Impact of protein source and vitamin stability on broiler performance

by

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Abstract

A study was carried out to determine the effect of replacing fish meal with either soybean meal or poultry by-product meal on broiler performance and total feed cost per kg of gain. A second study evaluated the effect of storage time and trace minerals on the stability of vitamins stored at high temperature and relative humidity and their subsequent effects on broiler performance, bone strength and ash. A third study consisting of two experiments was conducted to determine the effects of particle size, diet, method of analysis (laboratory, ground and unground) and feed form (mash and pellet) on the crude protein predictability of the near infrared reflectance spectroscopy while using standard calibrations installed with the instrument. In study 1, three dietary treatments, 1) SBM-FM diet, 2) SBM diet and 3) SBM-PBM diet, were allocated to 36 pens using a completely randomized design with 12 replicates per treatment. Replacing FM with SBM and PBM in broiler diets improved growth performance and reduced total feed cost per kg of gain. In study 2, seven experimental treatments, 1) 0 d VP, 2) 30 d VTMP, 3) 30 d VP, 4) 60 d VTMP, 5) 60 d VP, 6) 90 d VTMP and 7) 90 d VP, were stored for 0, 30, 60 and 90 days, respectively in an environmentally controlled chamber at 29.4°C and 75%. Samples of treatments were analyzed, and loss of vitamin activity was calculated after storage. Treatments were added to broiler diets to determine the effect of loss of vitamin activity on broiler performance. Dietary treatments were set up as randomized complete block design in four batteries. Storing vitamins with trace minerals for 90 days increased loss of vitamin activity as compared to when stored as vitamin premix. Loss of vitamin activity did not significantly affect overall broiler performance, bone strength and ash. In study 3, Exp. 1 was a 3 × 3 × 4 factorial with corn particles size (400, 600 and 800 µm), method of analysis (laboratory, unground and ground) and diet (SD, SFD, SFB and SB). Diets were formulated to contain 20% crude protein.

Subsamples were ground through a 0.5 mm sieve. Crude protein contents of ground and unground samples were analyzed using the Foss DS2500 NIRS (Model Foss DS2500 Monochromator, Foss NIRSystems, Laurel, MD) and compared to laboratory results from wet chemistry analysis. Interaction ($P \leq 0.05$) was observed between diet and method and particle size and method, but similar ($P \geq 0.05$) crude protein was observed for particle size. Diets and particle sizes were significantly different ($P \leq 0.05$) as unground samples but no differences ($P \geq 0.05$) were observed when ground and analyzed using the NIRS or wet chemistry. Exp. 2 was a 3 ×2 factorial with method of analysis (laboratory, unground and ground) and feed form (mash and pellet). Diets were formulated to contain 20% crude protein and manufactured with 600 µm corn particle size. Portions of diets were pelleted using a pellet mill and cooled. Ground and unground mash and pellets were analyzed as in Exp. 1. Interaction was found ($P \leq 0.05$) between feed form and method of analysis. Feed form and method of analysis significantly ($P \leq 0.05$) affected crude protein prediction from the NIRS. Crude protein content of ground mash and pellets were similar ($P \geq 0.05$) to that of laboratory results. Generally, analyzing finished feed samples in the unground form with the NIRS while using standard calibrations yielded less accurate predictions for crude protein, but samples in the ground form yielded similar ($P \geq 0.05$) results when analyzed with either the NIRS or wet chemistry.

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Dedication

I humbly dedicate this dissertation to the Lord God Almighty who shielded me with His love and perfect word. Who blessed me with wisdom and knowledge to complete this thesis. Also, there are several people without whom this thesis would not have been possible. To my parents, Miss Jennifer Hinton and Mr. Michael Frempong; siblings, Eugene Oduro-Nimo and Louisa Gyamfuah; and my Kansas State Ghanaian family.

Chapter 1 - Protein Sources, Vitamin Stability and the Near Infrared Reflectance Spectroscopy

Protein Sources

Fish Meal

What is fish meal, the types and how is it processed?

Fish meal is produced from undecomposed whole fish, fish cuttings, either or both that has been cleaned, dried, ground which is with or without the extraction of part of the oil (AAFCO, 2018). There are several types of fish meal available depending on the type of fish used, but the most common types on the market are the menhaden, anchovy and pilchard. Fish meal can be manufactured from all types of seafood; however, it is often produced from wild-caught, small marine fish that contain high amounts of bones and oil, which are usually not edible by humans directly (Miles and Chapman, 2006). Fish meal is manufactured through the processes of cooking, pressing, drying and grinding of whole fish or fish cuttings. During the processing, water and part or all of the oil is extracted (Miles and Chapman, 2006).

What is the chemical composition of fish meal?

Fish meal is among the "big three" sources of quality protein used in animal feed (FAO, 2016). Fish has large amounts of energy per unit weight, and it is also an excellent source of protein and lipids as well as minerals and vitamins. The crude protein of high-grade fish meal is usually between 62% and 70% (AAFCO, 2018). The amino acid content of fish meal makes it attractive when formulating diets to meet the amino acid requirements of poultry. The protein

digestibility of fish meal is above 95% (Beski et al., 2015), and it is consistent, unlike some plant proteins that can vary based on the amount of heat treatment used during processing. The energy content of fish meal is directly related to its protein and oil contents due to the little or no carbohydrate content. The lipids in fish meal may be between 6% to 10% by weight but can range from 4% to 20%. Lipids in fish meal have high digestibility and are sources of essential polyunsaturated fatty acids (Beski et al., 2015). Fish meal contain both the omega-3 and omega-6 fatty acid families, but it contains more omega-3 (Pike, 1999) compared to plant-based proteins which contain more omega-6 fatty acids. The digestibility of the lipids in fish meal is above 90% and easily digestible by monogastrics. Fish meal is a good source of minerals and vitamins (IFOMA, 2001) which are essential for animal performance. The average mineral composition of fish meal ranges between 17% and 25%. It contains high amounts of calcium, phosphorus and magnesium (Miles and Chapman, 2006). The phosphorus present in fish meal is highly available to poultry and pigs unlike the phosphorus in plant proteins which is bound in the form of phytic acid. Fish meal is rich in B-complex vitamins, particularly cobalamin (B_{12}), niacin, choline, pantothenic acid and riboflavin.

What are the benefits of adding fish meal to poultry diets?

Fish meal has a long history of use in poultry feeds (IFFO, 2017). The high protein content of fish meal provides a wide range of micronutrients such as amino acids, vitamins and minerals. Aside from the positive effect on growth, the nutritional content has been reported to have positive impacts on animal health (Pike, 1999) and the quality of meat and eggs (IFFO, 2017). Several studies have been conducted to evaluate the effect of including fish meal in poultry diets on performance. A review on the effect of fish meal on animal performance by Cho

and Kim (2011) reported improved growth performance for most of the articles reviewed. Herstad (1973) also reported improved feed conversion ratio in broilers when fish meal was added to diets. In this study, it was observed that increasing the level of fish meal resulted in improved feed conversion. A study by Karimi (2006) investigated the inclusion of different levels of fish meal on the performance of broiler chicks and reported an overall improvement in growth and feed conversion for diets with higher levels of fish meal. Vogt and Stute (1967) also observed better performance when soybean meal was supplemented with fish meal. They observed better performance for birds fed diets containing fish meal as compared to birds fed diets containing only soybean meal.

Soybean Meal

What is soybean meal, the types on the market and how are they processed?

Soybean meal is a by-product of the extraction of soybean oil from soybeans. It is obtained by grinding the remaining flakes after removing most of the oil from soybeans by a solvent extraction process (AAFCO, 2018). Soybean meal is widely available (Oil World, 2015; FAO, 2016) and has become the primary protein source for determining the price of other protein sources (Willis, 2003). Solvent extraction and mechanical expelling are the two primary methods used to extract oil from soybeans. The most commonly used method is the solvent extraction method. The process involves cracking, dehulling, heating, flaking, hexane oil extraction and drying. The addition of hulls back to the meal after drying reduces the protein content to standard trading specifications. The two most common types of dehulled soybean meal are referred to as high and low protein soybean meals (Johnson et al., 2004; NRC, 1994). The high protein dehulled soybean meal contains 47% to 49% crude protein and up to 1.5% fat, while the low

protein dehulled soybean meal contains 43% to 44% crude protein and a higher amount of crude fiber (Johnson et al., 2004).

What is the chemical composition of soybean meal?

Soybean meal that has been correctly processed contains high quality proteins which are a good source of both lysine (Stein et al., 2008) and tryptophan (Baker, 2000) that are required in poultry diets. Additionally, the essential amino acid profile of SBM complements cereal grains, and this makes it a preferred protein source for poultry (Ravindran, 2013; Yasothai, 2016). The amount of digestible lysine present in soybean meal exceeds the lysine requirement for chicks (per unit of protein) (Baker, 2000). The methionine content of soybean meal is lower, but this can be supplemented with the synthetic form of methionine (Willis, 2003). The digestibility of soybean meal protein is approximately 85% in monogastrics (Woodworth et al., 2001) and ranges from 82% to 94% for individual amino acids. The fat content of soybean meal may vary based on the method of processing. The fat content ranges from 0.55% to 5.5% (Banaszkiewicz, 2011). Soybean meal also contains small amounts of calcium, phosphorus, magnesium, potassium and sodium, vitamin E, thiamine, riboflavin, pantothenic acid, biotin, folic acid and niacin.

The nutritional composition of soybean meal has made it the most preferred protein source to replace fish meal in poultry diets. Some studies conducted to replace fish meal with soybean meal in broiler or chicken diets reported no difference in broiler performance after balancing for methionine. Hartel (1968) concluded that rations that are properly balanced with methionine, yielded similar broiler performance for soybean meal and fish meal diets. Damron et

al. (1971) did not report differences in growth rate or feed conversion of broilers when dehulled soybean meal was partly replaced with 3% anchovy fish meal.

What are the benefits of adding soybean meal to poultry diets?

Soybean meal serves as the primary protein source for monogastrics (Stein et al., 2008). It is considered the standard among intact protein sources used in the feed industry (Cromwell, 1999; Willis, 2003). Research has shown that proper thermal processing of soybean meal yields better broiler chick performance (Marsman et al., 1997). The major benefit of soybean meal in poultry diets is the reduction in feed cost. Including SBM instead of animal meals such as fish meal and poultry by-product meal helps to reduce the total feed cost, and this goes a long way to reduce the production cost.

Poultry by-product Meal

What is poultry by-product meal, the types and how is it processed?

Poultry by-product meal (PBM) is the ground, rendered, or clean parts of slaughtered poultry such as head, feet, undeveloped eggs and intestines, exclusive of feathers except in such amounts as might occur unavoidably in good processing practices (AAFCO, 2018). Poultry by-product meal can be obtained from culled laying hens and in such instances, it is referred to as spent hen meal. This is usually done in areas with poor or no market for spent hens (Kersey et al., 1997; Hertrampf et al., 2000). Compared to some other rendered products, PBM is not popular globally. It is primarily available in developed countries that have concentrated poultry production. The processing of PBM includes grinding, stabilization through fermentation, rendering, cooking, sterilization, drying and or fat extraction. The level of its inclusion in poultry diets may be limited due to diseases associated with animal meals (European Community, 2002).

What is the chemical composition of poultry by-product meal?

The nutritional content of poultry by-product meal may vary based on the source of raw materials (Johnson et al., 1997). The percentages of meat, entrails and bones affect the nutrient content of the final product (Watson, 2006; Dale et al., 2004). Processing plants may use parts of the chicken such as de-boned remains from further processing whereas other plants may sell whole birds and just render the entrails of the bird. Feed grade PBM contains 58% to 62% protein, 12% to 15% fats and 18% to 23% ash (Meeker, 2009). It is a palatable and high-quality ingredient due to the presence of essential amino acids such as lysine, fatty acids, vitamins and minerals. The protein digestibility of PBM is generally between 80% to 90% (Firman and Remus, 1993).

What are the benefits of adding poultry by-product to poultry diets?

Poultry by-product meal may be used as a protein source in formulating feeds for poultry, livestock, exotic animals, dogs and cats (AAFCO, 2018). Poultry by-product meal is an important protein source of animal protein in domestic animal feeds (Meeker, 2009). Poultry by-product meal can be included at 5% to 10% in broiler diets to provide essential energy and nutrients. In poultry diets, it is usually used to supplement other major protein sources like fishmeal and soybean meal. It can be used up to 10% in broiler diets without adversely affecting performance (Meeker, 2009). Research has reported positive results when included in the diets of chickens and other species of animals. Manzoor et al. (2014) reported improved weight gain and feed conversion in broilers when PBM diets were supplemented with amino acids. Escalona and Pesti (1987) reported improved growth for chicks fed diets containing 5% PBM. Also, Boling

and Firman (1997) suggested PBM as an alternate protein source in poultry diets when they observed no significant difference in toms' performance after the inclusion of PBM in their diets.

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Vitamin Stability

What are vitamins and their general effect on broiler nutrition?

Vitamins are a group of organic compounds that are essential for normal growth and development, but broilers cannot synthetize them; therefore, they must be included in their diets (Ekaidem et al., 2006; Coelho, 2002). Vitamins are primarily made up of combinations of molecules such as carbon, hydrogen, oxygen, nitrogen and sulfur. Vitamins play a variety of roles in the body. Some function as hormones, antioxidants or cofactors alone or in combination with minerals for growth, health or reproduction. Vitamins found in feedstuffs are in small quantities (Coelho, 2002), and hence synthetic forms must be included in diets.

Types and Importance of Vitamins in Broiler Nutrition

What are the different types of vitamins and their important in broiler nutrition?

Vitamins are generally grouped as either fat soluble or water soluble based on whether they dissolve in either lipids or water. The four fat soluble vitamins include: vitamin A, D, E, and K. There are nine water soluble vitamins. These include the B vitamins and vitamin C.

What effect does Vitamin A have on Broiler Performance?

Vitamin A is not found in plants but rather as precursors known as carotenes. Animals can convert these carotenoids into active forms of vitamin A. The presence of double bonds in vitamin A makes it possible for it to exist in several isomeric forms. It is required in poultry diets to support growth, health and life. It is also essential for organ development, cell proliferation and differentiation (McDowell, 2000; Esteban et al., 2010). The absence or inadequate amounts of vitamin A in poultry diets will lead to reduced growth, lowered resistance to disease, eye

lesions, muscular incoordination, lowered egg production and blood spots in eggs (Scott et al., 1982; Squires and Naber, 1993b).

What effect does Vitamin D have on Broiler Performance?

Vitamin D represents a group of closely related compounds which have antirachitic properties. The most common types of vitamin D are vitamin D_2 (ergosterol) and vitamin D_3 (cholecalciferol) (McDowell, 2012). Vitamin D_2 is obtained from a plant steroid whereas cholecalciferol is derived from the precursor, 7-dehydrocholesterol, which is from animal products. Vitamin D functions to enhance intestinal absorption and mobilization, retention and deposition of calcium and phosphorus in the bone to a level that supports normal bone mineralization and other body functions (McDowell, 2012). Vitamin D plays major roles in the regulation of the parathyroid gland, the immune system, the skin, cancer prevention (Lemire, 1992). The most prominent disease resulting from vitamin D is rickets, which is usually due to reduced concentration of calcium and phosphorus in the bone matrix. This is usually accompanied with severe leg weakness, lameness and stiff-legged gait. Insufficient amounts of vitamin D in poultry diets affects growth rate, egg quality and hatchability.

