24-27.

Balog, J. M., and Millard, R. J. (1989). Influence of the sense of taste on broiler chick feed consumption. *Poultry Science, 68*, 1519-1526.

Barlow, S. M., and Windsor, M. L. (1984). Fishery by-products. International Association of Fishmeal Manufacturers. International fishmeal and oil manufacturers association, 2 College Yard, Lower Dangnall Street, Herts, AL3 4PA, UK, Technical Bulletin No: 1.

Barteczko, J. A. N., Lasek, O., and Augustyn, R. (2008). Energy utilization and nutritive value of maize grain cultivars for Broiler chickens. *Journal of Central European Agriculture, 9*, 209-211.

Batterham, E. S. (1992). Availability and utilization of amino acid for growing pigs. *Nutrition Research Review, 5*, 1-18.

Bcic, A., Harris, P. J., and Stone, B. A. (1988). Structure and function of plant cell walls. Pages 297-372 in *The Biochemistry of Plants*. *Vol. 14, J. Preiss, ed. Academic Press, Inc., London*.

Bellaver, C., Costa, C. A. F., Avila, V. S., Fraha, M., Lima, G. J. M. M., Hackenhar, L., and Baldi, P. (2005). Substitution of animal by-product meal by vegetable ingredients in diets for broilers. *Ciência Rural, 35*, 671-677.

Bellaver, C., Brum, P. A. R., Lima, G. M. M., Boff, J., and Kerber, J. (2001). Substituição parcial do farelo de soja pela farinha de vísceras de aves em dietas balanceadas com base na proteína e em aminoácidos totais ou digestíveis para frangos de corte. *Revista Brasileira de Ciência Avícola, 3*, 233-240.

Berg, C., and Sanotra, G. S. (2003). Can a modified latency-to-lie test be used to validate gait-scoring results in commercial broiler flocks. *Animal Welfare, 12*, 655–659.

Bhat, R., Sridhar, K. R., and Yokotani, K. T. (2007). Effect of ionising radiation on anti-nutritional features of velvet seed bean (Mucuna pruriens). *Food Chemistry, 103*, 860-866.

Bhuiyan, M. M., Islam, A. F., and Iji, P. A. (2010). Response of broiler chickens to diets containing artificially dried high-moisture maize supplemented with microbial enzymes. *South African Journal of Animal Science, 40*, 348-362.

Bhuiyan, M. M., Clatworthy, G., and Iji, P. A. (2012a). Vegetable protein diets are adequate but broiler chickens prefer animal protein diets. *World's Poultry Science Journal, Vol. 68, Suppl.1, CD-ROM.*

Bhuiyan, M. M., Hossain, M. A., and Iji, P. A. (2012b). Preference of broiler chickens for animal and vegetable protein diets. *Australasian Medical Journal*, *5*, 657.

Bilgili, S. F., Alley, M. A., Hess, J. B., and Nagaraj, M. (2006). Influence of age and sex on foot pad quality and yield in broiler chickens paw (feet) reared on low and high density diets. *Journal of Applied Poultry Research, 15*, 433-441.

Black, J. L. (2001). Variation in nutritional value of cereal grains across livestock species. In: *Proceedings of the Australian Poultry Science Symposium, Sydney, Australia, 21*, 99-102.

Boekholt, H. A., Van der Grinten, P. H., Scheurs, V. V. A. M., Los, M. J. N., and Leffering, C. P. (1994). Effect of dietary energy restriction on retention of protein, fat and energy in broiler chickens. *British Poultry Science, 35*, 603–614.

Borchers, R., Anderson, C. W., and Mussehl, F. E. (1948). Trypsin inhibitor.VI. Effect of various heating periods on the growth promoting value of soybean oil meal for chicks. *Poultry Science, 27*, 601-604.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248-254.

Brannon, P. M. (1990). Adaptation of the exocrine pancreas to diet. *Annual Review of Nutrition, 10*, 88-105.

Bregendahl, K., Sell, J. L., and Zimmerman, D. R. (2002). Effect of low protein diets on growth performance and body composition of broiler chicks. *Poultry Science, 81*, 1156-1167.

Brickett, K. E., Dahiya, J. P., Classen, H. L., Annett, C. B., Gomis, S. (2007). The impact of nutrient density, feed form, and photoperiod on the walking ability and skeletal quality of broiler chickens. *Poultry Science, 86*, 2117-2125.

Café, M. B., Borges, C. A., Fritts, C. A., and Waldrop, P. W. (2002). Avizyme improves performance of broilers fed corn-soybean meal-based diets. *Journal of Applied Poultry Research, 11*, 29-33.

Caires, C. M., Fernandes, E. A., Fagundes, N. S., Carvalho, A. P., Maciel, M. P., and Oliveir, B. R. (2010). The use of animal by-products in broiler feeds. *Brazilian Journal of Poultry Science, 12*, 41-46.

Cancherini, L., Junqueira, O. M., Andreotti, M .O., Barbosa, M. J. B., and Oliveira, M. C. (2004). Utilização de subprodutos de origem animal em dietas para frangos de corte com base no conceito de proteínas bruta e ideal, no período de 43 a 49 dias de idade. *Revista Brasileira de Zootecnia, 33*, 2060-2065.

Castell, A. G., Guenter, W., Igbasan, F. A. (1996). Nutritive value of peas for non-ruminant diets. *Animal Feed Science and Technology, 60*, 209-227.

CEC. (2000). Council Regulation 2000/766 concerning certain protection measures with regard to transmissible spongiform encephalopathies and the feeding of animal protein. *Official Journal of European Communities, 43*, 32-33.

Chandler, N. J. (1994). A re-evaluation of protein meals from the rendering industry. 2. International Feed Congress and Exhibition. *April 6-8, Kuşadası-Turkey*.

Chen, P., Ji, P., and Li, S. L. (2008). Effects of feeding extruded soybean, ground canola seed and whole cottonseed on ruminal fermentation, performance and milk composition and fatty acid profile in early lactation dairy cow. *Asian-Australian Journal of Animal Sciences, 21*, 204- 213.

Choct, M., Hughes, R. J, Wang, J., Bedford, M. R., Morgan, A. J., and Annison, G. (1996). Increased small intestinal fermentation in partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science, 37*, 609-621.

Clandinin, D. R. (1973). Factors affecting the feeding value of rapeseed meal for chickens. *Research on rape seed meal and oil'' page 62, Publication number-32. Second progress report, Research Committee, Rapeseed Association of Canada*.

Clandinin, D. R., Robblee, A. R., Slinger, S. J., and Bell, J. M. (1981). Composition of canola meal. In: *Canola meal for Livestock and Poultry*. Pub. No. 59, 8-11, Canola Council of Canada, Winnipeg, Manitoba.

Classen, H. L., Campbell, G. L., and Grootwassink, J. W. D. (1988). Improved feeding value of Saskatchewan-grown barley for chickens with dietary enzyme supplementation. *Canadian Journal of Animal Science, 68*, 1253–1259.

Classen, H. L., and Bedford, M. R. (1999). The use of enzymes to improve the nutritive value of poultry feeds. In: *Recent Development in Poultry Nutrition* 2. Eds Wiseman, J. and Garnsworthy, P.C., Nottingham University Press, Nottingham, UK, pp. 285-308.

Coetzee, G. J. M., and Hoffman, L. C. (2002). Effects of various dietary n-2/n-6 fatty acid ratios on the performance and body composition of broilers. *South African Journal of Animal Science, 32*, 175-184.

Cohen, S. A., and Michaud, D. P. (1993). Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high performance liquid chromatography. *Analytical Biochemistry*, *211*, 279-287.

Cohen, S. A. (2001). Amino acids analysis using precolumn derivatisation with 6-aminoquinoly l-N-hydroxysuccinimidyl carbamate. In C. Copper, Packer, N. and Williams, K. (Ed.), *Molecular Biology* (*Vol.159*), USA: Humana Press, Totowa, N. J.

Colibar, O., Crainiceanu, E., Darabus, G., and Mot, M. (2001). Vegetable protein sources-a way to improve the performance of broilers. *Lucrai Stiinifice—Medicina Veterinaria, Universitatea de Stiinte Agricole si Medicina Veterinara "Ion Ionescu de la Brad"Iasi, 44*, 850-851.