What effect does Vitamin E have on Broiler Performance?

Vitamin E describes all tocol and tocotrienol derivatives which shows the qualitative biologic activity of alpha-tocopherol (IUPAC-IUB, 1973). Alpha-tocopherol is the primary and most biologically active vitamin E compound found in feedstuffs. Vitamin E plays an essential role the in optimum function of the reproductive, muscular, circulatory, nervous and immune systems (Meydani and Han, 2006) (Bendich, 1987; McDowell, 2000). It is an antioxidant that

protects tissues from damage caused by free radicals which can be harmful to body tissues and organs (Chow, 1979). Several evidences gathered over the years show that vitamin E is related to the immune response and it is required for its normal function. Da Silva et al. (2009) reported that high levels of supplementation of vitamin E had a positive impact on immune response, which increased the resistance of poultry to infectious diseases. The absence of vitamin E in animal diet may lead to severe deficiencies and even death from tissue damage. Scott et al. (1982) grouped vitamin E deficiency in poultry into at least three conditions. These are 1) exudative diathesis characterized by subcutaneous edema and blackening of the affected parts, lethargy and lack of appetite; 2) "crazy chick disease" (encephalomalacia) with signs of ataxia, head retraction and "cycling" of legs; and 3) muscular dystrophy (progressive weakness and loss of muscle mass).

What is Vitamin K and its effect on Broiler Performance?

Vitamin K is used to describe a group of quinone compounds which possess anti-hemorrhagic effects and are soluble in fat. Naturally occurring sources are fat soluble but vitamin K_3 (menadione) is water soluble. It is needed to reduce blood coagulation time. Vitamin K_1, the fat-soluble form, is not currently used in the feed industry because it is too expensive hence the water-soluble vitamin K_3 salts are used. The function of vitamin K is to help convert inactive blood-clotting protein precursors to biologically active proteins (Suttie and Jackson, 1977). Vitamin K also plays a role in the regulation of multiple enzymes involved in metabolism of lipids in the brain cells (Denisova and Booth, 2005). Vitamin K added to feed should be increased when fed with vitamins A and E to improve poultry performance (Abawi and Sullivan, 1989; Frank et al., 1997). Deficiencies of vitamin K in monogastrics occur when fed in high

levels of antagonist, dicoumarol or sulfonamides forms which can inhibit intestinal synthesis of vitamin K. Vitamin K deficiency is characterized by low levels of prothrombin, increased clotting time and hemorrhaging. Vitamin K deficiencies in poultry is characterized by small hemorrhagic blemishes on the breast, wings and legs as well as on the intestine surface. Chicks may also show signs of anemia due to blood loss.

What effect does Vitamin B_1 have on Broiler Performance?

Vitamin B_1 is commonly known as thiamin and is characterized by a sulfurous odor and slightly bitter taste. It is highly soluble in water, sparingly soluble in alcohol and insoluble in fats. The compound used in poultry feeds exist in the hydrochloric or mononitrate form. The vitamin serves as a coenzyme, co-carboxylase or TPP in cells. Vitamin B_1 is important in oxidative decarboxylation reactions in the tricarboxylic acid cycle (TCA), which occurs in the cell mitochondria and cytoplasm. These reactions aided by vitamin B_1 convert carbohydrates to energy needed by the body for proper growth and development. Vitamin B_1 deficiency in poultry is prominent and this is because they are more susceptible to the neuromuscular effects of vitamin B_1 deficiency than most mammals. Birds that are deficient in vitamin B_1 can rapidly detect and discriminate between feeds that are high in carbohydrates (Thornton and Shutze, 1960) and does not contain the vitamin (Hughes and Wood-Gush, 1971).

What effect does Vitamin B_2 have on Broiler Performance?

Vitamin B_2, also known as riboflavin, is naturally found in three forms. One is a free dinucleotide riboflavin and the other two are coenzyme derivatives. This vitamin is odorless, bitter and sensitive to high temperatures. It easily dissolves in diluted basic or strongly acidic

solutions and it is slightly soluble in water. Vitamin B_2 is a part of many enzymes necessary for the metabolism of carbohydrate, fat and protein. Riboflavin is also needed in diets for skin development, fast healing and tensile strength (Ward, 1993). Vitamin B_2 deficiency results in decreased growth rate and poor feed efficiency. In young chicks, the deficiency is characterized by "curled-toe" paralysis. Chicks suffering from riboflavin deficiencies may have inward curled toes when walking or resting on their hocks (Scott et al., 1982).

What effect does Niacin have on Broiler Performance?

Niacin consists of three antivitamins and antagonists. It is chemically the simplest among vitamins. Niacin in feedstuffs exists in coenzyme forms, which are hydrolyzed during digestion to form nicotinamide. In the body, the amino acid tryptophan serves as the precursor for the synthesis of niacin. The major function of niacin is to act as a coenzyme in the form of nicotinamide, NAD and NADP. These enzymes are critical in the metabolism of carbohydrates, proteins and lipids used to produce energy. Niacin deficiencies in poultry causes severe metabolic disorders of the skin and digestive organs. The deficiency is characterized by loss of appetite, retarded growth, weakness, digestive disorders and diarrhea. In chickens, niacin deficiency is characterized mainly by loss of appetite and slow growth.

What effect does Vitamin B_6 have on Broiler Performance?

Vitamin B_6 describes a group of three compounds: pyridoxol (pyridoxine), pyridoxal, and pyridoxamine. Vitamin B_6 plays an important role in the metabolism of amino acids, carbohydrates and fatty acid in the tricarboxylic acid cycle in energy production. Vitamin B_6

deficiency is characterized by retarded growth, dermatitis, epileptic-like convulsions, anemia and partial alopecia.

Factors affecting Vitamin Stability during Storage

What factors affect the Stability of Vitamins during Storage?

Vitamins are liable nutrients that are sensitive to several factors that affect their stability (Gadient, 1986; Schneider, 1986). They are biologically active biochemicals, which are generally sensitive to their physical and chemical environment (Coelho, 2002). Their concentration or potency can be reduced by several factors in premixes and finished feed (Gadient, 1986; Frye, 1994) over a period of time. Exposure of vitamins to multiple stress factors simultaneously will significantly affect their stability. For example, storing vitamins in a highly humid environment increases the rate of oxidation of vitamins (Christian, 1983). Storing vitamins under elevated temperatures has been reported to affect the rate of vitamin degradation in premixes and finished feeds. Finally, mixing vitamins with highly reactive minerals such as copper, iron and zinc and choline chloride also decreases their stability (Schneider, 1986).

What is the effect of Temperature on Vitamin Stability?

Heat is a catalyst that speeds up the rate at which particles collide with each other. Storing vitamins at high temperatures increases the rate of reaction and thus reduces vitamin concentration. This is because at high temperatures, particles tend to move faster increasing the rate of collision and therefore the rate of reaction. The effect of temperature or heat on vitamin deterioration has been reported by several researchers. Charles (1972) reported that vitamins were susceptible to destructive factors such as oxidizing agents, reducing agents and pH and these destructive forces were accelerated by heat. He observed that vitamins stored both alone

and within multiple vitamin premixes had minimal deterioration at low temperatures but started to deteriorate when the ambient temperature was increased beyond 24°C. Wornick (1968) also reported a minimal loss of vitamin activity in samples of feed products and additives stored in a deep-freeze, between -18 and -10°C. However, at temperatures between 16 and 27°C, some vitamin losses occurred. Gadient (1986) reported that temperature does not affect all vitamins. Vitamins A, B_6 and folic acid were among the vitamins which were highly sensitive to temperature, whereas D_3, E, K_3, B_1, B_{12} and calcium pantothenate were slightly sensitive to temperature, while B_2, nicotinic acid and biotin were unaffected by temperature. Quackenbush (1963) reported three-quarters loss in carotene activity after storing hybrid yellow corn high in carotene at 25˚C for years. However, loss of carotene was less when stored at a lower temperature, 7˚C. Chen (1990) reported 38% to 43% loss in activity in three commercial cross-linked vitamin A beadlets in feed pelleted at 93˚C and stored for three months. Zhuge and Klopfenstein (1986) reported 0.36 and 1.32 mg/g of premix loss in vitamin activity for riboflavin and niacin, respectively when stored at 43˚C temperature.

What is the effect of Relative Humidity on Vitamin Stability?

McDowell and Ward (2008) reported humidity as the primary factor that decreased the stability of vitamins in premixes and feedstuffs. They stated that the detrimental effects of trace minerals and choline chloride on vitamin stability were increased when stored in a humid environment. Vitamins stored with hygroscopic ingredients such as choline chloride will cause the premix to cake-up or clump, which binds vitamins and trace minerals together, speeding up reactions and the causing loss of vitamin activity (Zhuge and Klopfenstein, 1986). Christian (1983) also reported that humidity has a greater adverse effect on vitamin stability as compared

to temperature. He reported that storing vitamin trace mineral premixes under low humidity for 3 months resulted in a 14% loss of vitamin A activity; however, at a higher humidity, loss of vitamin activity increased to 88% for the same storage period. The presence of high amount of moisture may also lead to the growth of Mould and subsequently deterioration of vitamins and minerals.

What are the effects of Trace minerals on Vitamin Stability?

Vitamin stability is reduced in the presence of certain trace minerals (Zhuge and Klopfenstein, 1986) and choline chloride, which speed up oxidation (Shurson et al., 2011). Trace minerals produce redox reactions that cause oxidation of vitamins (Coelho, 2002). Trace minerals vary in their redox potential with copper, iron and zinc being the most reactive, while selenium, iodine and manganese are less reactive minerals (Coelho, 2002; Mavromichalis, 2016). Blending vitamins with trace minerals to form vitamin trace mineral premixes increases the loss of vitamin activity during prolonged storage periods under high temperatures and humidity (Mavromichalis, 2016). Adams (1982) found 55% and 0% loss of pyridoxine activity when he stored thiamin and pyridoxine with and without trace minerals for 3 months, respectively. Also, Shurson et al. (2011) reported higher losses in activity in vitamin premixes blended with inorganic trace minerals as compared to a vitamin premixes without inorganic trace minerals stored for 120 days. For example, he observed 9% and 8.6% loss of activity per month for vitamins A and $B_{6,}$ respectively. Zhuge and Klopfenstein (1986) also reported significant losses of 12.68 mg/g of premix in vitamin A activity when stored with trace minerals at three temperatures. He observed that vitamin A without trace minerals had similar losses of activity

even at different temperatures, but loss of activity was different for vitamin A that contained trace minerals. Frye (1978) also reported reduced K_3 stability when stored with trace minerals.

How does Trace minerals react with Vitamins?

Trace minerals reduce vitamin concentration or vitamin activity through friction and redox reaction. Reactive trace minerals reduce vitamin activity by oxidizing the vitamins. First, the metallic-like nature of trace minerals reduces crystals of vitamins to smaller particles by eroding their protective coating. The smaller particles increase the surface area of vitamins for reaction between the vitamin particles and vitamins and trace mineral particles. Redox is a chemical reaction in which the oxidation state of an atom changes. It is the exchange of electrons between two species. The charge of every atom is its oxidation state. It involves both reduction and oxidation processes. Oxidation is the loss of electrons by an atom or molecule where reduction is the gain of electrons by an atom or molecule. During oxidation, negatively charged atoms (anions) transfer their electrons to positively charged atoms (cations) (Haustein, 2014). Reduction is the opposite of oxidation. In vitamin trace mineral premixes, reactive trace minerals copper, iron or zinc removes or gives electrons from or to vitamins. The reaction may lead to the formation of a new compound. The resulting product may cause vitamins to be unstable reducing vitamin activity.

What are the effects of Storage Time on Vitamin Stability?

Storing vitamins for longer periods have been shown to have a significant effect on vitamin activity. Quackenbush (1963) reported one-half (1/2) loss of carotene in a hybrid yellow corn when stored for 8 months. However, after 3 years of storage, the loss of carotene activity

increased to three-quarters (3/4) of the total amount of carotene present in the corn. Dash and Mitchell (1976) also reported 50% loss of vitamin A activity in commercial feed containing 1,293 IU/kg after one year of storage.

Factors that increase Vitamin Stability during Storage

How can the stability of vitamins be maintained during storage?

The stability of vitamins can be maintained over time by adhering to good manufacturing and storage practices such as:

1) Minimize the time between the purchase and use of the premixes.
2) Store finished feeds and premixes in a cool, dry and dark area in an enclosed room.
3) Store vitamins and trace mineral premixes separately.
4) Avoid extreme acidity or alkalinity conditions.
5) Purchase a stabilized product forms of vitamins.
6) Minimize the use of inorganic trace minerals and reactive substances in premixes.

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Near Infrared Reflectance Spectroscopy

What is Near Infrared Reflectance Spectroscopy and how does it work?

Near-Infrared Reflectance Spectroscopy (NIRS) is a non-destructive analytical technique for measuring interactions between incident light and the surface of material. The method measures the absorption of electromagnetic radiations in the near infrared (NIR) region (wavelengths between 750 and 2500 nm) (Prieto et al., 2017). The NIRS uses the phenomena that light of specific frequencies in the NIR region are absorbed by molecular bonds vibrating at

similar frequencies. The spectra from the NIRS are influenced by the overlap of weak overtones and combinations of fundamental vibrational bonds for H-C, H-N and H-O bonds from the NIR region (van Kempen et al., 1996; Cozzolino and Moron, 2003). The spectroscopy in the NIR region provides information about the relative proportions of C–H, N–H and O–H bonds which are the primary constituents of organic molecules (Murray, 1993) present in a sample. The spectra from the NIRS are also affected by the physical and structural properties of the samples (Chang et al., 2001). Thus, the size and shape of particles, space between the particles and arrangement of the particles affects light transmission passing through a material which in turn affects light reflectance and the resulting spectra (Wetzel, 1983).

Spectra from the NIRS are difficult to interpret directly hence multivariate calibrations are necessary for quantitative analysis of sample constituents. A calibration is described as the correlation between spectral information and the composition values obtained from standard methods of analysis (van Kempen et al., 1996).

Advantages of the NIRS

What are the advantages associated with the NIRS?

The major advantage of NIRS is little or no sample preparation due to the ability of the near infrared region to penetrate material. Additionally, the method does not involve the use chemicals which eliminates soaking and the addition of chemicals for material digestion reducing the time used for analysis. Also, samples can be reused for repeated analysis for result verification.

Analysis cost; analysing samples with the NIRS is less expensive compared to wet chemistry analysis. The per sample cost associated with the NIRS is primarily the initial equipment cost along with routine preventive maintenance.

On-site measurements; hand-held NIRS can be used in the field to quickly take the measurements needed to make corrective actions during the manufacturing of feed.

Minimal training and skill are required to operate the NIRS. Once calibrations are installed, the instrument can be operated by feed mill operators.

Uses of the NIRS

What are the uses of the NIRS?

NIRS has gained popularity in developed countries and some developing countries due to its speed of analysis and low cost per analysis. NIRS has been employed in feed, food, pharmaceutical and petrochemical industries for identification and/or characterization of materials (Wetzel, 1983; Creaser and Davies, 1988; Murray and Cowe, 1992; Workman, 1996). The NIRS technique was initially developed 30 years ago for the rapid determination of grain moisture (Ben-Gera and Norris, 1968).

In the feed industry, it is largely used in feed ingredient, grain and forage quality assessment. It is used to measure the chemical composition of feed and feed ingredients. Swart et al. (2012) used the NIRS to accurately predict crude protein, crude fibre, acid detergent fibre, neutral detergent fibre and ether extract of mixed ostrich diets. The uses of NIRS in predicting the chemical composition of ingredients such as soybeans, soybean meal and canola meal have also been reported (Chen et al., 2009; Bastianelli et al., 2010; Delwiche et al., 2006; Clark et al., 1987; Shenk and Westerhaus 1994).

Factors affecting the Accuracy of the NIRS

What factors influence the accuracy of the NIRS?

The accuracy of the NIRS may be affected by the reference samples included in the calibration, poor sample preparation by the technician, physical and structural characteristics of the sample and instrument maintenance.

The results obtained with the NIRS can be no better than the reference samples used to develop the calibration and the wet chemistry values. Therefore, wet chemistry values must be run by a laboratory that can provide both accurate and precise values. When using the NIRS for finished feed analysis, it is important to determine whether existing or standard calibrations provided with the instrument contain samples that are similar to those being analysed with the instrument (Smith et al., 1991). New samples of feed or ingredients manufactured or received at the facility should be added to the equipment calibration (Undersander, 2006).

The ability of the NIRS to accurately analyse materials can be reduced over time by constant change in raw materials, and even some unknown factors. When using the NIRS it is important to conduct routine checks and maintenance to improve the predicting potential and to replace parts such as lights that are important to the functioning of the instrument. Daily instrument diagnostics are necessary to determine if there are any hardware (light source) or software problems (wavelength) with the instrument. Although the operation of the NIRS does not require specific skills, the technician performing the analysis must be able to follow basic laboratory procedures. Prior to analysis, the technician must inspect the bottom of the sample cup for scratches or cracks and clean cups to remove any residue from the previous sample.

The accuracy of the NIRS predictions also depends on the physical and structural characteristics such as particle sizes, density, shape, moisture, content and arrangement of the sample. These sample characteristics have been reported to influence the reflectance of the length of light transmission passing through the sample (Tamburini et al., 2017; Spragg and Green, 2013; Wetzel, 1983). The particle size of the sample has the greatest influence on the prediction of the results. This is because particle size affects the distance light travels through a sample before being reflected and scattered, and this affects the amount of light absorbed as well as spectra characteristics which may yield variable predictions (Spragg and Green, 2013). The bigger the particle size, the longer the distance light travels and the higher the absorption and vice versa.

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Chapter 2 - Evaluating the Effect of Replacing Fish Meal in Broiler Diets with either Soybean Meal or Poultry by-product Meal on Broiler Performance and Total Feed Cost per kilogram of Gain

Abstract

Farmers and researchers in some developing countries are evaluating the use of fish meal in broiler diets due to high cost and product variability. This study evaluated the effects of replacing fish meal with either soybean meal or poultry by-product meal on broiler performance and total feed cost per kg of gain. Three dietary treatments were manufactured to contain SBM and FM, SBM or SBM and PBM. Diets were balanced for lysine and fed in mash form. A total of 36 pens were randomly allocated to one of the three diets in a complete randomized design with 12 replications per treatment. A total of 900 1-day-old male Cobb 500 broilers were randomly assigned to pens with 25 chicks per pen and reared for 42 days. BW, ADFI and AdjFCR were determined at 14, 28 and 42 d of age. At d 14 and 28, broilers fed FM diets had poorer ($P \leq 0.05$) BW, ADFI and AdjFCR as compared to those fed SBM and PBM. Overall, 42 d broilers fed FM diets had lower BW ($P \leq 0.05$) compared to those fed SBM and PBM diets but AdjFCR was similar ($P \geq 0.05$) for all treatments. Total feed cost per kg of gain was significantly higher ($P \leq 0.05$) for FM diets as compared to SBM and PBM diets. Replacing FM with either SBM or PBM resulted in higher BW and ADFI but similar AdjFCR for all birds and reduced total feed cost per kg of gain.

Keywords: SBM: soybean meal, FM: fish meal, PBM: poultry by-product meal, BW: body weight, ADFI: average daily feed intake, AdjFCR: adjusted feed conversion ratio.

Introduction

In some developing countries, fish meal is the traditional protein source for broiler production, and its supply is mainly dependent on external sources, importation (Karimi, 2006). Among animal protein sources, fish meal is valued by farmers and nutritionists in these areas because of its nutrient profile and health enhancing nutrients. Fish meal contains high amounts of digestible crude protein and essential amino acids as well as fat, vitamins and minerals (Blair, 2008; Chadd, 2008). Because of this it is sometimes used to supplement vegetable protein sources such as soybean meal, canola meal and rapeseed meal. Research has demonstrated that including fish meal in balanced broiler diets resulted in better growth performance (Karimi, 2006; Cho and Kim, 2011; Mikulec et al., 2004; Herstad, 1973; Vogt and Stute, 1967). However, lack of availability, problems of product uniformity (Pike, 1999; Dale et al., 2004), higher cost in relation to plant proteins and the presence of trimethylamine in fish meal which causes a residual fish smell that taints the flavor of meat and eggs, limits its inclusion in broiler diets (Blair, 2008; Chadd, 2008). Some studies conducted to replace fish meal in broiler diets have used plant protein sources such as groundnut meal, cottonseed meal, full fat soybean meal, soybean meal canola meal, rapeseed meal and leaf protein concentrate (Oduguwa et al., 2004; Olomu and Offiong, 1985). However, soybean meal is the most commonly used and preferred plant protein source in broiler diets by farmers throughout the world (Saki et al., 2011).

Soybean meal has a high protein content as well as essential amino acids that meets the requirement of poultry (Yasothai, 2016). Soybean meal has the highest feeding value among plant protein sources (Olomu and Offiong, 1985). The well-balanced amino acid profile of soybean meal makes it suitable to balance most cereal-based diets (Cromwell, 1999; Ravindran, 2013; Beski et al., 2015). The similarities in its nutritional constituents with fish meal makes it

the most suitable plant protein to replace fish meal in broiler diets. DeGroote (1973) reported no differences in broiler body weight and feed conversion ratio when birds were fed a milo-soy diet with and without fish meal for six weeks, demonstrating that plant proteins can effectively replace fish meal in broiler diets. Aziz (2001) reported no differences in body weight gain, feed intake and feed efficiency when birds were fed diets with and without fish meal. He concluded that soybean meal successfully replaced fish meal in broiler diets when supplemented with methionine. Concerns on the negative effect of anti-nutritional factors (ANFs) present in soybean meal (trypsin inhibitors, lectins, phytic acid) (Swick, 1994; Petterson and Pontoppidan, 2013) on broiler growth performance (Marsman et al., 1997) may influence its inclusion in broiler diets as a major protein source in some developing countries. However, this problem can be eliminated or minimized by proper thermal processing (Mehri et al., 2010; Adeyemo and Longe, 2007) making this concern less important. Unlike fish meal, soybean meal is less expensive and largely and widely available all year round (Banaszkiewicz, 2011) making it a more accessible protein source for poultry production.

Poultry by-product meal (PBM) is another protein meal that is used in monogastric (poultry and pigs) (Liener, 1994) and aquaculture feeds and as well as pet food (Boling and Firman, 1997). Although it is usually variable in its nutritional composition depending on the substrate being processed (Meeker and Hamilton, 2006; Watson, 2006), its protein composition, digestibility, feed conversion ratio and feed intake in broilers is comparable to fish meal (Dale et al., 1993; National Renderers Association 2nd edition; Nengas and Davies, 1999; Takagi et al., 2000). Gerry (1956) and Fuller (1956) reported improved broiler growth performance when diets containing poultry by-products meal were fed.

The objective of this study was to evaluate the effect of replacing fish meal in broiler diets with either soybean meal or poultry by-product meal on growth performance and total feed cost per kilogram of gain.

Materials and Methods

Feed Formulation and Manufacturing

Three experimental diets were formulated to meet or exceed the minimum nutritional requirements (NRC, 1994) of broilers and fed over three growth phases (Table 2.1). The three protein sources used in the experiment were fish meal (FM), soybean meal (SBM) and poultry by-products meal (PBM). All diets were manufactured at the O.H. Kruse Feed Technology Innovation Center at Kansas State University using Current Good Manufacturing Practices (CGMPs). Diets were mixed for three minutes in a double shaft ribbon Hayes-Stolz mixer (Model 2261905, Hayes and Stolz Industrial Manufacturing Company, Burleson, TX). All diets were fed in mash form.

Sampling and Analysis

Composite samples of feed were collected from the mixer and during bagging. Samples were analyzed for crude protein and crude fat. Crude protein content was determined according to the Dumas Combustion method (AOAC 990.03).

Birds and Management

The care of the animals used in the trial conformed to the Guide for Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010). The experiment was conducted at

the Poultry Teaching and Research Facility at Kansas State University. A total of 900 1-day-old male Cobb 500 broiler chicks were placed in a curtain-sided house with forced air heat and reared to 42 d of age. There were 25 chicks per pen and 36 pens in total. The pens were randomly assigned to one of three treatments with a total of 12 replications per treatment. On the day of placement, birds were weighed and randomly allocated to the pens. The pens were 1.5 m x 1.5 m and had new pine shavings litter. Each pen had a plastic tube feeder and six nipple waterers. At the time of placement, birds were provided feed on floor pen trays. Chicks were allotted 0.9 kg starter, 2.3 kg grower and 2.0 kg finisher diets. Feed amounts were adjusted in each phase for mortality. Birds had *ad-libitum* access to feed and water throughout the study. Feeders were shaken daily to reduce variability in feed intake due to the poor flowability of the mash. Lighting was provided for 24 h daily from d 1 to 42. The room temperature was 33.5˚C from d 1 to 8 and then lowered to 29.4˚C for the remainder of the experiment.

Data Collection

Feed disappearance and pen body weight (BW) was measured on 42 d of age. Mortality was removed, weighed and recorded daily. Feed conversion ratio (FCR) was adjusted by adding the weight of the dead birds to the weight of live birds in each pen. The pen weights and feed disappearance were then used to calculate average daily feed intake (ADFI) and adjusted feed conversion ratio (AdjFCR).

Determination of Feed Cost per kilogram of Gain

Most recent prices of ingredients at the time (August 17th, 2018) of feed cost estimation were used. Feed cost was obtained by multiplying the price per kg of each ingredient by the

proportion of ingredient in the diet. Total feed cost was then estimated as the product of total feed consumed for 42 d and the sum of ingredient cost per treatment. Total feed cost per kg of gain of each treatment was calculated as the total feed cost divided by the total body weight of birds per treatment. The total feed cost per kg of gain ($/kg) equals total feed cost divided by total body weight.

Statistical Analysis

Treatments were allocated to pens using complete randomized design and the pens were the experimental units.

Data was analyzed using the GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC). Statements of statistical differences were based on $P \leq 0.05$. Differences between means were separated by the Tukey's studentized pairwise analysis.

Results

Effect of Protein Source on Growth Performance

Mortality for the experiment was 2.1% and this was not due to treatment effect. The effect of protein source on broiler performance is stated in Table 2.2.

Protein sources affected BW throughout the 42 d of the study. At d 14, 28 and 42, BW of birds fed SBM and PBM diets were greater ($P \leq 0.05$) than those fed FM diets. Birds fed SBM and PBM had similar BW throughout the study. Replacing FM with either SBM or PBM in broiler diets significantly ($P \leq 0.05$) influenced ADFI. At d 14, 28 and 42, ADFI was significantly different for birds fed SBM and FM diets. Feeding birds with SBM diets improved ($P \leq 0.05$) ADFI throughout the study as compared to birds fed FM diets. At d 42, ADFI of birds fed FM

and PBM diets was similar ($P \geq 0.05$). Birds fed SBM and PBM diets had similar ADFI ($P \geq 0.05$) over the whole experimental period (0 to 42).

AdjFCR was significantly different ($P \leq 0.05$) for treatments at d 14 and 28. At d 14 and 28, AdjFCR of birds fed FM diets was poorer than birds fed SBM and PBM diets. AdjFCR of SBM and PBM fed birds were similar at 14 and 28 d. However, at d 42, AdjFCR of birds fed diets containing the different protein sources was similar ($P \geq 0.05$).

Effect of Protein Source on Feed Cost per kg of Gain

Total feed cost per kg of gain was significantly different ($P \leq 0.05$) for diets containing different protein sources (Table 2.3). The total feed cost per kg of gain was significantly higher ($P \leq 0.05$) for diets containing FM ($1.75/kg) as compared to SBM ($1.23/kg) and PBM ($1.40/kg) diets. The SBM diets had the lowest total feed cost per kg of gain.

Discussion

Effect of Protein Source on Growth Performance

The results of this study indicated that birds fed SBM and PBM had similar or better growth performance as compared to birds fed FM. The improved ADFI and BW observed for the birds fed SBM (100%) and PBM (7.5 to 10%) indicated that these protein sources when properly balanced can support broiler growth performance and therefore can be used in place of FM without any adverse effect on broilers. Researchers have reported the use of FM in broiler diets resulted in improved ADFI and BW for birds fed such diets (Karimi, 2006, Cho and Kim, 2011; Mikulec et al., 2004; Vogt and Stute 1967; Herstad 1973). The researchers attributed their findings to the nutritional profile of FM, which impacted its palatability. Beski et al. (2015)

reported that the high digestibility of FM (>90%) improves FCR and support faster growth (IFFO, 2017). However, the present study observed lower overall ADFI and BW for birds fed FM diets. A possible explanation could be due to the quality of the FM used in the study, which may have influenced digestibility and palatability (Aksnes and Mundheim, 1997). The quality of FM can be impacted by both the starting raw materials as well as the method of processing. The appearance and smell of the fish meal obtained for the study were normal. Additionally, analysis carried out on FM at the start of the study showed that the nutritional content of the meal was within the normal range for crude protein, fat, and lysine. Since processing of FM involves, cooking, pressing, drying and grinding. The cooking and drying processes which involve the use of heat (FAO, SIFAR, 2001) can easily cause heat damage when temperature is not carefully controlled. de Koning (2002) suggested that in instances where overheating occurs, the resulting meal may be less digestible. Overheating have been shown to reduce true digestibility of FM (Jensen et al., 1990). Pike (1990) reported FM dried with heat above 100°C reduced digestibility and lowered animal performance. Gulbrandsen and Hjertnes (1986) also reported lower true nutrient digestibility when FM was overheated. While Fernandez et al., (1994) observed overheating lowered the true digestibility and biological value of essential nutrients such as amino acids. The lower BW observed in the current study may have been due to lower digestibility of the FM used in the feed or the lower ADFI observed in birds on the FM treatment.