Comb, G. F. (1998). Vitamins Fundamental Aspects in Nutrition and Health. 2nd Edn., Academic Press, San Diego, USA, pp: 430-438.

Consleg. (2004). Serviço das Publicações Oficiais das Comunidades Européias. Texto consolidado Regulamento no 1774/2002 do parlamento Europeu e do Conselho da União Européia; 2004. p.154 [citado em: 2005 mar] Disponível em: http://europa.eu.int/eur-lex /pt /consleg/pdf /2002 /pt_2002 R1774do_001. Pdf.

Coon, C. N., Leske, K. L., Akavanichan, O., and Cheng, T. K. (1990). Effect of oligosaccharide -free soybean on true metabolizable energy and fibre digestion in adult roasters. *Poultry Science*, *69*, 787-793.

Cowieson, A. J., Acamovic, T., and Bedford, M. R. (2003). Supplementation of diets containing pea meal with exogenous enzymes: effect on weight gain, feed conversion, nutrient digestibility and gross morphology of the gastrointestinal tract of growing broiler chicks. *British Poultry Science*, *44*, 427-437.

Cowieson, A. J., Acamovic, T., and Bedford, M. R. (2006). Phytic acid and phytase: Implications for protein utilization by poultry. *Poultry Science*, *85*, 878-885.

Cowieson, A. J., and Ravindran, V. (2008). Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy, minerals and amino acids. *British Poultry Science*, *49*, 37-44.

Cowieson, A. J., and Bedford, M. R. (2009). The effect of phytase and carbohydrase on ileal amino acid digestibility in mono-gastric diets: complementary mode of action? *World's Poultry Science Journal*, *65*, 609-624.

Cowieson, A. J. (2010). Strategic selection of exogenous enzymes for corn-based poultry diets. *Journal of Poultry Science*, *47*, 1-7.

Crenshaw, T. D., Peo, E. R., Lewis Jr., A. J., and Moser, B. D. (1981). Bone strength as a trait for assessing mineralization in swine. A critical review of techniques involved. *Journal of Animal Science*, *53*, 827-835.

Crèpon, K. (2007). Nutritional value of legumes (pea and faba bean) and economics of their use. In P. C. Garnsworthy and J. Wiseman, eds. *Recent Advances in Animal Nutrition*. pp. 331–366. Nottingham, UK, Nottingham University Press.

Cruz, V. C., Ducatti, C., Araújo, P. C., Sartori, J. R., and Piccinin, A. (2009). Effect of poultry viscera meal inclusion in broiler diets in different rearing periods on performance, carcass and parts yields. *Brazilian Journal of Poultry Science*, *11*, 161-165.

Cruze, V. C., Pezzato, A. C., Pinheiro, D. F., Goncalves, J. C., and Sartori, J. R. (2005). Effects of free-choice feeding on the performance and ileal digestibility of nutrients in broilers. *Brazilian Journal of Poultry Science, 7,* 143-150.

Cumming, R. B., and Mastika, I. M. (1987). Why choice feeding trials have failed. In: *Proceedings of the Poultry Husbandry Research Foundation Symposium, Sydney, Australia,* 23-29.

Dale, N. (1996). Variation in feed ingredients quality: Oilseed meals. *Animal Feed Science and Technology*, *59*, 129-135.

De Toledo, T. C. F., Canniatti-Brazaca, S. G., Artur, V., and Piedade, S. M. S. (2007). Effects of gamma radiation on total phenolics, trypsin and tanin inhibitors in soybean grains. *Radiation Physics and Chemistry*, *76*, 1653-1656.

Decuypere, E., Bruggeman, V., Barbato, G. F., and Buyse, J. (2003). Growth and reproduction problems associated with selection for increased broiler meat production. Pages 13-28 in *Poultry Genetics, Breeding and Biotechnology*; W. M. Muir, and S. E. Aggrey, ed. CABI Publ., Oxon, UK.

Dilger, R. N., and Baker, D. H. (2008). Cystine imbalance and its effect on methionine precursor utilization in chicks. *Journal of Animal Science, 86*, 1832-1840.

Donkoh, A., Anang, D. M., Atuahene, C. C., Koomson, B., and Oppong, H. G. (2001). Further studies on the use of solar-dried blood meal as a feed ingredient for poultry. *Journal of Animal Feed Science*, *10*, 159 – 167.

Douglas, M. W., Parsons, C. M., and Bedford, M. R. (2000). Effect of various soybean meal sources and Avizyme on chick growth performance and ileal digestible energy. *Journal of Applied Poultry Research*, *9*, 74-80.

Dublecz, K. (2003). Poultry nutrition without animal protein sources. *Allattenyesztes es Takarmanyozas*, *52*, 479-489.

Duke, G. E. (1986). Alimentary canal: secretion and digestion, special digestive functions and absorption. In P. D. Sturkie (Ed.), *Avian Physiology* (pp.289-302): Springer-Verlag, New York.

Eichner, G., Vieira, S. L., Torres, C. A., Coneglian, J. L. B., Freitas, D. M., and Oyarzabal, O. A (2007). Litter moisture and footpad dermatitis as affected by diets formulated on an all-vegetable basis or having the inclusion of poultry by-product. *Journal of Applied Poultry Research, 16*, 344-350.

El Boushy, A. R. Y., and van der Poel, A. F. B. (1994). *Poultry feed from waste*. 1st Edn Chapman and Hall, p.408.

Emmans, G. C. (1977). The nutrient intake of hens given a choice of diets in relation to their protein requirement. *British Poultry Science, 18*, 227-236.

FAO. (2000). Food and Agriculture Organization of the United States (http://aps.fao.org/default.htm).

Faria F. D. E., Rosa, P. S., Viera, B. S., Macari, M., and Furlan, R. L. (2005). Protein levels and environmental temperature effects on carcass characteristics, performance, and nitrogen excretion of broiler chickens from 7 to 21 days of age. *Brazilian Journal of Poultry Science, 7*, 247-253.

Farkas, J. (2006). Irradiation for better foods. *Trends in Food Science and Technology*, *17*, 148-152.

Fasuyi, A. O., Aletor, V. A. (2005). Varietal composition and functional properties of cassava leaf meal and leaf protein concentrates. *Pakistan Journal of Nutrition, 4*, 43-49.

Fleet, J. C., and Saylor, W. W. (1983). Interaction of dietary electrolytes and coccidiostats in broilers. *Poultry Science, 62*, 1422-1423.

Forbes, J. M., and Sharitatmadari, F. S. (1994). Diet selection for protein by poultry. *World's Poultry Science Journal*, *50*, 7--24.

Forbes, J. M. (1995). Voluntary food intake and diet selection in farm animals. CAB International, p.532.

Forbes, J. M. (2006). Food choice and intake in chickens. In V. Bels, ed. *Feeding in domestic vertebrates from structure to behaviour,* pp. 108–119, Wallingford, UK, CABI.

Francesch, M., and Brufau, J. (2003). Nutritional factors affecting excreta/litter moisture and quality. In: *Proceeding of 14th European Symposium on Poultry Nutrition, Norway*, 266-273.

Francesch, M., and Brufau, J. (2004). Nutritional factors affecting excreta/litter moisture and quality. *World Poultry Science, 60*, 64-75.

Gharaghani, H., Zaghari, M., Shahhoseini, G., and Moravej, H. (2008). Effect of gamma irradiation on anti-nutritional factors and nutritional value of canola meal for broiler chickens. *Asian-Australasian Journal of Animal Sciences, 21*, 1479-1485.

Ghazi, S., Rooke, J. A., and Galbraith, H. (2003). Improvement in nutritive value of soybean meal by protease and α-galactosidase treatments in broiler cockerels and broiler chicks. *British Poultry Science, 44*, 410-418.

Giang, V. D., Chinh, B.V., Huyen, D.V., and Ha, N. N. (2001). Potential and characteristics of animal feeds in Vietnam. *Composition and Nutritive Values of Animal Feeds in Vietnam*. Agricultural Publishing House, Hanoi, Vietnam, pp. 22- 36.