The palatability of feed depends on the quality of the ingredients as well as how an animal is first introduced to a feed (Provenza, 1995; Tantikitti, 2014). In the current study, starting with 10% FM may have decreased the palatability of the feed as seen by the lower ADFI. The lower ADFI observed in subsequent phases of the current study suggests the FM diets

were not as palatable as the SBM and PBM diets. However, since the overall AdjFCR was similar across all treatments by the end of the study it suggests there may have been an improvement in digestibility of the FM in the diets which may have been due to changes in the intestinal physiology of birds.

Contrary to the findings of this study, Aziz et al. (2001), Gerry (1956) and Fuller (1956) reported similar growth performance for birds fed diets with and without FM and SBM and PBM. Aziz et al (2001) compared 14% FM in broiler diets with different levels of SBM (25, 50, 75 and 100%) replacement and found no difference in broiler live weight gain, feed intake and FCE. Based on his findings he concluded that FM could be replaced completely in broiler diets by SBM when supplemented with methionine. Gerry (1956) and Fuller (1956) investigated the effect of replacing FM with PBM on broilers and observed similar performance for FM and PBM diets. In their study, they replaced FM with PBM and recorded no difference in BW and FCR. In other studies, Karimi (2006) and Mikulec et al. (2004) observed better performance for birds fed FM diets as compared to birds fed SBM diets. Karimi (2006) varied the levels of FM from 0 to 5% in broiler corn-soy diets and reported improved performance for higher levels of FM. Mikulec et al (2004) included 6% FM in broiler starter diets and reported improved live weight and live weight gain for birds fed diet containing FM.

Effect of Protein Source on Feed Cost per kg of Gain

Replacing FM with either SBM or PBM significantly reduced total feed cost per kg of gain. The price of FM per kg was greater than that of SBM and PBM. The cost of fish meal resulted in a higher total feed cost per kg of gain for the FM diets as compared to SBM and PBM diets. The high cost of fish meal is driven by the high demand and seasonal or limited production

of fish. Fish meal can be produced from all species of fish but is usually produced from wild caught marines such as menhaden, anchovies and sardines (Durand, 1998; Miles and Chapman, 2006). It is largely produced by only a few countries (Ecuador, Peru, Chile, Denmark, Iceland and Norway). Demand for fish meal is constantly increasing but supply is not growing accordingly, and this affects producer and supplier behavior (Delgado et al., 2003) increasing the cost of FM. SBM, on the other hand, is less expensive although there is high demand for this commodity all year round. The primary reason is the higher worldwide production of soybeans year-round in several parts of the world (Banaszkiewicz, 2011).

Conclusion

In conclusion, replacing fish meal with either soybean meal or poultry by-products meal in broiler diets yielded better broiler body weights and average daily feed intake and reduced total feed cost per kg of gain. The data from this study indicated that replacing FM with a lower cost protein source resulted in a low cost of gain.

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Figures and Tables

Table 2.1 Diet composition and chemical analysis of experimental diets

	Starter			Grower			Finisher		
	Protein Sources[1]								
Ingredients, %	FM	SBM	PBM	FM	SBM	PBM	FM	SBM	PBM
Corn	65.40	54.98	64.15	67.01	59.60	66.75	63.14	60.58	62.20
DDGS	2.00	2.00	2.00	2.00	2.00	2.00	6.00	6.00	6.00
Soybean meal	18.00	33.00	18.00	16.00	28.00	16.00	16.00	23.00	16.00
Fish meal	10.00	-	-	8.50	-	-	7.50	-	-
Poultry by-products meal	-	-	10.00	-	-	8.50	-	-	7.50
Choice white grease	2.40	5.80	2.80	4.30	6.40	3.84	5.70	6.90	6.00
Monocal P, 21%	0.50	1.82	1.01	0.54	1.70	1.00	0.35	1.36	0.73
L-Lysine	0.22	0.18	0.20	0.18	0.19	0.19	0.08	0.27	0.10
DL-Methionine	0.38	0.42	0.37	0.32	0.37	0.32	0.10	0.31	0.24
L-Threonine	0.30	0.30	0.30	0.20	0.30	0.25	0.23	0.20	0.20
Limestone	0.35	0.75	0.55	0.40	0.74	0.50	0.45	0.78	0.58
Salt	0.10	0.40	0.27	0.20	0.35	0.30	0.10	0.25	0.10
Vitamin TM premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100	100	100	100	100
Calculated analysis									
ME, Kcal/kg	13.00	12.60	12.30	13.00	12.90	12.90	13.00	13.20	13.30
Crude protein, %	21.23	21.24	21.21	19.08	19.05	19.48	18.74	17.80	18.97
Crude Fat, %	6.17	8.34	6.82	7.92	9.04	7.73	9.24	9.66	9.76
Avail lysine, %	1.18	1.18	1.18	1.07	1.07	1.07	0.87	0.87	0.87
Avail methionine, %	0.59	0.55	0.56	0.53	0.50	0.51	0.46	0.45	0.45
Analyzed results									
Crude protein, %	19.35	23.20	20.22	19.13	20.34	19.68	18.92	17.58	22.00
Crude fiber, %	2.06	2.21	2.08	2.21	2.49	2.32	2.34	2.23	2.70
Fat, %	5.64	7.96	6.82	7.92	9.04	7.73	9.24	9.66	9.76

[1]Protein Sources: FM: Fish Meal, SBM: Soybean Meal, PBM: Poultry by-product Meal.
[2]Supplied the following minimum supplements per kilogram: vitamin A, 635,600 IU; vitamin D3, 22,7000 ICU; vitamin E, 1,362 IU; menadione, 68.1 mg; riboflavin, 544.8 mg; thiamine, 90.8 mg; d-pantothenic acid, 544.8 mg; niacin 2.270 mg; vitamin B6, 113.5 mg; folic acid, 56.75 mg; choline, 31,780 mg, biotin, 3.632 mg; Mn, 40,000 mg; Zn, 40,000 mg; Fe, 20,000 mg; Cu, 4,500 mg; I, 500 mg; and Se, 60 mg.

Table 2.2 Effect of protein source on body weight, feed intake and feed conversion ratio on broilers reared for 42 days[1]

	BW, g			ADFI, g			AdjFCR		
	Age (d)								
Protein source	14	28	42	14	28	42	14	28	42
Fish meal	372[b]	1349[b]	2879[b]	32[b]	66[b]	101[b]	1.36[a]	1.42[a]	1.52
Soybean meal	429[a]	1468[a]	3018[a]	34[a]	69[a]	108[a]	1.25[b]	1.37[b]	1.53
Poultry by-product meal	413[a]	1448[a]	2931[a]	34[a]	68[a]	103[ab]	1.27[b]	1.36[b]	1.50
SEM[2]	6	18	30	0.27	0.56	1.40	0.01	0.02	0.01
P-value	<0.0001	<0.0001	0.0101	0.0001	0.0005	0.0067	<0.0001	0.0147	0.3149

[1]A total of 900 1-day old Cobb 500 broilers were used with 25 chicks per pen and 12 replicates per treatment.
[2]SEM: Standard error of means.
[ab]Mean values within a column with different superscripts are significantly different ($P<0.05$).

Table 2.3 Effect of replacing fish meal with soybean meal and poultry by-products meal on total feed cost/kg of gain[1,2]

Treatment	Total feed cost/kg of gain
Fish meal	1.75[a]
Soybean meal	1.23[c]
Poultry by-products meal	1.40[b]
SEM	0.0161
P-value	<0.0001

[1]Ingredient prices used in feed cost estimation were obtained from August 17, 2018.
[2]Feed cost per metric: FM:$448, SBM: $329, PBM: $352.
[a-c]Mean values within columns are significantly different ($P \leq 0.05$).

Table 2.4 Chemical analysis of protein sources (as-fed basis) used in diets[1]

	Protein Sources[2]		
Analyzed results, %	FM	SBM	PBM
Crude protein,	57.04	47.19	65.92
Fat	10.40	1.39	13.40
Lysine	3.91	3.02	4.06
Methionine	1.49	0.63	1.30
Lysine digestibility	87.4	89.1	84.3
Methionine digestibility	90.8	93.3	87.4
Digestible lysine	3.41	2.93	3.43
Digestible methionine	1.35	0.59	1.13
Ash	23.29	6.41	13.80

[1]Ingredients were analyzed by Adisseo NIR Service.
[2]Protein Sources: FM: Fish Meal, SBM: Soybean Meal, PBM: Poultry by-products Meal.

Chapter 3 - Effect of Storage Time and Trace Minerals on the Stability of Vitamins stored at High Temperature and Relative Humidity and Their Subsequent Effect on Broiler Performance

Abstract

Storage of vitamins with trace minerals as premixes at high temperature and relative humidity affects vitamin stability. The objective of this study was to determine the effect of storage time and trace minerals on the stability of vitamins stored at high temperature and relative humidity and their subsequent effects on broiler growth, bone strength and ash. Vitamin and trace mineral premixes were manufactured using a cross flow blender and mixed for three minutes. Separate batches of vitamins and trace minerals were mixed at 30 days intervals to create seven treatments, 0 d VP, 30 d VTMP, 30 d VP, 60 d VTMP, 60 d VP, 90 d VTMP and 90 d VP. Treatments were stored in an environmentally controlled chamber at a temperature of 29.4°C and relative humidity of 75%. Treatments were used to manufacture diets and allocated to batteries using a randomized complete block design. A total of 280 1-day old male Cobb 500 broilers were randomly allocated to 56 cages. Diets used in the study were balanced for amino acids and metabolizable energy. Loss of vitamin activity was greater in VTMP as compared to VP without trace minerals. VP stored for 90 d had higher losses of vitamin activity as compared to those stored for 30 days. Vitamin B_1 had the highest loss of activity for all the storage periods. At d 7 and 14, BW of birds fed 0 d VP and 60 d VTMP was significantly different ($P \leq 0.05$) from birds fed diets containing 90 d VTMP. However, ADFI of birds fed the different treatments were not significantly different ($P \geq 0.05$). AdjFCR was better for birds fed 90 d VTMP and 90 d VP than birds fed 0 d VP. Overall, no significant difference was observed in BW, ADFI and

AdjFCR. Bone-breaking strength and ash of birds were not significantly affected ($P \leq 0.05$) by treatments.

Keywords: VTMP: vitamin trace mineral premix, VP: vitamin premix, BW: body weight, AdjFCR: adjusted feed conversion ratio, ADFI: average daily feed intake, broilers.

Introduction

Vitamins are organic compounds required by animals for proper growth and development (Ekaidem et al., 2006; Coelho, 1991) that cannot be synthesized in the body; therefore, they must be included in diets. Vitamins and trace minerals are essential co-factors in many metabolic processes for effective utilization of nutrients in animal diets (Shurson et al., 2011). Also, they are active biochemicals, which are generally sensitive to their physical and chemical environments (Coelho, 1991). The type of matrix used in a premix or finished feed in which vitamins are added greatly affects their stability. Mixing vitamins with trace minerals reduces the vitamins' stability as vitamins are labile nutrients and can easily be oxidized (Gadient, 1986; Schneider, 1986). When mixed with premixes, highly reactive trace minerals such as copper, zinc and iron, oxidize vitamins and increases deterioration (Shurson et al., 2011). Coelho (1991) reported that friction is an important factor in vitamin stability because it causes erosion of protective vitamin coatings and reduces the vitamin crystals to smaller particles, which increases the surface area of vitamins. This increases the vitamins and trace minerals reactions and results in loss of vitamin activity.

Individual vitamins have different degrees of sensitivity to environmental conditions under which they can be degraded (Shurson et al., 2011). Vitamins A, D_3, K_3, C and B_1 are

affected by temperature and longer periods of storage whereas riboflavin, vitamin B_6 and folic acid are easily degraded when exposed to light, although they can withstand high temperatures and longer periods of storage (McDowell, 2012).

Conditions such as humidity, temperature, pH and storage time have been shown to affect the stability of vitamins with trace mineral premixes (Coelho, 1991; Gadient, 1986; Killeit, 1988). Storage of vitamins at high temperatures and relative humidity for a long period in the presence of trace minerals has been shown to have adverse effects on vitamin stability (Coelho, 1991; Zhuge and Klopfenstein 1986). For example, Adams (1972) reported 55% loss of vitamin B_6 activity after storing a multi-vitamin premix containing inorganic trace minerals for three months at 36.7°C as compared to a 24% loss in a similar premix with no trace minerals. Quackenbush (1963) also reported 50% loss of carotene (Vitamin A precursor) in a hybrid yellow corn when stored for eight months. However, after three years of storage loss of carotene activity increased to 75% of the total amount of carotene present in the corn. Frye (1994) observed 20% loss of vitamin E activity when he stored vitamin trace minerals premix for three months.

Grain and oilseed-based diets, such as corn-soybean meal, fed to chickens do not contain adequate vitamin levels and therefore must be fortified with vitamins. However, the addition of vitamin trace mineral premixes at concentrations lower than the minimal NRC requirements of broilers may cause deficiencies, which in turn may affect feed intake, feed efficiency and growth. Poultry raised under intensive production systems are particularly susceptible to vitamin deficiencies (Ward, 1996) as compared to other livestock. This is due to the limited ability of the gut flora of broilers to synthesize vitamins, the high vitamin requirements by broilers and the increased stress from modern high-density production. Therefore, vitamin and vitamin trace

mineral premixes to be used in broiler diets must be monitored during storage. The objective of the study was to determine the effect of storage time and trace minerals on the stability of vitamins stored at high temperature and relative humidity and their subsequent effects on broiler growth performance, bone strength and ash.

Materials and Methods

Premix Formulation, Manufacturing and Storage

Vitamin trace mineral premixes (VTMP) were prepared at Kansas State University using a cross-flow blender (V-blender) (Patterson Kelly cross-flow blender, Stroudsburg, PA). A single lot of broiler trace minerals (TM) and vitamin premix (VP) was used. The TM was stored at room temperature and VP was held in cold storage between manufacturing periods. The VP (0.227 kg) was added to the TM (0.908 kg) and blended for three minutes to create the VTMP. Premixes were prepared at 30 d intervals to create the 30, 60 and 90 d treatments. VP treatments were created by weighing and storing VP in 3-ply paper bags that were sealed at the time of each mixing period. VTMP and VP were stored in an environmentally controlled chamber with a data logger to record daily low and high temperature and relative humidity fluctuations. Room temperature and humidity were maintained between 29.4 and 31.7°C and 74 and 79%, respectively throughout the storage period.

Sampling and Assays

A 50 g sample of each treatment was sent to the laboratory to determine vitamin activity. Vitamin A was determined using AOAC 974.29 mod method with an UPC^2 instrument. Vitamin D_3 and E were analyzed using LC/MS and HPLC instruments according to the modified AOAC official method 2011.12 and AOAC official method 971.30 α-tocopherol and α-tocopherol

acetate in foods and feeds (modified), respectively. Vitamins B_1 and B_6 were determined with the HPLC through the modified ACQuity UPLC H-Class system. Percent loss of vitamin activity was calculated using the difference between vitamin concentrations before and after storage.

Feed Formulation and Manufacturing

Seven experimental diets were manufactured to meet or exceed the minimum nutritional requirement of broilers (NRC, 1994) and fed for 22 d (Table 3.2). All diets were manufactured at the O.H. Kruse Feed Technology Innovation Center using Current Good Manufacturing Practices (CGMPs). Diets were mixed for three minutes in a Hayes-Stolz ribbon mixer (Model 2261905, Burleson, TX) and fed in mash form. The VTMP was mixed with 2.27 kg of feed before adding it to the mixer to enhance distribution.

Bird Management

The care of the animals used in the trial conformed to the Guide for Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010). The experiment was conducted at the Poultry Teaching and Research Facility at Kansas State University. A total of 280 Cobb 500 male 1-d old broilers were randomly allotted to four batteries with each battery serving as a block and reared for 22 d. There were five birds per cage and 56 cages in total. The cages within each block were randomly assigned to treatments with two replications per block and eight replications per treatment in total. Chicks were introduced to feed and water at the time of placement. Chicks were allotted a total 0.9 kg starter diet. Chicks were allowed *ad libitum* access to water and feed throughout the study. Chicks were provided 24 hours lighting from d 1 to 22. Brooding temperature was maintained at 32°C throughout the study. Birds were observed daily,

and dead and weak birds were removed and weighed to calculate adjusted feed conversion ratio (AdjFCR).

Data Collection

Feed disappearance and cage body weight (BW) were recorded subsequently at d 7, 14 and 22. Mortality was removed, weighed and recorded daily. Feed conversion ratio was adjusted for each cage by adding mortality weight to live bird weights. Cage weights and feed disappearance were used to determine ADFI and AdjFCR.

Bone Breaking Strength and Ash Analysis

On d 22, two birds were randomly selected from each cage after weighing. Birds were euthanized, and the right leg was removed. Collected legs were bagged and stored in a freezer at 4°C. The legs were thawed for a day, the flesh was then removed from the tibia and the tibia placed in plastic sample bags to minimize moisture loss. The sample bags were frozen for 24 hours. Sample bags were then removed, thawed and tibia weights recorded. Tibia breaking strength (breaking force divided by bone weight expressed as kgf/g) was measured using an Instron Universal Testing Machine (model 6656, Instron Corporation, Canton, MA, USA) with 100 kg-load cell range at a crosshead speed of 50 mm/min with the tibia supported on a 3.49 cm span. Moisture free ash was determined by ashing the tibia in tared ceramic crucibles for 24 h at 600°C. The percentage of tibia ash was determined by dividing tibia ash weight by dry tibia weight and multiplying by 100.

Statistical Analysis

Vitamin and vitamin trace mineral premixes were arranged in a 2 × 3 factorial with trace minerals (with and without TM) and storage time (30, 60 and 90 days) plus a control.

For the bird study, treatments were allocated to blocks using a randomized complete block design. Battery cages were the experimental unit and battery was the block. The data was analyzed using GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC) as a randomized complete block design. Significant differences were based on $P \leq 0.05$.

Results

Loss of Vitamin Activity

Average monthly temperature and relative humidity in the environmental chamber were 29.9°C and 76.5%, respectively. Temperature and relative humidity ranged between 29.9 ± 3°C and 76.5 ± 1.6 over the 90 d storage period, respectively. Figures 3.1 to 3.5 represents vitamin retention levels used to estimate percent loss of vitamin activity.

Generally, loss of vitamin activity was greater for all vitamins stored as VTMP versus vitamins stored as VP (Figures 3.1 to 3.5). Loss of vitamin activity in VTMP and VP for 30 d were 79.6% and 15.8%, respectively. At 60 d, loss of vitamin activity in the VTMP increased to 81.4%, but lower loss was observed for VP. Loss of vitamin activity at 90 d storage was similar to that of 60 d for the VTMP, but higher VP (19.6%) loss was observed. Thus, storing vitamins in the presence of TM for 90 d (81.4%) resulted in higher loss of activity as compared to when stored for 30 d (79.6%).

Individual losses in vitamin activity ranged from 5% to 31% for VP and 79% to 83% for VTMP over the whole experimental period (Table 3.1). Loss of vitamin A activity was 79%,

82% and 82% for VTMP and 16%, 14% and 30% for VP at 30, 60 and 90 d, respectively. Loss of vitamin D_3 activity was 78%, 81% and 82% for VTMP and 10%, 9% and 17% for VP at 30, 60 and 90 d respectively. Loss of activity in vitamin E was 79%, 81% and 82% for VTMP and 9%, 6% and 5% for VP at 30, 60 and 90 d, respectively. Loss of vitamin B_1 activity was 80%, 83% and 83% for VTMP and 29%, 25% and 31% for VP at 30, 60 and 90 d, respectively. Loss of vitamin B_6 activity was 82%, 82% and 81% for VTMP and 15%, 14% and 15% for VP at 30, 60 and 90 d, respectively.

Effect of Loss of Vitamin Activity on Broiler Performance

Mortality was 3.21% for the 22 d bird study and was not due to treatment effects. The levels of VP and VTMP added to complete diets are shown in Table 3.3. The subsequent effect of loss of vitamin activity on broiler growth performance is shown in Table 3.4.

At d 7 and 14, BW of birds fed diets containing treatments were significantly different ($P \leq 0.05$). Birds fed diets containing 0 d VP and 60 d VTMP had lower BW as compared to birds fed 90 d VTMP diets. BW of birds fed 30 d VP and VTMP, 60 d VP and 90 d VP were intermediate between the 0 d VP, 60 d VTMP and 90 d VTMP. But the differences in BW of birds fed treatments disappeared at d 22.

Similar ADFI was observed for birds fed treatments at d 7 14 and 22. Thus birds fed different treatments consumed similar amounts of feed over the whole experimental period.

AdjFCR at d 7 and 14 was impacted by treatments with birds fed 0 d VP having poorer AdjFCR as compared to those fed diets containing 90 d VP and VTMP. But overall AdjFCR was similar ($P \geq 0.05$) for birds fed treatments.

Effect of Loss of Vitamin Activity on Bone breaking strength and Ash

No leg abnormalities were observed in the study. At the end of the study, bone strength and ash were not significantly different ($P \geq 0.05$) for birds fed diets containing different concentrations of vitamins (Table 3.5). This was not unexpected since the loss of vitamin D_3 activity was not high enough in any of the treatments to cause a deficiency that could produce leg abnormalities such as rickets.

Discussion

Loss of Vitamin Activity

The results of this study suggest that loss of vitamin activity can be reduced when stored as VP rather than as VTMP under high temperature and relative humidity for 90 d. The high loss of vitamin activity observed for the VTMP was due to the presence of TM, which increased redox reaction. The metallic nature of some TM such as copper, zinc and iron erode the protective coatings of vitamins through friction and reduces vitamins to smaller particles, which increases the surface area for reaction between vitamin particles and vitamin and trace mineral particles (Coelho, 1991). In VTMP, TM reacts with vitamins by gaining electrons from vitamins causing them to be oxidized. At high temperatures and relative humidity, reaction between particles of vitamins and trace minerals increase and this is because temperature and relative humidity act as catalysts. For particles to react, they must first collide. At high temperatures, particles move faster, increasing the rates of collision and reaction.

Losses in vitamin activity for this study were higher than the results observed by Shurson et al. (2011) and did not conform to some of his ranking on the effect of trace minerals on some vitamins (Shurson et al., 1996). He ranked vitamin D_3 as more stable in commercial premix than

vitamins A, E, B_1 and B_6. However, the current study observed similar loss of vitamin activity for vitamins A, D_3, E and B_1 after 90 d of storage. The difference between the current study and his study could be due to the differences in relative humidity. In their study treatments were stored for 120 d at lower relative humidity (38, 34, 24 and 22%) compared to the 90 d storage and 76.5% relative humidity used in the current study.

The loss of vitamin A activity observed for the present study was expected because vitamin A stability is highly affected by temperature, relative humidity and TM (McDowell, 2012). Also, previous studies have reported high loss of vitamin activity when vitamin A was stored at similar or higher conditions than ones used in the current study. A study conducted by Cort et al.(1975) observed a 28% loss of vitamin A activity when wheat flour was stored for three months at 45°C. Chen (1990) also reported 20% to 70% loss of vitamin A activity when three commercial cross-linked vitamin A were stored for three months at high temperature and relative humidity. The loss of vitamin A activity in the current study was slightly lower (81%) than those reported by Christian (1983) who observed a 98% loss of vitamin A activity when stored in a base-mix containing inorganic TM for three months in an environment similar to those used in the current study. Quackenbush (1963) reported three-quarters loss in carotene (precursor of vitamin A) activity after storing hybrid yellow corn high in carotene at 25˚C for years. However, loss of carotene was less when stored at a lower temperature, 7˚C

Stability of vitamin D is reported to be affected most by high temperature, relative humidity as well as TM (McDowell, 2012). Storing vitamin D_3 with TM at high temperature and relative humidity reduced vitamin D stability resulting in high loss of vitamin activity. Loss of vitamin D_3 activity in the current study was higher than the results observed by Hirsch (1982) after storing a conventional vitamin D_3 product with trace mineral premix at 22°C for 12 weeks.

In his study the presence of TM resulted in 66% loss of vitamin activity. The temperature used in his study was lower than the temperature used in the current study. The lower loss of vitamin activity observed in his study as compared to the current study could be due the lower temperature (22°C) the VTMP was stored at. Schneider (1986) also reported 10% to 30% loss in vitamin D_3 activity after storing vitamins for either four or six months at 22°C. This indicated that storing vitamin D_3 at a low temperature may reduce loss of vitamin activity and vice versa.

The lower loss of vitamin E activity observed in the VP in the current study was not unexpected because vitamin E is described as one of the most stable vitamins and usually ranked among the vitamins less sensitive to temperature and relative humidity (Gadient, 1986; Shurson et al, 2011). However, the presence of TM reduced their stability through friction by eroding their protective coatings resulting in high loss of vitamin E in the VTMP.

Vitamin B_1 is described as one of the most unstable B vitamins. It is highly unstable at high temperature and relative humidity conditions. Therefore, the high losses (83%) observed in this study were not surprising as this study stored VTMP and VP at high temperature and relative humidity. Vitamin B_1 has been reported to be less stable in the presence of moisture. Hoffman reported 12% loss of activity when flour containing 12% moisture was fortified with vitamin B_1 and stored for five months. However, no losses were observed when the flour moisture level was reduced to 6%. He also recorded 50% loss of vitamin B_1 activity when foods fortified with vitamin B_1 were pasteurized or boiled.

Loss of vitamin B_6 was lower when stored as VP because of its ability to withstand high temperature and relative humidity. But in the presence of TM they were rapidly degraded, and this was because the stability of Vitamin B_6 is highly affected by the presence of TM (Adams, 1982). The loss of vitamin B_6 activity (81%) in the current study was similar to the results

reported by Adams (1982) who observed an 81% loss after storing pyridoxine in a vitamin premix that contained inorganic trace minerals for three months.

Effect of Loss of Vitamin Activity on Broiler Performance

The growth performance observed at d 7 and 14 of the study were unexpected, as the ADFI was similar but there were differences in BW and AdjFCR. The BW of birds at d 7 and 14 were 74% and 85% of target weight respectively. The differences observed in BW could be due to differences in bird metabolism as weight variations were observed within cages. Bottje et al. (2006) reported that male broilers of the same genetic line fed the same diet may exhibit different feed efficiency phenotypes due to differences in mitochondrial metabolism. Birds may consume similar amounts of feed but the conversion of the feed to gain may differ. Birds fed the diets continued to improve as their age advanced and this resulted to similar performance at d 22. The poor performance which was observed for some birds in the early stages of the study may have been due to issues at the hatchery which may have affected the metabolism of these birds. However, reasons for the lower BW observed for birds fed 0 d VP are unclear; therefore, further studies should be conducted to determine the effect of feeding high concentration of VP to young birds.

The overall no difference in growth performance at d 22 suggested that although there were significant losses in vitamin activity in the premixes with the greatest loss of vitamin activity occurring in the VTMP stored for 90 d, the amounts of vitamins retained were either equal to or slightly higher than the NRC (1994) requirements for broilers (Table 3.3). This indicated that the losses of vitamin activity for the storage periods and conditions were not extreme enough to result in poorer performance in the 22 d chick assay. Similarities observed in

growth performance of birds fed the different treatments agreed with Moravej et al. (2013) who observed no differences in BW, FI and FCR when birds were fed diets containing different levels of VP. Han et al. (2013) also found no significant differences in the growth performance of birds fed diets containing varying levels of vitamin D_3.

Effect of Loss of Vitamin Activity Bone Breaking Strength and Ash

Bone quality is measured using several factors such as bone ash, bone mineralization and bone-breaking strength. Bone-breaking strength and bone ash are affected by the amount of calcium and phosphorus deposited in the bone. The absorption of calcium and phosphorus to the bone matrix increases bone thickness (Peterson et al., 2011) which in turn increases the amount of bone ash and bone-breaking strength (Cromwell et al., 1972). The absorption of calcium and phosphorus across the intestinal epithelium is affected by the amount vitamin D in diets (Han et al., 2013; NRC, 1994). Thus, vitamin D helps mobilize calcium and phosphorus to mineralize the bone matrix which in turn increases bone tissue synthesis. Therefore, the sufficient amounts of vitamin D_3 in the diets fed to chickens enhanced calcium and phosphorus absorption and did not affect bone thickness and in turn bone breaking strength and bone ash. The results observed for bone-breaking strength and bone ash suggested the loss of Vitamin D3 was not in excess to impair the uptake of minerals to the bone matrix.

Conclusion

Vitamins stored in the presence of trace minerals at high temperature and relative humidity for 90 d affected vitamin stability. However, the loss of vitamin activity did not affect BW, ADFI and AdjFCR of broilers fed for 22 d. This study indicated that storing vitamins in the

presence of trace minerals at high temperature and relative humidity for 90 d led to higher loss of vitamin activity but did not affect overall BW, ADFI and AdjFCR of birds fed for 22 days.