Graham, K. K., Kerley, M. S., Firman, J. D., and Alle, G. l. (2002). The effect of enzyme treatment of soybean meal on oligosaccharide disappearance and chick growth performance. *Poultry Science, 81*, 1014-1019.

Grastilleur, D. (2003). Substitution of animal and fish protein meals with other protein sources. In: *Proceedings of 14th European Symposium on Poultry Nutrition*, *Norway*, pp. 261-265.

Gatel, F. (1994). Protein quality of legume seeds for non-ruminant animals: A literature review. *Animal Feed Science and Technology, 45*, 317– 348.

Hamilton, C. R. (2002). Real and perceived issues involving animal proteins. In: FAO Animal production and Health Proceedings "Protein sources for animal the Animal Feed Industry'. Expert Consultation and Workshop, Bangkok, 29/04 to 03/05. FAO Animal Production and Health Proceedings.

Hargis, P. H., and Creger, C. R. (1980). Effects of varying dietary protein and energy levels on growth rate and body fat in broilers. *Poultry Science*, *59*, 1499-1504.

Havenstein, G. B., Ferket, P. R., Scheidler, S. E., and Larson, B. T. (1994). Growth, liveability and feed conversion of 1957 vs 1991 broilers when fed ’’typical’’1957 and 1991 broilers diets. *Poultry Science*, *73*, 1785-1794.

Hill, F. W., and Totsuku, K. (1964). Studies of the metabolizable energy of cotton seed meals for chicks with particular reference to the effects of gossypol. *Poultry Science, 43*, 362-370.

Hooge, D. M., and Rowland, L. O. (1978). Effect of dietary sand on feed conversion of broilers and laying hens. *Poultry Science, 57*, 1145.

Hossain, M. A., Islam, A. F., and Iji, P. A. (2011a). Performance of broiler chickens fed diets based on all-vegetable ingredients. In: *Recent Advances in Animal Nutrition-Australia*, *18*, 99-100.

Hossain, M. A., Islam, A. F., and Iji, P. A. (2012a). Energy utilization and growth responses of broiler chickens on vegetable protein diets. In: *Proceedings of Australian Poultry Science Symposium, Sydney, Australia*, *23*, 276- 279.

Hossain, M. A., Islam, A. F., and Iji, P. A. (2012b). Energy Utilization and Performance of Broiler Chickens Raised on Diets with Vegetable Proteins or Conventional Feeds. *Asian Journal of Poultry Science, 6*, 117-128.

Hossain, M. A., Islam, A. F., and Iji, P. A. (2013a). Impact of exogenous enzymes on micronutrient digestibility, tissue protein contents, and internal enzyme activities of broilers fed on all-vegetable diets. In: *Proceedings of the 8th International Poultry Show and Seminar, WPSA-BB, Bangladesh*, pp:135-141.

Hossain, M. A., Islam, A. F., and Iji, P. A. (2013b). Growth responses, excreta quality, nutrient digestibility, bone development and meat yield traits of broiler chickens fed on vegetable or animal protein diets. *South African Journal of Animal Science, 43(In press).*

Hruby, M., Hamre, M. L., and Coon, C. N. (1995). Free-choice feeding and three temperature treatments. *Journal of Applied Poultry Research, 4*, 356-365.

Hughes, B. O. (1984). The principles underlying choice-feeding behaviour in fowls-with special reference to production experiments. *World's Poultry Science Journal, 40*, 141-150.

Hughes, R. J., and Choct, M. (1999). Chemical and physical characteristics of grains related to variability in energy and amino acid availability in poultry. *Australian Journal of Agricultural Research, 50*, 689-701.

Hossain, M. A., Islam, M. M, Islam, A. F., and Iji, P. A. (2011b). Constraints to use of all-vegetable feed ingredients and strategies to improve such diets for poultry birds: A Review. *Bangladesh Research Publication Journal, 6*, 120-135.

Hofacre, C. L., White, D. G., Maurer, J. J., Morales, C., Lobsinger, C., and Hudson, C. (2001). Characterization of antibiotic-resistant bacteria in rendered animal products. *Avian Disease, 45*, 953-961.

Huisman, J., and Tolman, G. H. (1992). Anti-nutritional factors in the plant proteins of diets for non-ruminants. In: *Recent Advances in Animal Nutrition* 1992 (P.C. Garnsworthy, W. Haresign and D. J. A. Cole, editors), Butterworth Heinemann Ltd, Oxford, UK, pp. 3-31.

Iji, P. A., Saki, A., and Tivey, D. R. (2001a). Body and intestinal growth of broiler chicks on a commercial starter diet. 2. Development and characteristics of intestinal enzymes. *British Poultry Science, 42*, 514-522.

Iji, P. A., Saki, A., and Tivey, D. R. (2001b). Body and intestinal growth of broiler chicks on a commercial starter diet. *British Poultry Science, 42*, 505.

Iji, P. A., Khumalo, K., Slippers, S., and Gous, R. M. (2004). Intestinal function and body growth of broiler chickens on maize-based diets supplemented with mimosa tannins and a microbial enzyme. *Journal of the Science of Food and Agriculture, 84*, 1451-1458.

Ikeme, A. I. (Ed.). (1990). *Meat Science and Technology. A comprehensive approach. African FEP Publication Ltd., p. 320*.

Iris, G. G., and Balnave, D. (1993). Poor performance of broilers fed diets containing soybean meal as the sole protein concentrates. *Australian Journal of Agricultural Research, 44*, 1467-1481.

Jackson, S., Summers, J. D., and Lesson, S. (1982). Effect of dietary protein and energy on broiler performance and production cost. *Poultry Science, 61*, 2232-2240.

Jan, B., Olga, L., and Romana, V. (2008). Energy utilization and nutritive value of maize grain cultivars for Broiler chickens. *Journal of Central European Agriculture, 9,* 209-211.

Janocha, A., and Milczarek, A. (2006). The effects of vegetable diets in slaughter yield and fatty acid profile of meat in broiler chickens. *Roczniki Instytutu Przemysu Miesnego I Tuszczowego, 44,* 71-79.

Jeroch, H., and S., Danicke, S. (1996). Barley in poultry feeding: a review. *World's Poultry Science Journal, 51*, 271–291.

Jiang, Z., Y., Zhou, Y., Lu, F., Han, Z., and Wang, T. (2008). Effects of different levels of supplementary-alpha-amylase on digestive enzyme activities and pancreatic amylase mRNA expression of young broilers. *Asian-Australasian Journal of Animal Sciences, 21*, 97-102.

Jensen, L. S., Bartov, I., Beirnie, M. J., Veltmann, J. R., and Fetcher, D. L. (1980). Reproduction of the oily bird syndrome in broilers. *Poultry Science, 59,* 2256-2266.

Johnson, D. E. (2007). Contributions of animal nutrition research to nutritional principles: energetics. *Journal of Nutrition, 137,* 698–701.

Karunajeewa, H. (1978). The performance of cross-bred hens given choice feeding of whole grains and a concentrate mixture and the influence of source of xanthophylls on yolk colour. *British Poultry Science, 19*, 699.

Kermanshahi, H., and Abbasi-Pour, A. R. (2006). Replacement value of soybean meal with rapeseed meal supplemented with or without a dietary NSP-degrading enzyme on performance, carcass traits and thyroid hormones of broiler chickens. *International Journal of Poultry Science, 5*, 932-937.

Kestin, S. C., Kowles, T. G., Tinch, A. E., and Gregory, N. G. (1992). Prevalence of leg weakness in broiler chicken and its relationship with genotype. *Veterinary Record*, *131*, 190-194.

Kestin, S. C., Su, G., and Sorensen, P. (1999). Different commercial broiler crosses have different susceptibilities to leg weakness. *Poultry Science, 78*, 1085–1090.

Khajali, F., Rafiei, F., and Faraji, M. (2007). Replacement of canola meal for soybean meal in female broiler diets with or without addition of enzyme supplementation. In: *Proceedings of the 16th European Symposium on Poultry Nutrition, France,* pp.55-57.

Kocher, A. (2001). Enzymatic degradation of non-starch polysaccharides in vegetable proteins in poultry diets. In: *Recent Advances in Animal Nutrition-Australia, 13*, 163-168.