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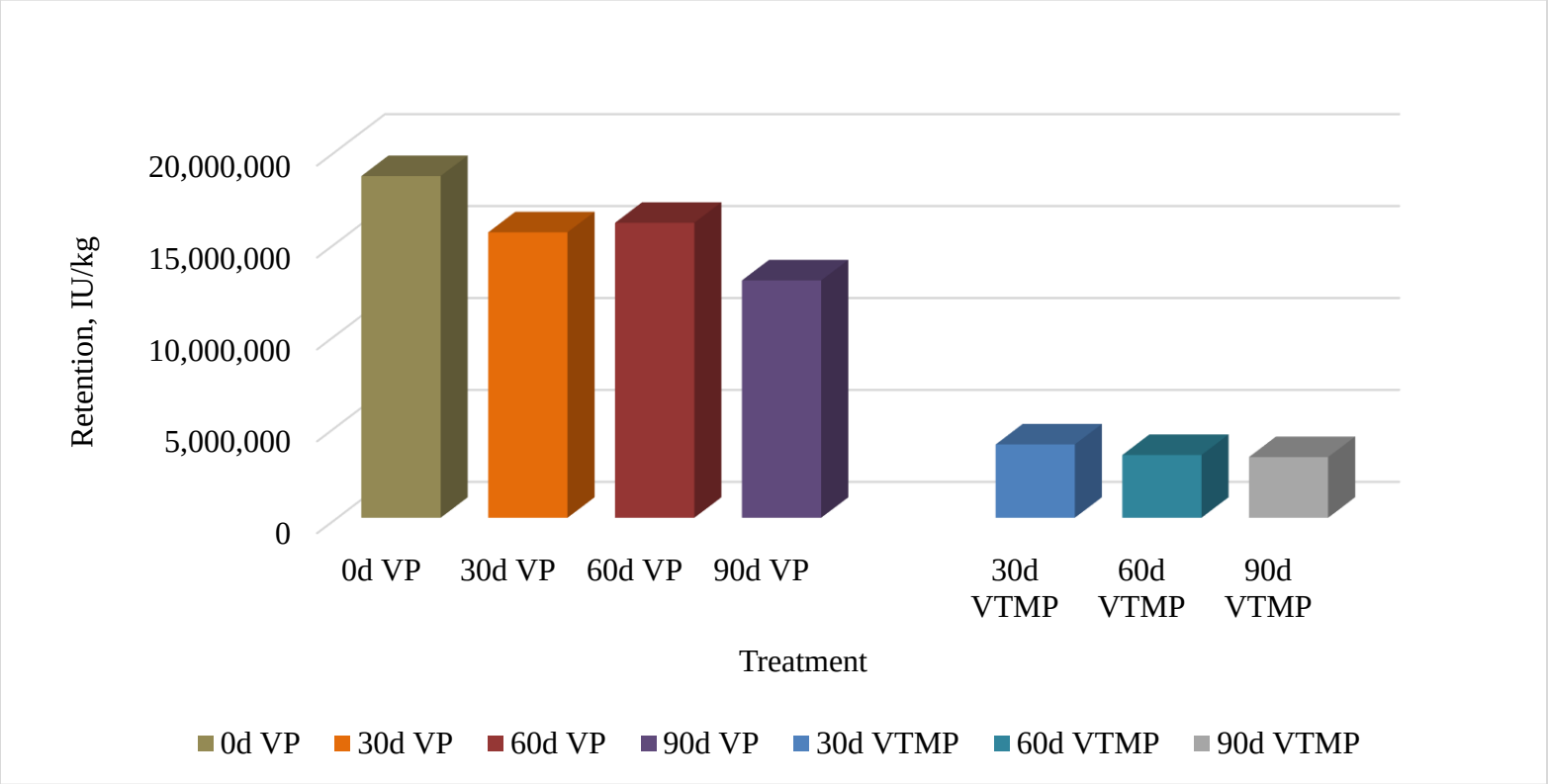
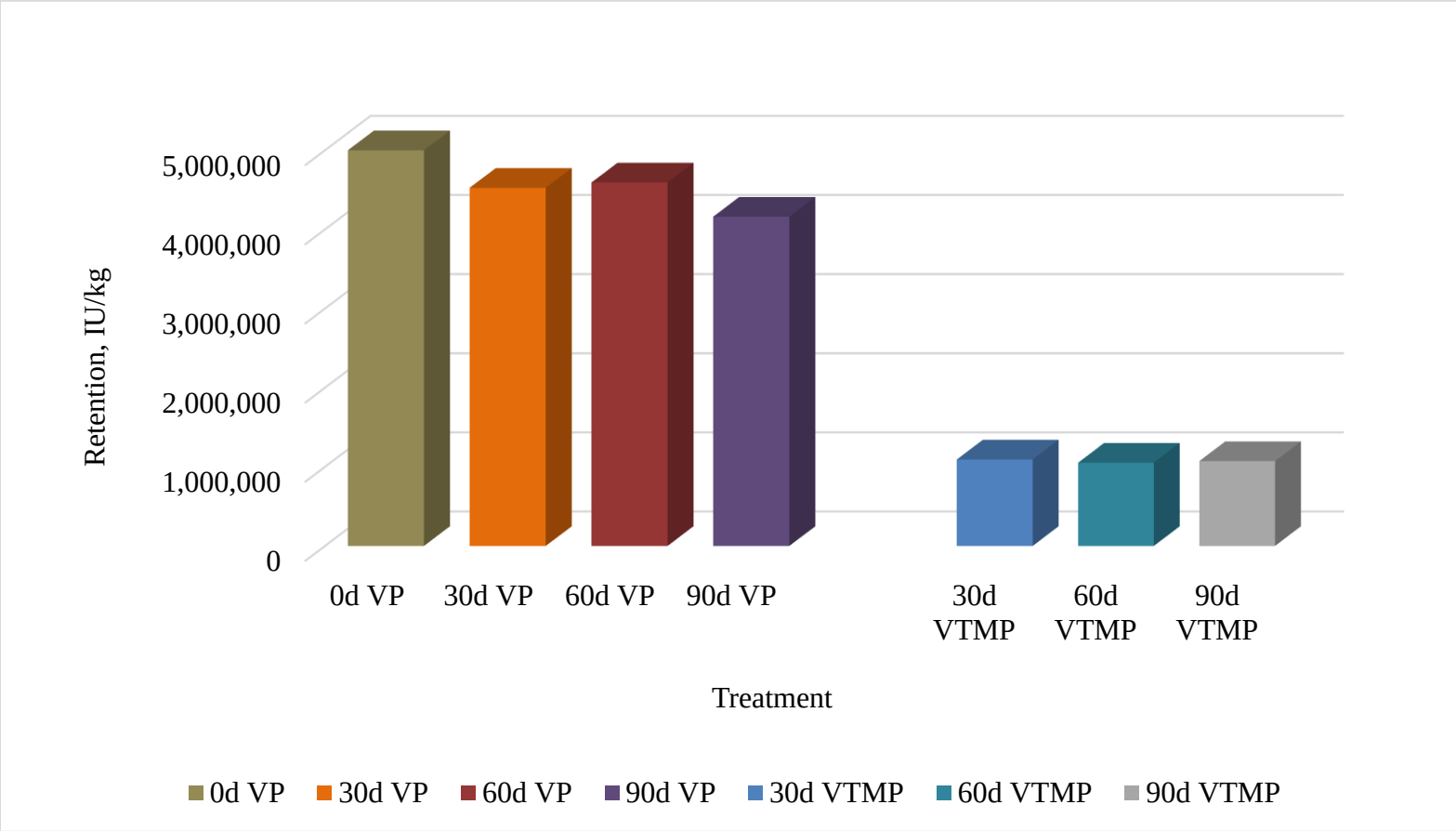
Figures and Tables

Figure 3.1 Effect of storage time and trace minerals on vitamin A retention level



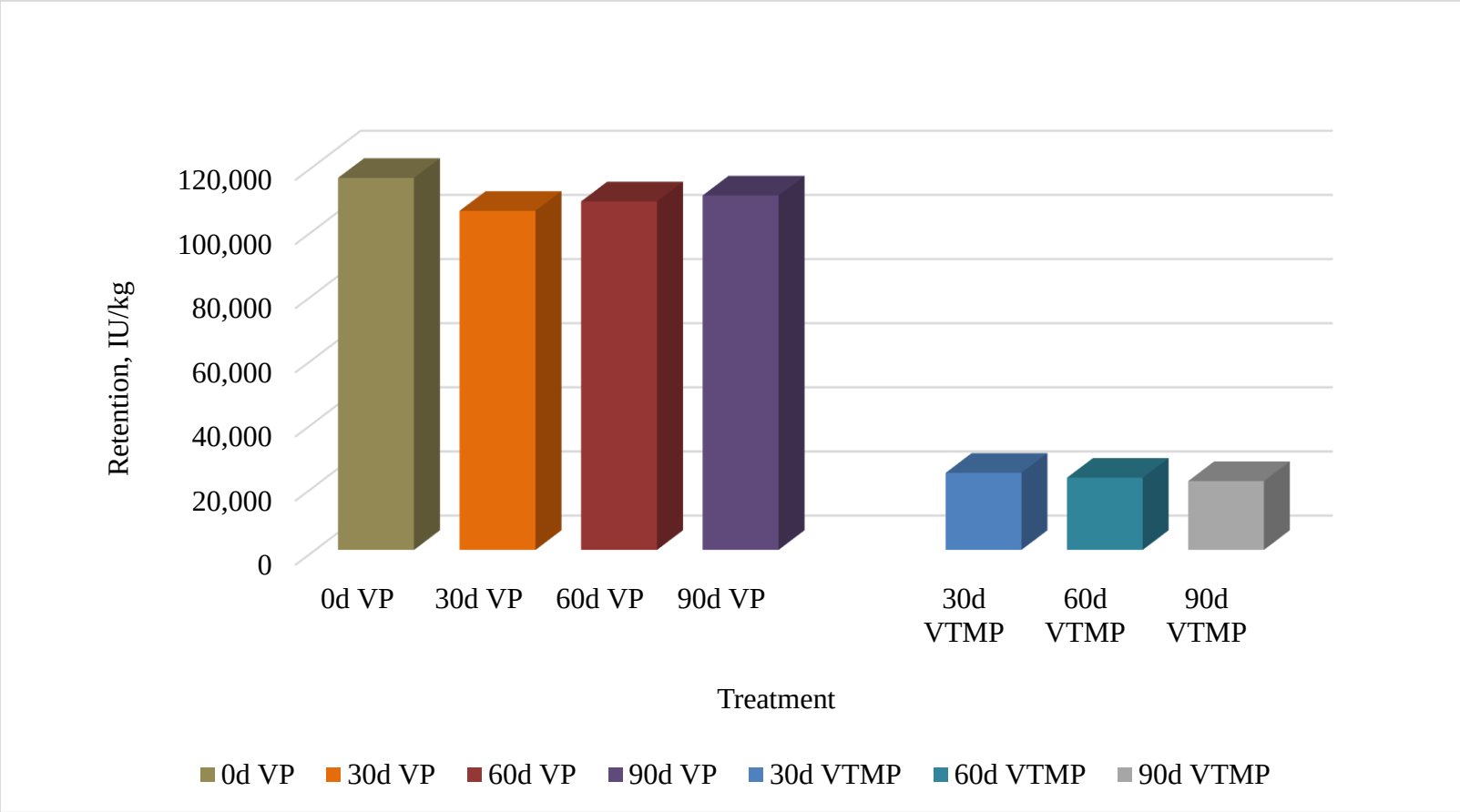
Vitamins premix (VP) and vitamin trace minerals (VTMP) were stored in an environmentally controlled chamber at a temperature of 29.4°C and 75% humidity.

Figure 3.2 Effect of storage time and trace minerals on vitamin D_3 retention level



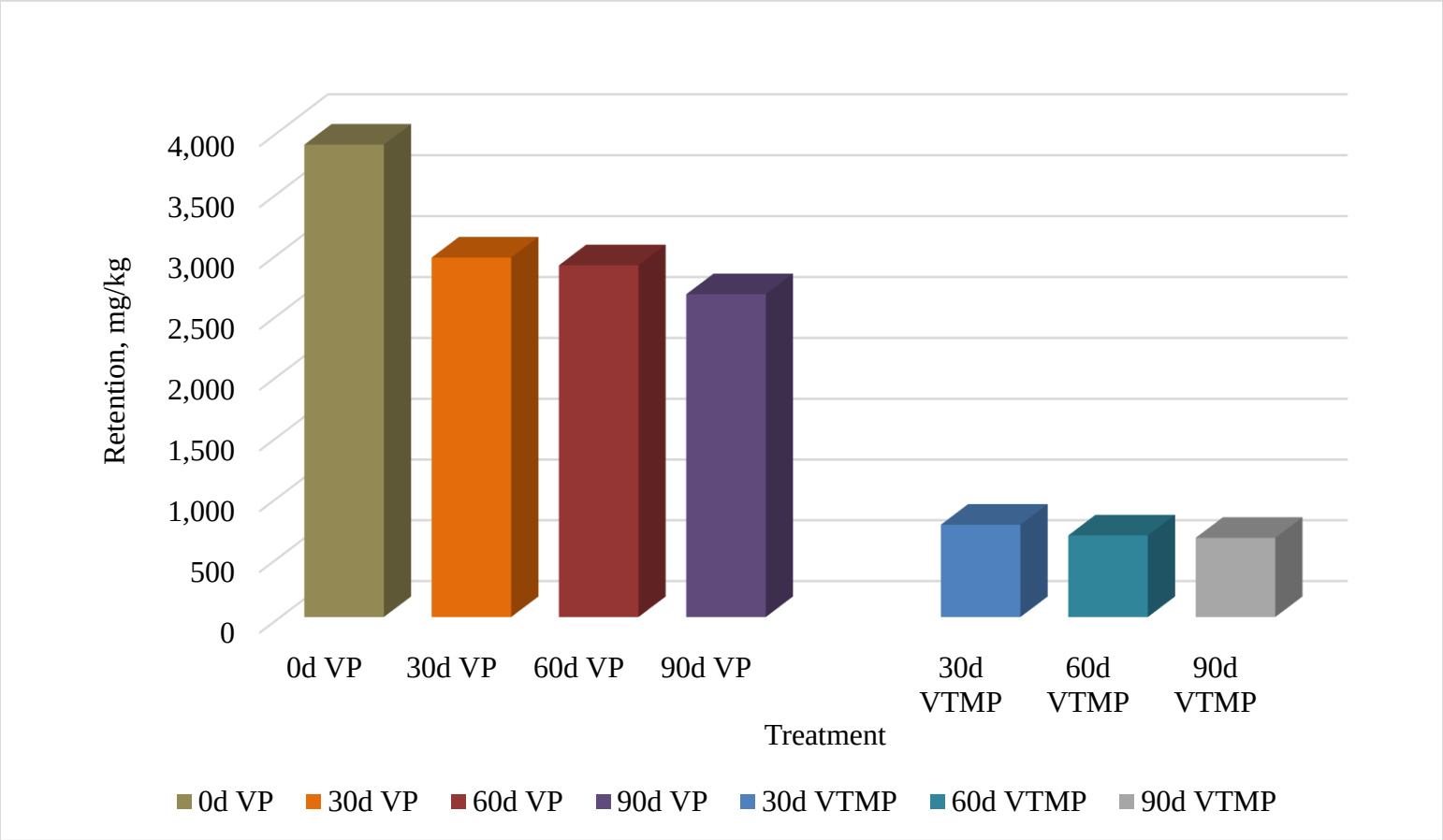
Vitamins premix (VP) and vitamin trace minerals (VTMP) were stored in an environmentally controlled chamber at a temperature of 29.4°C and 75% humidity.