Kocher, A. (2002). Use of oilseed meals in broiler diets: effects of feed enzymes. In: *Proceedings of the Australian Poultry Science Symposium, Sydney, Australia, 14*, 89-96.

Kralik, G., Ivankovic, S., and Skrtic, Z. (2002). Changing the profile of fatty acid of muscle tissue of broilers. Krmiva, *44*, 297-305.

Kristensen, H. H., and Wathes, C. M. (2000). Ammonia and poultry welfare: a review. *World's Poultry Science Journal*, *56*, 235-245.

Kritchevsky, D. (1990). Protein and atherosclerosis. *Journal of Nutrition Science and Vitamin*, *36* (suppl), S81-S86.

Krogdahl, A., Lee, T. B., and Olli, J. J. (1994). Soybean protease inhibitors affect intestinal trypsin activities and amino acid digestibility in rainbow trout (Oncorhynchus mykiss). *Comparative Biochemistry and Physiology, 110*, 215 -219.

Karimi, A. (2006). The effects of varying fishmeal inclusion levels (%) on performance of broiler chicks. *International Journal of Poultry Science, 5*, 255-258.

Larbier, M., and Leclercq, B. (1992). Energy metabolism. In: *Nutrition and Feeding of Poultry*, pp. 47–73 [J Wiseman, editor], Nottingham: Nottingham University Press.

Larbier, M. L. (1992). Metabolism of water and minerals. In: *Nutrition and Feeding of Poultry*, pp.101-118 [M. L. Larbier, editor], Nottingham University Press.

Lautner, V., and Zenisek, Z. (1965). Estimation of the energy value of components of feed mixtures for chickens. *Nutrition Abstract Review, 35,* 540.

Lazaro, R. M., Latorre, A., Medel, P., Garcia, M., and Mateos, G. G. (2004). Feeding regimen and enzyme supplementation to rye-based diets for broiler. *Poultry Science, 83*, 152-160.

Le Goff, G., and Noblet, J. (2001). Comparative digestibility of dietary energy and nutrients in growing pigs and adult sows. *Journal of Animal Science, 79*, 2418-2427.

Leeson, S., Atteh, J. O, and Summers, J. D. (1987). The replacement value of canola meal for soybean meal in poultry diets. *Canadian Journal of Animal Science, 67*, 151-158.

Leeson, S., Caston, L., and Summers, J. D. (1996). Broiler response to energy and protein dilution in the finisher diets. *Poultry Science, 75*, 522-528.

Leeson, S., and Summers, J. D. (1997). Feeding programs for broilers. In *Commercial Poultry Nutrition*, 2nd ed., pp. 207–254 [S Leeson and JD Summers, editors], Guelph: University Books.

Leeson, S., Summers, J. D. (Ed.). (2001). *Nutrition of the Chicken. 4th ed. Ontario: University Books, p. 413.*

Leske, K. L., Jevne, C. J., and Coon, C. N. (1993). Effect of oligosaccharide addition on nitrogen corrected true metabolizable energy of soy protein concentrates. *Poultry Science, 72*, 664-668.

Lesson, S. (1995). New concepts in feeding broiler. XIV Congreso Latinoamericano de Chile, pp. 30-40.

Lewis, J., Fenwick, G. R. (1987). Glucosinolate content of Brassica vegetables: Analysis of twenty-four cultivars of calabres. *Food Chemistry, 25*, 259-268.

Liener, I. E. (1953). Sotin, a toxic protein from the soybean. 1. Inhibition of rat growth. *Journal of Nutrition*, *49*, 527-539.

Liener, I. E. (1980). *Toxic constituents of plant foodstuffs*. Second edition, New York and London, Academic Press, p.502.

Liener, I. E. (1989). In: Proc. Recent Advances of Research in Anti-nutritional Factors in Legume Seeds, Eds. J. Huisman, A. F. B. van der Poel and J. E. Liener, pp. 6-13.

Liu, H., Luo, P., Chen, S., and Shang, S. (2011). Effects of squid ink on growth performance, antioxidant functions and immunity in growing broiler chickens. *Asian-Australasian Journal of Animal Sciences*, *24*, 1752-1756.

Lodhi, G. N., Renner, R., and Glandinin, D. R. (1969). Studies on the metabolizable energy of rape seed meal for growing chickens and laying hens. *Poultry Science, 48*, 964-970.

Lopez, G., and Leeson, S. (2005). Utilization of metabolizable energy by young broilers and birds of intermediate growth rate. *Poultry Science, 84*, 1069–1076.

Lung, B. D., and Man, L. H. (1999). *Poultry feeds and feeding*. Agricultural Publishing House, Hanoi, Vietnam, p. 211.

Mahmoudnia, N., Boldaji, F., Dastar, B., and Zerehdaran, S. (2011). Nutritional evaluation of poultry by-product meal in broiler chickens. *Animal Biology and Animal husbandry International Journal of the Bio-flux Society*, *3*, 55-61.

Maiorka, A., da Silva, A.V. F., and Santin, E. (2004). Broiler breeder age and dietary energy level on performance and pancreas lipase and trypsin activities of 7 days old chick. *International Journal of Poultry Science, 3*, 234-237.

March, B. E., Smith, T., and EI-Lankany, S. (1973). Variation in estimates of the metabolizable energy of rapeseed meal determined with chickens of different ages. *Poultry Science, 52*, 614-618.

Mounteney, G. F. (Ed.). (1975). *Poultry Production Technology. 2nd edn. Avian Publication, USA*, pp.*1-25*.

March, B. E., and Biely, J. (1971). An evaluation of supplementary protein and metabolizable energy value of rape seed meal for chicks. *Canadian Journal of Animal Science, 51*, 749-756.

March, B. E., and Biely, J. (1973). Chemical, physical and nutritional characteristics of different samples of wheat. *Canadian Journal of Animal Science, 53,* 569–577.

McCleary, B. V., Solah, V., and Gibson, T. S. (1994). Quantitative measurement of total starch in cereal flours and products. *Journal of Cereal Science, 20*, 51-58.

McDonald, P., Edwards, R. A., and Greenhalgh, J. F. D., (Ed.). (1992). *Animal Nutrition* (ed.): Published in the United States with John Wiley and Sons. Inc. NewYork, 4th Edn, pp. 455-483.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D, and Morgan, C. A. (2002). *Animal Nutrition*, Six Edition, Pearson Education Limited, Edinburgh Gate, Harlow, Essex CM20 2JE, England.

Mcpherson, G. A. (1985). Lowry program: Elsevier-BIOSOFT, 68 Hills Rd, Cambridge, CB2, ILA, UK.

Medeiros, D. M., Ilich, J., Ireton, J., Matkovic, V., Shiry, L., and Wildman, R. (1997). Femurs from rats fed diets deficient in copper or iron have decreased mechanical strength and altered mineral composition. *The Journal of Trace Elements in Experimental Medicine, 10*, 197–203.

Mendes, A. A. (2003). Rastreabilidade oficial avícola em discussão [citado em 2005 jan]. Disponível em: http: //www. dipemar.com.br/carne/315/materia_especial_carne.htm.

Mendonca, C. X., and Jensen, L. S. (1989). Effect of formulating diets with different assigned energy data for poultry by-products meal on the performance and abdominal fat content of finishing broilers. *Poultry Science, 68*, 1672-1677.

Meng, X., Slominiski, B. A., Nyachoti, C. M., Campbell, L. D., and Guenter, W. (2005). Degradation of cell wall polysaccharides by combination of carbohydrase enzymes and their effect on nutrient utilization and broiler chickens performance. *Poultry Science, 84*, 37-47.

Meng, X., and Slominski, B. A. (2005). Nutritive values of corn, soybean meal, canola meal and peas for broiler chickens as affected by a multi-carbohydrases preparation of cell wall degrading enzymes. *Poultry Science, 84*, 1242-1251.

Mihailovic, V., Matic, R., Mikic, A., and Cupina, B. (2005a). Possibilities for utilization of spring vetch for grain. *Contemporary Agriculture, LIV, 3-4*, 318-322.

Moran, E. T. (1985). Digestion and absorption of carbohydrates in fowl and events through perinatal development. *Journal of Nutrition, 115*, 665.

Miles, R. D., and Nelson, T. S. (1974). The effect of enzymatic hydrolysis of phytate on the available energy content of feed ingredients for chickens and rats. *Poultry Science, 53*, 1714-1717.

Mihailovic, V., Mikic, A., Eric, P., Vasiljevic, S., Cupina, B., and Katic, S. (2005b). Protein pea in animal feeding. *Biotechnology in Animal Husbandry, 21*, 281-285.

Mikić, A., V. Perić, V. Đorđević, M. Srebrić, and Mihailović, V. (2009). Anti-nutritional factors in some grain legumes. *Biotechnology in Animal Husbandry, 25*, 1181-1188.

Monin, G. (1998). Recent methods for predicting quality of whole meat. Meat Science 1998; 49: S231-S243.

Moran, E. T., and Summers, J. D. (1968). Keratins as sources of protein for the growing chick, 5: practical application of feather and hog hair meals in broiler diets; effects on growth, feed utilisation and carcase quality. *Poultry Science, 47*, 940-945.

Moran, E. T. J., Summers, J. D. and Slingers, S. J. (1966). Keratin as a source of protein for the growing chicks. *Poultry Science, 45*, 1257-1264.

Moura, B. H. S. (2003). *Desempenho e composição da carcaça de frangos de corte alimentados com diferentes níveis energéticos com e sem óleo [dissertação]*. Belo Horizonte: Escola de Veterinária, UFMG.

Murakami, A. E., Oviedo-Rondon, E. O., Martins, E. N., Pereira, M. S., and Scapinello, C. (2001). Sodium and chloride requirements of growing broiler chickens (21 to 42 days of age) fed corn-soybean diets. *Poultry Science, 80*, 289-294.

Narcy, A., Letourneau-Montminy, M. P., Magnin, M., Lescoat, P. Jondreville, C. Sauvant, D., and Nys, Y. (2009). Phosphorus utilization in broilers. *Paper presented in the 17th European Symposium on Poultry Nutrition, Edinburg, Scotland*, pp.14-20.

Nesse, L. L., Nordby, K., Heir, E., Bergsjoe, B, Vardund, T., Nygaard, H., and Holstad, G. (2003). Molecular analyses of Salmonella enterica isolates from fish feed factories and fish feed ingredients. *Application of Environmental Microbiology, 69*, 1075-1081.

Newkirk, R. W., Classen, H. L., and Tyler, R. T. (1997). Nutritional evaluation of low glucosinolate mustard meals (Brassica juncea) in broiler diets. *Poultry Science, 76*, 1272-1277.

Noble, D. O., Picard, M. L., Dunnington, E. A., Uzu, G., Larsen, A. S., and Siegel, P. B. (1993). Food itake adjustment of chicks: Short term reactions of genetic stocks to deficiencies in lysine, methionine, or tryptophan. *British Poultry Science, 34*, 725-735.

Newkirk, R. W., and Classen, H. L. (2001). The non-mineral nutritional impact of phytate in canola meal for broiler chicks. *Animal Feed Science and Technology, 9*, 115-128.

Newkirk, R. W., and Classen, H. L. (2002). The effects of toasting canola meal on body weight, feed conversion efficiency, and mortality in broiler chickens. *Poultry Science*, *81*, 815-825.

Nitsan, Z., Dror, Y., Nir, I., and Shapira, I. (1974). The effects of force-feeding on enzymes of the liver, kidney, pancreas and digestive tract of chicks. *British Journal of Nutrition, 32*, 241-247.

Nitsan, Z., Avraham, G. B., Zerof, Z., and Nir, I. (1991). Growth and development of the digestive organs and some enzymes in the broiler chicks after hatching. *British Poultry Science*, *32*, 515-523.

Noblet, J. (2006). Energy evaluation of feeds for pigs: Consequences on Diet Formulation and Environment Protection, *Lohmann Information, Vol. 41*, pp. 38-50.

Noy, Y., and Sklan, D. (1995). Digestion and absorption in the young chicks. *Poultry Science*, *74*, 366-373.

Noy, Y., and Sklan, D. (2001). Nutrient use in chicks during the first week post hatch. *International Journal of Poultry Science, 81*, 391-399.

NRC. (1994). *Nutrient Requirement of Poultry*, 9th Revised Edition, National Research Council. National Academy Press, Washington, D.C., USA, p.70.

Ojewola, G. S., and Ewa, U. E. (2005). Response of growing broiler to varying dietary plant protein. *International Journal of Poultry Science, 4*, 765-771.

Olomu, J. M. (Ed.). (1995). *Mono-gastric Animal Nutrition, 2nd edn. Principles and Practice. Jachem. Publication,* p. 320.

Olukosi, O. A., Cowieson, A. J., and Adeola, O. (2008). Energy utilization and growth performance of broilers receiving diets supplemented with enzymes containing carbohydrase or phytase activity individually or in combination. *British Journal of Nutrition, 99*, 682–690.

Olukosi, O. A., Cowieson, A. J., and Adeola, O. (2010). Broiler responses to supplementation of phytase and admixture of carbohydrases and protease in maize-soybean meal diets with or without maize distillers dried grain with soluble. *British Poultry Science, 51*, 434-443.

Oluyemi, J. A., and Roberts, F. A. (2000). *Poultry production in warm wet climates*. Macmillan Press Ltd. 2nd Edn. Spectrum Books Ltd., Ibadan, Nigeria.

Omenka, R. O., and Anyasor, G. N. (2010). Vegetable-based feed formulation on poultry meat quality. *African Journal of Food, Agriculture, Nutrition and Development, 10,* 2001-2011.

Orban, J. I., Adeola, O., and Stroshine, R. (1999). Microbial phytase in finisher diets of white Pekin ducks: Effect on growth performance, plasma phosphorus concentration, and leg bone characteristics. *Poultry Science, 78*, 366–377.

Park, C. S., Kwon, S. K., and Min, T. H. (1983). The effect of seedling pelleted diets on energy metabolism and nitrogen relation in growing chickens. *Research Reports of Agriculture Science and Technology, 10*, 206-211.

Parsons, C. M., Potter, L. M., and Brown Jr, R. D. (1980). True metabolizable energy and amino acid digestibility of dehulled soybean meal. *Poultry Science*, *60*, 2687.

Parsons, C. M., Castanon, F., and Han, Y. (1997). Protein and amino acid quality of meat and bone meal. *Poultry Science, 76,* 361-368.

Parsons, C. M., and Wang, X. (1998). Dietary formulation with meat and bone meal on a total versus a digestible or bioavailable amino acid basis. *Poultry Science*, *77*, 1010-1015.

Pawlak, A., Gornowiccz, E., and Dziadek, K. (2005). Effect of protein type in feed mixtures on performance and meat quality of broiler chickens. *Roczniki Naukowe Zootechniki*, *32*, 115-125.

Payne, R. L. (2007). The potential for using low crude protein diets for broilers and turkeys. Degussa Amino News, 8, 2–13.

Peng, Y. L., Guo, Y. M., and Yuam, J. M. (2003). Effects of microbial phytase replacing partial inorganic phosphorus supplementation and xylanase on the growth performance and nutrient digestibility in broiler fed wheat-based diets. *Asian-Australasian Journal of Animal Science*, *16*, 239-247.

Perez-Maldonado, R. A., Blight, G. W., and Pos, J. (2001). Upper limits of inclusion of canola meal and cottonseed meal in diets formulated on a digestible amino acid basis for broiler. In: *Proceedings of the Australian Poultry Science Symposium*, *Sydney*, *Australia*, *13*, 156-159.

Park, G. H., and Choi, H. S. (1997). Effect of dietary taurine on growth of broiler chicks and its physiological mechanisms. *Korean Journal of Animal Science, 39*, 124-134.

Peron, A., and Partridge, G. G. (2010). Other Enzyme Applications Relevant to the Animal Feed Industry. In: *Enzymes in Farm Animal Nutrition*, Bedford, M. R. and G. G. Partridge (Eds.). 2nd Ed., CAB International, Wallingford, UK., p: 243.