Figure 3.3 Effect of storage time and trace minerals on vitamin E retention level



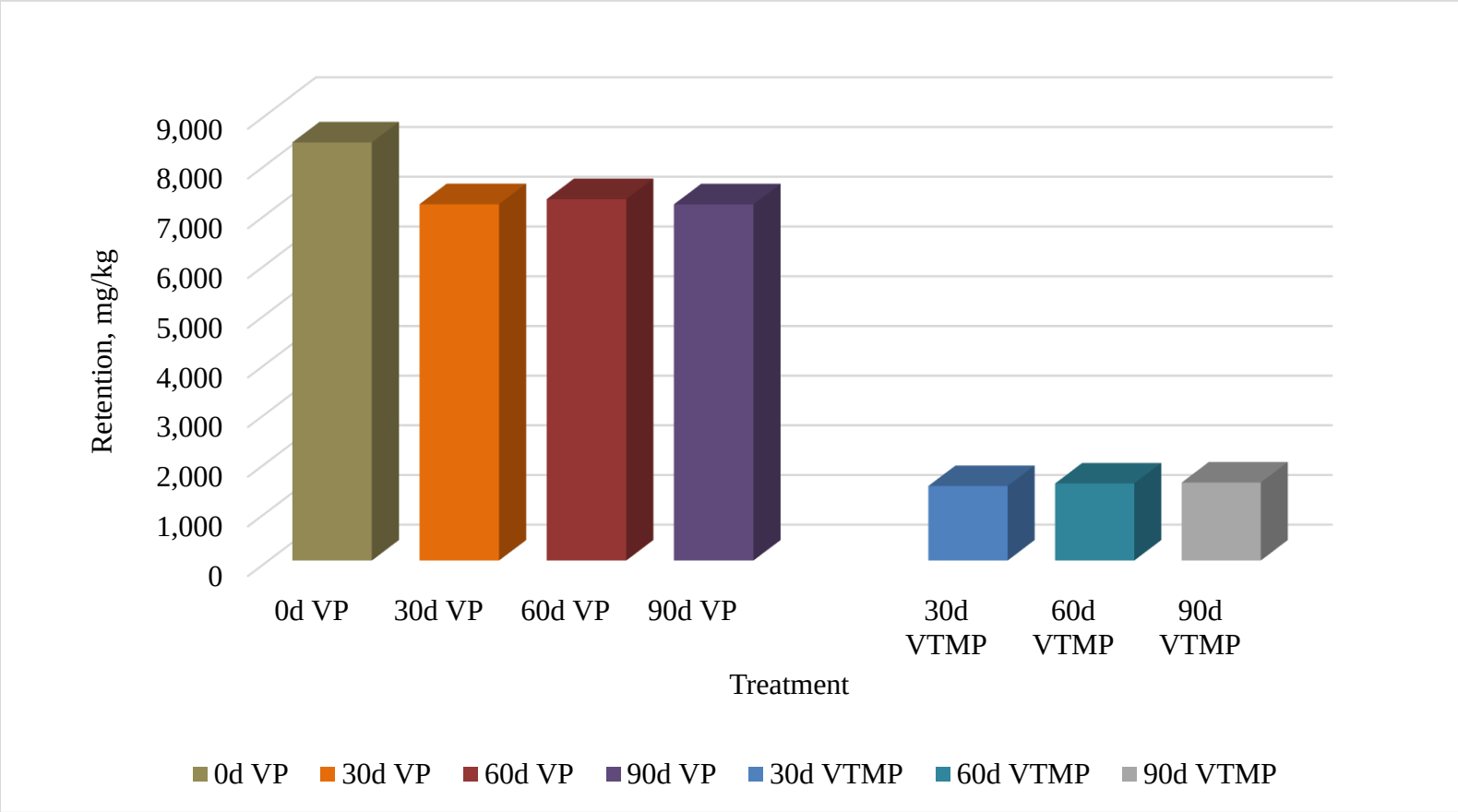
Vitamins premix (VP) and vitamin trace minerals (VTMP) were stored in an environmentally controlled chamber at a temperature of 29.4°C and 75% humidity.

Figure 3.4 Effect of storage time and trace minerals on vitamin B_1 retention level



Vitamins premix (VP) and vitamin trace minerals (VTMP) were stored in an environmentally controlled chamber at a temperature of 29.4°C and 75% humidity.

Figure 3.5 Effect of storage time and trace minerals on vitamin B_6 retention level



Vitamins premix (VP) and vitamin trace minerals (VTMP) were stored in an environmentally controlled chamber at a temperature of 29.4°C and 75% humidity.

Table 3.1 Effect of storage time and trace minerals on loss of vitamin activity

	30d VP[1]	60d VP	90d VP	30d VTMP[2]	60d VTMP	90d VTMP
Vitamin, unit	Loss of Activity, %					
A, IU/kg	16	14	30	79	82	82
D_3, IU/kg	10	9	17	78	79	79
E, IU/kg	9	6	5	79	81	82
B_1, mg/kg	29	25	31	80	83	83
B_6, mg/kg	15	14	15	82	82	81

[1]VP: Vitamin Premix stored without trace minerals.
[2]VTMP: Vitamin Trace Mineral Premix.

Table 3.2 Composition of experimental diet

Ingredient, %	
Corn	59.70
Soybean meal	34.60
Soy oil	1.00
L-Lysine	0.15
DL-Methionine	0.28
L-Threonine	0.08
Monocalcium phosphate 21%	2.10
Limestone	1.40
Salt	0.23
Vitamin TM premix[1]	0.25
Sodium bicarbonate	0.20
Choline chloride	0.01
Total	100
Calculated analysis	
ME, MJ/kg	12.38
Total ME, MJ/kg	12.72
Crude protein, %	22.10
Crude fat, %	3.37
Avail lysine, %	1.20
Avail methionine, %	0.58
Avail threonine, %	0.78
Analyzed results	
Crude protein, %	21.90
Crude fiber, %	2.80
Fat, %	4.00

[1]Supplied the following minimum supplements per kilogram: vitamin A, 18,586,982 IU; vitamin D_3, 5,039,290 IU; vitamin E, 115,990 IU; vitamin B_{12}, 26 mg; biotin, 331 mg; menadione, 3,968 mg; thiamine, 3,883 mg; riboflavin, 8,414 mg; d-pantothenic acid, 22,046 mg; vitamin B6, 7,055 mg; niacin, 82,672 mg; folic acid, 2,205 mg; phytase, 1,000,000 FYT; Ca, 15.75 %; Mn, 6.0 %; Zn, 6.0 %; Fe, 4.0 %; Mg, 2.68 %; Cu, 5000 ppm; I, 1,250 ppm; Co, 500 ppm.

Table 3.3 Comparison of vitamin levels in complete feed versus NRC and Cobb 500 requirements

	Calculated Complete Feed[3]							NRC[4]	Cobb[5]
Vitamin, unit/kg	0 d VP[1]	30 d VP	60 d VP	90 d VP	30 d VTMP[2]	60 d VTMP	90 d VTMP		
A, IU/kg	9,323	7,788	8,047	6,536	2,000	1,715	1,656	1,500	13,000
D3, IU/kg	2,527	2,270	2,303	2,087	548	527	537	200	5,000
E, IU/kg	55	58	53	55	12	11	11	5	80
B1, mg/kg	2	2	1	1	0.4	0.3	0.3	2	3
B6, mg/kg	4	4	4	4	1	1	1	4	4

[1]VP: Vitamin premix.
[2]VTMP: Vitamin trace mineral premix.
[3]Source: Vitamin analysis by DSM laboratory.
[4]Source: National Research Council, nutritional requirements of broilers 1994.
[5]Source: Cobb-vantress.com Broiler performance and nutrition supplement.

Table 3.4 Effect of storage time and trace minerals on broiler performance[1]

	BW, g			ADFI, g			AdjFCR		
	Age, d								
Treatment	7	14	22	7	14	22	7	14	22
0 d VP	134^{bc}	380^{b}	835	19	33	52	1.42^{a}	1.41^{a}	1.42
30 d VTMP	141^{ba}	425^{a}	868	19	36	54	1.33^{ab}	1.35^{abc}	1.39
30 d VP	137^{ba}	399^{ba}	819	19	34	54	1.36^{ab}	1.31^{bc}	1.40
60 d VTMP	133^{c}	380^{b}	819	18	32	51	1.36^{ab}	1.35^{abc}	1.41
60 d VP	136^{bc}	392^{ba}	827	18	33	53	1.37^{ab}	1.37^{ab}	1.42
90 d VTMP	144^{a}	428^{a}	880	17	34	54	1.27^{c}	1.26^{c}	1.34
90 d VP	140^{bac}	411^{ba}	873	17	33	54	1.34^{bc}	1.28^{c}	1.39
SEM[2]	3.6755	10.4925	20.4863	0.8442	1.0020	1.3846	0.0577	0.0363	0.0209
P-value	0.0017	0.0087	0.1114	0.1751	0.1823	0.5802	0.0010	0.0419	0.1026

[1]A total of 280 1-day old Cobb 500 broilers were used in a vitamin study with 5 chicks per cage and 8 replicates per treatment.
[2]SEM: Standard error of means.
[a-c]Mean values within a column with different superscripts are significantly different ($P \leq 0.05$).
BW at 0 d was 48g. BW of birds were 74%, 85% and 87% of Cobb 500 target weight at d 7, 14 and 22 respectively.

Table 3.5 Effect of storage time and trace minerals on vitamins and its subsequent effect on bone-breaking strength and ash[1]

Treatment	Bone-breaking strength, kgf/g[2]	Bone Ash, %
0 VP	2.02	42.63
30 VTMP	1.86	40.25
30 VP	2.02	40.14
60 VTMP	1.76	41.06
60 VP	2.01	39.91
90 VTMP	1.80	41.69
90 VP	1.86	42.05
SEM[3]	0.1002	1.0060
P-value	0.3239	0.3628

[1]Data are means of 8 replications per treatment.
[2]kgf/g: kilogram force per gram of bone weight. Bone breaking strength was determined using an Instron Universal Machine with 100 kg load cell range at a crosshead speed of 50 mm/min and supported on a 3.49 cm span.
[3]SEM: Standard error of means.

Chapter 4 - Determining the Influence of Particle Sizes, Diets, Methods of Analysis and Feed Form on the Predictability of the Near Infrared Reflectance Spectroscopy

Abstract

The near infrared reflectance spectroscopy (NIRS) is a rapid and non-destructive technique used to evaluate the chemical composition of complete feed and ingredients. The accuracy of prediction is not only affected by calibrations but sample particle size, shape and arrangement. The purpose of this study was to determine the accuracy of the near infrared reflectance spectroscopy in predicting the crude protein content of broiler diets of different corn particle sizes, methods of analysis (laboratory, NIRS-ground and NIRS-unground) and feed form (mash and pellet) using standard calibrations provided with the instrument. In Exp. 1, treatments were arranged in a 3 × 3× 4 factorial with diets (Soybean meal and DDGS, Soybean meal Fish meal and DDGS, Soybean meal Fish meal and Wheat bran and Soybean meal and Wheat bran) manufactured using different corn particle sizes (400, 600 and 800 µm) to contain 20% protein content and analyzed with three methods (laboratory, NIRS-ground and NIRS-unground). Exp. 2 was a 3 × 2 factorial with method of analysis (laboratory, NIRS-ground and NIRS-unground) and feed form (mash and pellet). Diets were pelleted and cooled in a counter-flow cooler for 10 minutes. Prior to NIRS analysis, subsamples of mash and pellets were ground through a 0.5 mm sieve. Ground and unground mash and pellet samples for Exp. 1 and 2 were scanned on a Foss NIRS D2500 with a wavelength range of 400 to 2,500 nm at a reflectance of log (1/R) at 2 nm intervals for each sample. Laboratory values from wet chemistry analyses were obtained using a standard method and these were compared to results from the NIRS. In Exp. 1, an interaction

(P≤0.05) was found between diets and method and particle size and method. A significant difference *(P*≤0.05) was recorded for diets but not for particle sizes. Results of NIRS-ground samples were more accurate as compared to NIRS-unground samples. In Exp. 2, an interaction was observed between feed form and method of analysis. Unground feed samples were significantly different (*P*≤0.05) for the feed forms but grinding samples yielded similar results for both NIRS and laboratory analyses. In conclusion, analyzing unground feed samples using standard calibrations yielded less accurate results compared to samples analyzed as ground with either NIRS or wet chemistry (laboratory).

Keywords: near infrared spectroscopy, prediction, chemical composition, ground, unground, wet chemistry

Introduction

Chemical analyses of feedstuffs are essential for nutritionists to formulate diets to meet the nutrient requirements of livestock, (USDA, January 2012; Goedhart, 1990; Lerman and Bie, 1975). However, traditional methods available for ingredient and feed analysis are expensive, time consuming and skill intensive. The near infrared reflectance spectroscopy (NIRS) on the other hand provides rapid and accurate information from high resolution spectra for solid and liquid samples with minimal sample preparation. The NIRS technique is economical, facilitates qualitative and quantitative analyses as well as being non-destructive to samples. Samples analyzed with NIRS require no chemicals and therefore eliminate chemical and disposal costs. NIRS allows the determination of multiple values (crude protein, fat, moisture and fiber) in a single scan unlike wet chemistry analysis where most nutrients are analyzed separately with different methods. The NIRS technique measures light absorption of a feed or ingredient sample when scanned using wavelengths in the near-infrared region (750 to 2500 nm). Spectrum

absorption in the NIR region depends on the chemical bonds (C-H, N-H, S-H or O-H) as well as the physical and structural characteristics of the sample. The chemical bonds make it possible to identify specific regions of the spectrum associated with sample constituents such as starch, crude protein or fiber (Mould, 2003).

Although the instrument is highly efficient, variations can sometimes be observed in results (Undersander, 2006). These variations could be due to factors such as technician, cross-contamination, analysis of a sample that is not present in the installed calibrations, physical form of feed (mash or pellet), particle size, shape, distribution of samples (Norris et al., 1989; Williams and Sobering 1986) and spaces between sample. The use of NIRS in the prediction of ingredient chemical composition is generally more accurate as compared to when used to predict the nutritional contents of compound feeds (Swart et al., 2012). This is because compound feeds fed to poultry are usually made from a wide range of ingredients and by-products of different particle sizes from different sources like cereal grains, animal products and plant products. The use of different quantities of ingredients in different diets may yield different spectral properties (absorption bands and wavelength) even for diets with similar chemical compositions (Givens and Deaville, 1999). Because of this it is important to make bias adjustments to standard calibrations for new samples routinely. Smith and Flinn (1991) suggested that calibrations must be verified for their ability to predict accurate results for new population analysis (samples containing materials from new regions and new diets formulations).