Picard, M. L., Uzu, G., Dunnington, E. A., and Siegel, P. B. (1993). Food intake adjustments of chicks: short-term reactions to deficiencies in lysine, methionine, and tryptophan. *British Poultry Science, 34*, 737-746.

Pierson, E. E., Potter, L. M., and Shelton, J. R. (1979). Response from Menhaden Fish Meal in Diets of Young Turkeys. *Poultry Science, 58*, 616-625.

Pierson, E. E. M., Potter, L. M., and Brown Jr, R. D. (1980). Amino acid digestibility of dehulled soybean meal by adult turkeys. *Poultry Science, 59*, 845.

Pike, I. H. (1976). Formulation of poultry diets for maximum protein requirements for protein. *Feedstuffs, 48*, 25-33, 52.

Pike, I. H. (1979). Unidentified growth factor of fish meal: Possible nutritional explanations. *Feedstuffs*, *51*, 32-33.

Pike, I. H. (1999). Health benefit from feeding fish oil and fishmeal: The role of long chain omega-3 polyunsaturated fatty acids in animal feeding. International fishmeal and oil manufacturers association, 2 College Yard, Lower Dangnall Street, Herts, AL3 4PA, UK, Technical Bulletin No : 28.

Pinchasov, Y., Nir, I., and Nitsan, Z. (1990). Metabolic and anatomical adaptation s of heavy-bodied chicks to intermittent feeding. 2. Pancreatic digestive enzymes. *British Poultry Science, 31*, 769-777.

Pirgozliev, V., Bedford, M. R., Acamovic, T., Mares, P., and Allymehr, M. (2011). The effects of supplementary bacterial phytase on dietary energy and total tract amino acid digestibility when fed to young chickens. *British Poultry Science, 52*, 245-254.

Pirzgoliev, V., and Rose, S. P. (1999). Net energy systems for poultry feeds: a quantitative review. *World's Poultry Science Journal, 55*, 23-36.

Rabie, M. H., and Szilagyi, M. (1998). Effects of L-carnitine supplementation of diets differing in energy levels on performance, abdominal fat content, and yield and composition of edible meat of broilers. *British Journal of Nutrition, 80,* 391-400.

Rakariyatham, N., and Sakorn, P. (2002). Biodegradation of glucosinolates in brown mustard meal (Brassica juncea) by *Aspergillus spp.* NR-4201 in liquid and solid culture. *Biodegradation,* 3, 395- 409.

Rama Rao, S. V., Raju, M.V. L. N., Reddy, M. R., and Pavani, P. (2006). Interaction between dietary calcium and non-phytate calcium levels on growth, bone mineralization and mineral excretion in commercial broilers. *Animal Feed Science and Technology, 131*, 133-148.

Rassmussen, P. B., and Petterson, D. (1997). A multi-component carbohydrase improves the production performance of broilers fed diets containing soybean meal in mixtures or soybean and canola meal as plant protein sources. In: *Proceedings of the 9th Australian Poultry Science Symposium, Sydney, Australia, 9*, 157-160.

Ravindran, G., and Bryden, W. L. (2005). Tryptophan determination in proteins and feedstuffs by ion change chromatography. *Food Chemistry, 89*, 309-314.

Ravindran, V., Cabahug, S., Bryden, W. L., and Selle, P. H. (1999). The influence of microbial phytase on the bioavailability of protein and energy in broiler chickens. In: *Phyatse in Animal Nutrition and Waste Management* (editors M. B. Coelho and E.T. Kornegay), BASF Monograph Series, BASF Corporation, Parsippany, New Jersey, USA, pp. 573-584.

Ravindran, V., Cabahug, S., Ravindran, G., Bryden, W. L., and Selle, P. H. (2000). Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on nutrient digestibility and retention. *British Poultry Science, 41*, 193-200.

Radhakrishnan, L., Kadirvel, R., and Vijayakumar, R. (2001). Replacement of fishmeal with vegetable protein sources in broiler diets. *Indian Journal of Poultry Science, 36*, 300-304.

Ravindran, V., Selle, P. H, Ravindran, G., Morel, P. C. H., Kies, A. K., and Bryden, W. L. (2001). Microbial phytase improves performance, apparent metabolizable energy and ileal amino acid digestibility fed a lysine-deficient diets. *Poultry Science, 80*, 338-344.

Ravindran, V., Sands, J. S., Hruby, M., and Partridge, G. G. (2005). Influence of phyzyme tm XP phytase on nutrient utilization in broilers fed diets containing graded levels of phytate. In: *Proceedings of the Australian Poultry Science Symposium, 17*, 238-241.

Rechant, M. (1995). Effect of B-gluconase on performance of broiler chicks and laying hens. *Canadian Journal of Animal Science, 73*, 941-951.

Reddy, N. R., Sathe, S. K., and Salunkhe, D. K. (1982). Phytases in legumes and cereals. *Advances in Food Research, 28*, 1-91.

Reddy, V. R., and Eshwaraiah (1989). Effect of graded replacement of fishmeal with vegetable proteins in broiler starter rations. *Indian Journal of Animal Nutrition, 6*, 166-169.

Reddy, V. R., and Eshwaraiah (1991). Performance of broilers on vegetable sources of proteins. *Indian Journal of Animal Nutrition, 8*, 301-304.

Rehman, A., Bhatti, B. M., Hameed, S., and Afzal, S. (2002). Effects of substitution of soybean meal with canola and sunflower meals on the performance of broilers. *Pakistan Veterinary Journal, 22*, 52-55.

Remignon, H., and Le Bihan-Duval, E. (2003). Meat quality problems associated with selection for increased production. Pages 53-66 in *Poultry Genetics, Breeding and Biotechnology*. W. M. Muir, and S. E. Aggrey, ed. CABI Publ., Oxon, UK.

Renner, R., and Hill, F. W. (1960). Studies of the effect of heat treatment on the metabolizable energy value of soybean and extracted soybean flakes for the chicks. *Journal of Nutrition, 70*, 219-225.

Ricketts, J., and Brannon, P. M. (1994). Amount and type of dietary fat regulate pancreatic lipase gene expression in rats. *Journal of Nutrition, 124*, 1166-1167.

Ringel, J., Lemme, A. Knox, A., McNab, J., and Redshaw, M. S. (2007). Effects of graded levels of creatine and guanidino acetic acid in vegetable-based diets on performance and biological parameters in muscle tissue. In: *Proceedings of the 16th European Symposium on Poultry Nutrition, France,* pp.387-390.

Ristic, M., and Damme, K. (2001). Changing from animal to vegetable protein at the fattening. Consequences on carcass and meat quality of broiler. *Fleischwirtschaft, 81*, 114-116.

Robinson, D., and Singh, D. N. (2001). Rural Industries Research and Development Corporation. *Queensland Poultry Research and Development Centre, March Publication N° DAQ-241*.

Rojas, S. W., and Scott, M. L. (1969). Factors affecting the nutritive value of cotton seed meal as a protein source in chick diets. *Poultry Science, 48*, 819-835.

Rose, S. P., and Kyriazakis, I. (1991). Diet selection of pigs and poultry. In: *Proceedings' of the Nutrition Society, 50*, 87-98.

Rosen, G. (2006). Exogenous enzymes as pro-nutrients in broiler diets. In J. Wiseman and P.C. Garnsworthy, eds. *Recent Developments in Non-ruminant Nutrition*, Nottingham, UK, Nottingham University Press, pp. 163–191.

Routman, K. S., Yoshida, L., Frizzas de Lima, A. C., Macari, M., and Pizauro Jr., J. M. (2003). Intestinal and pancreas enzyme activity of broilers exposed to thermal stress. *Brazilian Journal of Poultry Science, 5*, 23-27.

Roy, B. C., Ranvig, H., Chowdhury, S. D., Rashid. M. M., and Chwalibog, A. (2004). Evaluation of compound broiler feeds manufactured in Bangladesh. *Livestock Research for Rural Development, 16,* 11.

Sadagopan, V. R., Shyam S. G., and Dange, M. (1993). Response of broilers to vegetable protein sources combination diets. *Indian Journal of Poultry Science, 28,* 249-252.

Sakomura, N. K., Silva, R, Couto, H. P., Coon, C., and Pacheco, C. R. (2003). Modelling metabolizable energy utilization in broiler breeder pullets. *Poultry Science, 82*, 419–427.

Saur, W. C., Cichon, R., and Misir, R. (1982). Amino acid availability and protein quality of canola and rapeseed meals for pigs and rats. *Journal of Animal Science, 54*, 292-301.

Sauvant, D., Perez, J. M., and Tran, G. (2004). Tables of composition and nutritional values of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses, fish; D. Sauvant, J. M., Perez, and Tran, G. (Eds). Wageningen Academic Publishers, Wageningen and INRA Editions, Versailles.

Scaife, J. R., Moyo, J., Galbraith, H., Michie, W., and Campbell, V. (1994). Effect of different dietary supplemental fats and oils on the tissue fatty acid composition and growth of female broilers. *British Poultry Science, 35*, 107-118.

Sebastian, S., Touchburn, S. P., Chavez, E. R., and Lague, P. C. (1997). Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. *Poultry Science, 76*, 1760–1769.

Selle, P. H., and Ravindran, V. (2007). Microbial phytase in poultry nutrition. *Animal Feed Science and Technology, 135*, 1-14.

Selle, P. H., Ravindran,V., and Bryden, W. L. (2000). The role of dietary enzymes in improving amino acids digestibility. *Asian-Australian Journal of Animal Science, (Suppl.C) 13*, 75.

Selle, P. H., Ravindran,V., Ravindran, G., Caldwell, R. A., and Bryden, W. L. (2003). Phytate and phytase: consequences for protein utilization. *Nutrition Research Review, 13*, 255-278.

Senkoylu, N., and Dale, N. (1999). Sunflower meal in poultry diets: A review. *World's Poultry Science Journal, 55*, 154–174.

Shawrang, P., Nikkhah, A., Zare-Shahneh, A., Sadeghi, A. A., Raisali, G., and Moradi-Shahrebabak, M. (2008). Effects of gamma irradiation on chemical composition and ruminal protein degradation of canola meal. *Radiation Physics and Chemistry, 77,* 918-922.

Shirazi-Beechey, S. P., Smith, M. W., Wang, Y., and James, P. S. (1991). Post-natal development of lamb intestinal digestive enzymes is not regulated by diet. *Journal of Physiology, 437*, 691-698.

Shirley, R. B., and Parsons, C. M. (2001). Effect of ash content on protein quality of meat and bone meal. *Poultry Science, 80*, 626-632.

Short, F. J., Gorten, P., Wiseman, J., and Boorman, K. N. (1996). Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology, 59*, 215–221.

Smits, C. H. M., and Annison, G. (1996). Non-starch polysaccharides in broiler nutrition-towards a physiologically valid approach to their determination. *World's Poultry Science Journal*, *52*, 203-221.

Shrivastava, H. P., Sadagopan, V. R., Johri, T. S., and Chand, S. (1981). Sunflower seed meal for poultry-a review. *Indian Poultry Gazette*, 65: 100-112.

Sibbald, I. R. (1982.). Measurement of bioavailable energy in poultry feeding stuffs: a review. *Canadian Journal of Animal Science, 62*, 983–1048.

Siddhuraju, P., Makkar, H. P. S., and Becker, K. (2002). The effect of ionising radiation on anti-nutritional factors and the nutritional value of plant materials with reference to human and animal food. *Food Chemistry, 78*, 187-205.

Singh, K. S., and Panda, B. (1990). Proceedings of the FAO expert consultation on the substitution of imported concentrate feeds in animal production systems in developing countries. FAO regional office for Asia and Pacific, Bangkok, Thailand.

Singh, K. S., and Panda, B. (1992). *Poultry Nutrition*. Kalyani Publishers, New Delhi, India, pp. 57-61.

Sinurat, A. P., and Balnave, D. (1986). Free choice feeding of broiler at high temperature. *British Poultry Science, 27*, 577-584.

Sirtori, C. R., Lovati, M. R., Manzoni, C., Gianazza, E., Bondioli, A., Staels, B., and Auwers, J. (1995). Reduction of serum cholesterol by soy proteins. *Nutritional, Metabolic and Cardiovascular Diseases, 8*, 334–340.

Slominski, B. A., Meng, X., Campbell, L. D., Guenter, W., and Jones, O. (2006). The use of Enzyme Technology for improved energy utilization from full–fat oilseeds. Part II: flaxseed. *Poultry Science, 85*, 1031-1037.

Smith, A., Rose, S. P., Well, R. G., and Pirgozliev, V. (2000). Effect of excess dietary sodium, potassium, calcium, and phosphorus on excreta moisture of laying hens. *British Journal of Nutrition, 41*, 598-607.

Smits, H. M., Veldman, A.,Verstegen, M..W. A., and Beynen, A. C. (1997). Dietary carboxylmethyl cellulose with instead of low viscosity reduces macronutrient digestion in broiler chickens. *Journal of Animal Nutrition, 127*, 483-487.

Smith, N. (2005). The effect of the dietary inclusion of canola oilcake, full-fat canola and sweet lupins on the production performance and fat composition of broilers and pigs. *M S Thesis, Department of Animal Science, University of Stellenbosch, South Africa*.

Solangi, A. A., Memon, A., Qureshi, T. A., Leghari, H. H., Baloch, G. M., and Wagan, M. P. (2002). Replacement of fishmeal by soybean meal in broiler ration. *Journal of Animal and Veterinary Advances, 1*, 28-30.

Steenfeldt, J. S., Knudsen, K. E. B., Borsting, C. F., and Eggun, B. O. (1995). The nutritive value of decorticated mill fractions of wheat. 2. Evaluation with raw and enzyme treated fractions using adult cockerels. *Animal Feed Science and Technology, 54*, 249-265.

Stefanidou, M., Maravelias, A., and Dona, A. (2006). Zinc: a multipurpose trace element. *Arch Toxicology, 80*, 1-9.

Summers, J. D., Slingers, S. J., and Ashton, G. C. (1965). Evaluation of meat meal and feather meal for the growing chickens. *Canadian Journal of Animal Science, 45*, 63-70.

Swatson, H. K., Iji, P. A., and Gous, R. M. (2003). Body growth, visceral organ weight and intestinal digestive enzyme of chickens on diets varying in energy and protein contents. *Journal of Animal and Veterinary Advances, 2*, 305-311.

Sweeney, R. A. (1989). Generic combustion method for determination of crude protein in feeds: Collaborative study. *Journal of Association Official Analytical Chemist*, *72*, 770-774.

Swiatkiewicz, S., and Arczewska-wlosek, A. (2011). Prospects for the use of genetically modified crops with improved nutritional properties as feed materials in poultry nutrition. *World's Poultry Science Journal, 67*, 631-642.

Senkoylu, N., and Dale, N. (2006). Nutritional evaluation of a high-oil sunflower meal in broiler starter diet. *Journal of Applied Poultry Research 15*, 40–47.

Swick, R. A., and Tan, P. H. (1997). Characteristics of protein meal: Considerations in using common Asian proteins meal. *Rev. Zootecnica International*, 14-25.

Tacon, A. G. J. (1992). Nutritional fish pathology: morphological signs of nutrient deficiency and toxicity in farmed fish. FAO Fisheries Technical Paper No. 330, FAO, Rome, p:75.

Takahashi, K., Jensen, L. S., and Bolden, S. (1984). Diet composition, environmental temperature and exogenous estradiol effects on hepatic lipid deposition in growing chicks. *Journal of Poultry Science, 63*, 524-531.

Thorpe, J., and Beal, J. D. (2000). In: *Enzymes in farm animal nutrition*. Eds. M. R. Bedford and G. G. Partridge, CABI Publishing, Wallingford, UK, pp.125-143.

Teguia, A., and Beynen, A. C. (2005). Alternative feedstuffs for broilers in Cameroon. *Livestock Research for Rural Development*, *17,* 5.

Takahashi, K., Matsushita, K., and Akiba, Y. (2004). Effect of substitution of fish meal with soybean meal on growth performances and excreta moisture contents during immunological stimulation in male broiler chicks. *Journal of Poultry Science, 41*, 241-247.

Thanaseelaan, V., Viswanathan, K., Chandrasekaran, D., Prabakaran, R. and Chellapandian, M. (2007). Chemical composition, amino acids, minerals and anti-nutritional factors of rapeseed meal. *Journal of Veterinary and Animal Science, 3*, 101-105.

Thompson, L. U., and Yoon, J. H. (1984). Starch digestibility as affected by polyphenol and phytic acid. *Journal of Food Science, 49*, 1228-1229.

Troche, C., Sun, X., McElory, A. P., Remus, J., and Novak, C. L. (2007). Supplementation of Avizyme 1502 to corn-soybean meal-wheat diets fed to turkey tom poults: the first fifty-six days of age. *Poultry Science, 86*, 496-502.

Uberoi, S. K., Vadhera, S., and Soni, G. L. (1992). Role of dietary fibre from pulses and cereals as hypocholesterolemic and hypolidemic agent. *Journal of Food Science Technology, 29*, 281–283.

Ullah, M. S., Pasha,T. N., Ali, Z., Saima, F., Khattak, F. M., and Hayat, Z. (2012). Effects of different pre-starter diets on broiler performance, gastrointestinal tract morphometry and carcass yield. *The Journal of Animal and Plant Sciences, 22*, 570-575.

United States Department of Agriculture (USDA) Economic Research Services. (2010). Soybeans and Oil Crops: Canola. http://www.ers.usda.gov/Briefing /Soybeans Oilcrops / Canola. htm.

Van der Heide, D., Huisman, E. A., Kanis, E., Osse, J. W. H., and Verstegen, M. W. A. (1999). Regulation of feed intake. Wallingford, UK, CABI Publishing.

Van Der Klis, J. D., Kwakernaak, C., Jansman, A., and Blok, M. (2010). Energy in poultry diets: Adjusted AME or Net Energy. In: *Proceedings of Australian Poultry Science Symposium, Sydney, Australia, 21*, 44-49.

Veldman, A., Vahl, H. A., Borggreve, G. J., and Fuller, D. C. (1995). A survey of the incidence of Salmonella species and Enterobacteriaceae in poultry feeds and feed components. *Veterinary Research, 136*, 169-172.

Vieira, S. L., Penz Jr., A. M., Leboute, E. M., and Corteline, J. (1992). A nutritional evaluation of a high fiber sunflower meal. *Journal of Applied Poultry Research, 1,* 382-388.

Vieira, S. L., Lima, I. L., Borges, C. A. Q., Fernandes, L. M., Quadros, V. R. (2003). Broiler utilization of vegetarian diets. *Poultry Science, 82,* 38.

Vieira, S. L., and Lima, I. L. (2005). Live performance, water intake and excreta characteristics of broiler fed all-vegetable diets based on corn and soybean meal. *International Journal of Poultry Science, 4,* 365-368.

Vieira, S. L., Viola, E. S., Berres, J., Coneglian, J. L. B., Freitas, D. M., and Bortolini, T. C. K. (2006). Water intake and digestive metabolism of broilers fed all-vegetable diets containing acidulated soybean soapstock. *Brazilian Journal of Poultry Science, 8,* 159-165.

Vig, A. P., and Walia, A. (2001). Beneficial effects of Rhizopus oligosporus fermentation on reduction of glucosinolates, fibre and phytic acid in rapeseed (Brassica napus) meal. *Bio-resource Technology, 78,* 309-312.

Villamide, M. J., and San Juan, L. D. (1998). Effect of chemical composition of sunflower seed meal on its true metabolizable energy and amino acid digestibility. *Poultry Science, 77*, 1884–1892.

Waldroup, P. W., Kersey, J. H., Saleh, E. A., Fritts, C. A., Yan, F., Stillborn, H. L., Crum Jr., R. C., and Raboy, V. (2000). Nonphytate phosphorus requirements and phosphorus excretion of broilers chicks fed diets composed of normal or high available phosphate corn with and without phytase. *Poultry Science, 79,* 1451-1459.

Wang, X., and Parsons, C. M. (1998). Dietary formulation with meat and bone meal on a total versus a digestible or bioavailable amino acids basis. *Poultry Science*, *77*, 1010-1015.

Wappnir, R. W. (1989). Protein digestion and absorption of mineral elements. In: Mineral absorption in the mono-gastric GI tract, pp. 95-115 [R. D Frederick and Laszlo, J. A., editors], New York and London: Plenum press.

Warenham, C. N., Wiseman, J., and Cole, D. J. A. (1994). Processing and anti-nutritive factors in feedstuffs, In: *Principles of Pig Science*, Eds. D. J. A. Cole, J. Wiseman and M. A. Varley Vol.1, pp. 141-167 (Nottingham, Nottingham Press).

Webster, A. B., Fairchild, B. D., Cummings, T. S., and Stayer, P. A. (2008). Validation of a three point gait scoring system for field assessment of walking ability of commercial broilers. *The Journal of Applied Poultry Research*, *17*, 529–539.

Windsor, M., and Barlow, S. (1981). Introduction to Fishery By-Products. *Fishing News Books Ltd., Farnham, England*.

Wiseman, J., and Lewis, C. E. (1998). Influence of dietary energy and nutrient concentration on the growth of body weight and carcass components of broiler chickens. *Journal of Agricultural Science*, *131*, 361-371.

Woodham, A. A., and Deans, P. S. (1977). Nutritive value of mixed proteins. I. In cereal-based diets for poultry. *British Journal of Nutrition, 37*, 289-308.

Xavier, S. A. G., Stringhini, J. H., de Brito, A. B., Cafe, M. B., Leandro, N. S. M., Andrade, M. A., and Laboissiere, M. (2012). Poultry viscera and bone meal in broiler pre-starter and starter diets. *Brazilian Journal of Animal Science, 41*, 934-940.

Xavier, S. A. G., Stringhini, J. H., de Brito, A. B., Andrade, M. A., Cafe, M. B., and Leandro, N. S. M. (2011). Feather and blood meal in pre-starter and starter diets for broilers. *Brazilian Journal of Animal Science, 40*, 1745-1752.

Yan, F., Kersey, J. H., Fritts, C. A., Waldroup, P. W., Stilborn, H. L., Crum Jr., D. C., Rice, D. W., and Raboy, V. (2000). Evaluation of normal yellow dent corn and high available phosphorus corn in combination with reduced dietary phosphorus and phytase supplementation for broilers grown to market weights in litter pens. *Poultry Science, 79*, 1282-1289.

Yo, T., Siegel, P. B., Guerin, H., Picard, M. (1997). Self-selection of dietary protein and energy by broilers grown under a tropical climate: Effect of feed particle size on the feed choice. *Poultry Science*, *76*, 1467-1473.

Yousri, R. M., Hilali, E. A., EI-Hakeim, N. F., and Farag, M. D. E. H. (1991). Studies on chicks fed irradiated animal protein by-products: Part-1: Biological studies. *Isotopenpraxis, 27*, 108-114.

Yuan, F. J., Zhu, D. H, Deng, B., Fu, X. J., Deng, D. K., Zhu, S. L., Li, B. Q., and Shu, Q. Y. (2009). Effects of two low phytic acid mutations on seed quality and nutritional traits in soybean (*Glycine max L. Merr*). *Journal of Agricultural Feed Chemistry*, *57*, 3632-3638.

Zanella, I., Sakomura, N. K., Silversides, F. G., Fiqueirdo, A., and Pack, M. (1999). Effect of enzyme supplementation of broiler diet based on corn and soybeans. *Poultry Science*, *78*, 561-568.

Zilkifli, I., Iman, R. H. S., Alimon, A. R., Vidyadaran, M. K., and Babjee, S. A. (2001). Response of choice-fed Red Jungle Fowl and commercial broiler chickens offered a complete diet, corn and soybean. *Asian-Australasian Journal of Animal Science, 14*, 1758-1762.

Zyla, K., Koreleski, J., Swiatkiewicz, S., Ledoux, D. R., and Pironen, J. (2001). Influence of supplemental enzyme on the performance and phosphorus excretion of broiler fed wheat-based diets to 6 weeks of age. *Animal Feed Science and Technology*, *89*, 113-118.