The NIRS has been in existence and use for quite a while and its popularity in the feed industry is on the increase due to its advantages over wet chemistry and the availability of mathematical and statistical evaluation tools and software (Chang et al., 2001). Since the development of the NIRS, several researchers have demonstrated its ability to predict the

chemical composition of feedstuffs and feeds (Garrido, 2000; Garrido et al., 2002; Tillman et al., 2002; Xiccato et al., 2003), but limited research is available in feeds with different particle sizes, feed forms (mash and pellet), diets and physical sample form (ground and unground). Most of the studies done indicated that all samples were ground before NIRS analysis (Swart et al., 2012; Abram, 1989). However, NIRS equipment marketers indicate that samples require limited or no preparation prior to analysis of feed. Therefore, the objective of this study was to determine the accuracy of the NIRS in predicting the crude protein content of compound feeds with different corn particle sizes, different ingredients, methods of analysis (laboratory, NIRS-unground and NIRS-ground) and feed form (mash and pellets) using standard calibrations provided with the instrument.

Material and Methods

Experiment 1

Treatments were arranged as a 3 ×3 ×4 factorial with corn particle size (400, 600 and 800 µm), method of analysis (laboratory, NIRS-unground and NIRS-ground) and diet (Soybean meal and DDGS (SD) Soybean meal, Fish meal and DDGS (SFD), Soybean meal, Fish meal and Wheat bran (SFB) and Soybean meal and Wheat bran (SB)) (Table 4.1).

Corn was ground using a hammermill (Model 2215, Bliss industries, LLC, Ponca City, OK) to obtain the different corn particle sizes. All diets were mixed for six minutes using a Hayes-Stolz mixer (Model 2261905, Burleson, TX). Three replicates were manufactured for each diet. Samples were collected from the replicates of each diet after mixer discharge using a sampling probe. Each sample was divided and ground with a centrifugal mill (Model ZM-200, Retsch GmbH, Retsch-Allee, 42871 Haan, Germany) through a 0.5 mm sieve. Unground and

ground samples were analyzed with the NIRS and compared with laboratory results from wet chemistry analysis.

Experiment 2

Treatments were arranged in a 2 ×3 factorial design with method of analysis and feed form as factors. A corn-SBM wheat bran diet was formulated to contain 20% crude protein and manufactured using 600 µm corn particle size. The diet was manufactured the same way as in Exp. 1. Mash samples were collected during mixer discharge. The diet was pelleted in the Feed Safety Research Center at the O.H. Kruse Feed Technology and Innovation Center using a CPM pellet mill (Model CL-5, California Pellet Co., Crawfordsville, IN). Diets were conditioned to 85°C in a 37 cm × 98 cm conditioner and pelleted using a die with an L/D ratio of 5.55 (diameter= 4.0 mm, effective thickness= 22 mm). Samples of each treatment were collected and cooled in an experimental counter-flow cooler for 10 minutes. Pellet samples were collected after cooling.

Samples of the mash and pellets were ground in the same way as in Exp. 1 Ground and unground mash and pellet samples were analyzed with the NIRS and compared with laboratory results from wet chemistry.

NIRS Analysis

Ground and unground samples of the mash and pellets were scanned with a NIRS (Model, DS2500 Monochromator, Foss NIRSystems, Laurel, MD) using a large ring cup. All near infrared spectra were collected at wavelength between 400 and 2,500 nm registering absorbance values log (1/R) (where, r = reflectance) at 2 nm interval for each sample. Samples were analyzed for crude protein using the factory calibrations provided with the instrument.

Particle Size and Chemical Analysis

Particle size method

Particle sizes were analyzed using the 13-sieve method (ANSI/ASAE S319.4). A 100 ± 5 g of representative sample of ground corn was obtained after collecting and splitting sample. Sieve agitators added to the sieve stack and 0.5 g of flow agent (Model SSA-58, Gilson Company, Inc., Lewis Center, OH) was weighed and mixed with the 100 ± 5 g sample. The mixture was then placed on top of the sieve stack and the sieve stack was placed in the Ro-tap sieve shaker (Model RX-29, W. S. Tyler Industrial Group, Mentor, OH) and run for ten minutes. Each sieve together with its content was weighed and recorded. Weights of sieves before and after analyses were entered into a spreadsheet with formula to determine the mean (dgw) and standard deviation (Sgw).

Chemical Analysis

Portions of the ground samples were analyzed for crude protein using wet chemical reference methods. Protein was determined using a Leco Nitrogen Analyzer (TruMac N, Leco Corporation, St Joseph, MI) according to the Dumas Combustion method (AOAC 990.03). A 0.5 g of each sample was weighed into a tared crucible and placed on the carousel in the machine. Calculations of the crude protein content of the samples were based on the instrument control software. Results were reported as % protein using the nitrogen factor, 6.25 (AOAC, 2003).

% Protein = % Nitrogen x 6.25.

Statistical Analysis

Diets were the experimental unit in Exp.1 and feed form (mash and pellet) were the experimental units in Exp. 2. Data from the study was analyzed using the GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC) and significant statements were based on $P \leq 0.05$. Differences between means were separated using Tukey's studentized pairwise analysis. In Exp.1 particle size versus methods of analysis versus diets were compared, while in Exp.2 methods of analysis vs feed form were compared.

Results

Experiment 1

There was no particle × diet ×method interaction; therefore, these results were not presented. There were significant interactions ($P \leq 0.05$) between diet (SD, SFD, SFB and SB) and methods of analysis (laboratory, NIRS-ground and NIRS-unground) (Table 4.3) and particle size (400, 600 and 800 µm) and methods of analysis (laboratory, NIRS-ground and NIRS-unground) (Table 4.4). The interaction between particle size and methods of analysis was driven by the methods of analysis as NIRS-unground samples of the diets were significantly different ($P \leq 0.05$) (Table 4.3). The interaction between diet and method was also due to methods of analysis. The CP content of NIRS-unground samples of diets were significantly different ($P \leq 0.05$) but once samples were ground and analyzed with the NIRS or wet chemistry, the differences disappeared (Table 4.4). Average CP content of NIRS-ground samples (19.74%) were about 0.7% lower whereas NIRS-unground samples (18.65%) were about 1.8% than laboratory results (20.43%). Overall, correlation between CP content of laboratory and NIRS-ground samples was higher (0.30) than correlation observed between laboratory and NIRS-

unground samples (0.19) (Fig.4.1). However, both NIRS-ground and NIRS-unground CP results were lowly correlated with laboratory results.

Significant differences ($P \leq 0.05$) were also found in diets and the methods of analysis (Table 4.5). However, CP content of particle sizes was similar ($P \geq 0.05$) (Table 4.5).

Experiment 2

There was an interaction ($P \leq 0.05$) between feed form and methods of analysis (Table 4.6). Feed form and method of analysis significantly affected ($P \leq 0.05$) crude protein prediction (Table 4.7). For the differences observed in feed form, CP content of mash (19.69%) was significantly higher than that of pellet (18.80%). Also, CP content predictions from the NIRS-unground samples (17.84%) were significantly lower ($P \leq 0.05$) than NIRS-ground (19.50%) and laboratory results (20.30%). Thus, grinding mash and pellet samples to similar particle size, 0.5 mm eliminated the differences in feed form when analyzed with either wet chemistry method (laboratory) or with the NIRS.

Discussion

Experiment 1

The interaction observed between particles and method and diets and method was due to differences in the NIRS-unground method of sample analysis. The impact of diets on the CP predictability may have been due to differences in particle sizes of diets as the diets contained ingredients of different particle sizes. The different particles of the unground samples of diets may have caused variation in the surface area, which in turn may have affected the amounts of light transmitted, absorbed or reflected (Tamburini et al., 2017) leading to differences in spectral characteristics (absorbance bands, wavelength etc.) (Wetzel, 1983) and CP content predictions

from the NIRS. Particle size affects the distance light travels inside a sample before it is scattered or reflected to the surface. The larger the particle size, the smaller the amount of light that reappears from within the sample causing higher absorption and vice versa, which affects spectra resulting in differences in CP content of samples of variable particle sizes (Spragg and Green, 2013). The difference observed was eliminated when samples were ground and analyzed with either NIRS or wet chemistry. This is because grinding samples to similar particle sizes resulted in similar surface area and therefore similar spectra characteristics. The findings of this study agreed with the report of Abram (1989) who suggested that it was important to analyze mixed feed samples as ground rather than unground samples due to variability associated with unground samples when using the NIRS. Differences between NIRS-ground, NIRS-unground and laboratory values of samples were expected because bias adjustments were not performed on standard calibrations for the sample set. The diets analyzed in the current study may have been different from the diets used in the instrument calibrations. Ingredients used may have also been of different origins, harvesting seasons or processing methods. Results from this study were less accurate as compared to the findings of Swart et al. (2012), Pérez-Marín et al. (2004) and González-Martín et al. (2006) who accurately predicted the CP content of finished feeds using the NIRS. This was primarily due to calibrations that were specifically developed for their sample sets before analyses as compared to the current study where standard calibrations installed on the NIRS were used. This indicated that it is important to verify the ability of standard calibrations to accurately predict CP content of new samples prior to analysis.

Experiment 2

Analyzing unground samples of mash and pellets with the NIRS produced different CP content but grinding samples resulted in similar CP content, for both laboratory and NIRS methods. The differences observed between feed form could be due to differences in spaces between particles and shape of particles (Wetzel, 1983). As compared to mash, the particle size of pellets were much larger causing spaces between particles to be larger than those between mash particles. The differences in spaces between particles might have influenced light transmission, absorption and reflection, affecting spectral response and predicted CP results. Nonetheless, grinding feed samples to similar finer particle sizes eliminated differences in spaces between particles for mash and pellet samples and yielded similar results for both laboratory and NIRS-ground. These results indicated that NIRS-unground samples of mash and pellets may adversely affect the accuracy of CP predictability but grinding samples prior to analysis will produce similar results for both the NIRS and laboratory methods.

Conclusion

In conclusion, analyses of unground mash or pellet samples may result in greater variation in NIRS results but grinding samples prior to analysis may help improve results. Additionally, samples should be routinely analyzed with reference methods and the calibrations adjusted based on the feed manufactured at the feed mill.

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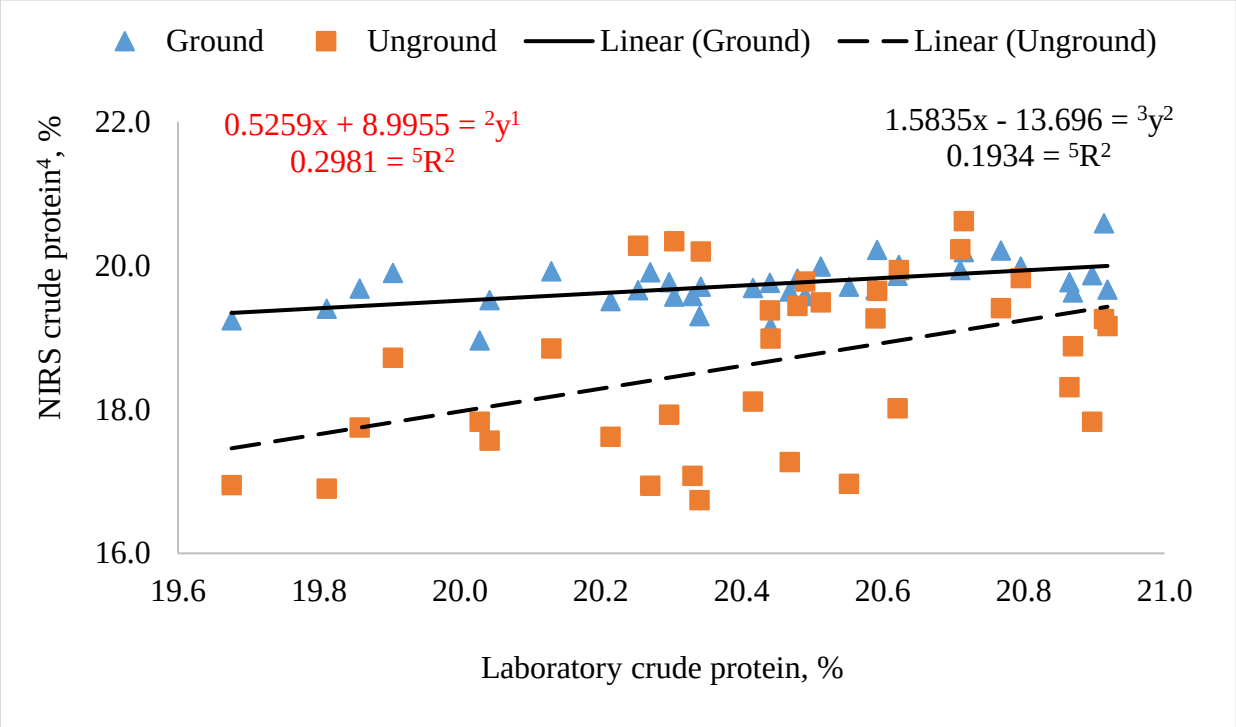
Figures and Tables

Figure 4.1 Correlation between NIRS predictions and laboratory crude protein[1]



[1]36 samples of each NIRS method (ground and unground) and laboratory method were used in the correlation.
[2]y[1]: Regression equation for correlation between NIRS-ground crude protein
[3]y[2]: Regression equation for correlation between NIRS-unground crude protein.
[4]Crude protein from NIRS-ground and NIRS-unground.
[5]R[2]: Coefficient of determination.

Table 4.1 Composition of experiment 1 diet[1]

	Diet[1]			
Ingredient, %	SD	SFD	SFB	SB
Corn	57.58	66.15	62.92	54.01
Soybean meal	30.50	18.00	17.00	30.10
Fish meal	-	9.50	9.70	-
DDGS	2.00	2.00	-	-
Wheat bran	-	-	6.00	6.00
Soy oil	5.80	2.40	2.40	5.80
L-Threonine	0.30	0.30	0.30	0.30
L-Lysine	0.18	0.16	0.18	0.18
DL-Methionine	0.42	0.29	0.30	0.39
Monocal P, 21%	1.82	0.50	0.50	1.82
Limestone	0.75	0.35	0.35	0.75
Salt	0.40	0.10	0.10	0.40
Vitamin TM premix[2]	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated analysis				
ME, MJ/kg	12.70	12.60	12.33	12.36
CP, %	20.10	20.10	20.02	20.02
Crude fat, %	8.40	6.13	6.20	8.43
Fiber, %	2.45	2.31	2.64	2.78
Lysine, %	1.25	1.25	1.25	1.25
Methionine, %	0.63	0.60	0.60	0.60

[1]Diets were manufactured for three different particle sizes, 400, 600, and 800 μm. SD: Soybean Meal and DDGS, SFD: Soybean meal-Fish meal-DDGS: SFB: Soybean meal-Fish meal-Wheat bran, SB: Soybean meal-Wheat bran.

[2]Supplied the following minimum supplements per kilogram of diet; vitamin A, 635,600 IU; vitamin D_3, 22,7000 ICU; vitamin E, 1,362 IU; menadione, 68.1 mg; riboflavin, 544.8 mg; thiamine, 90.8 mg; d-pantothenic acid, 544.8 mg; niacin 2.270 mg; vitamin B_6, 113.5 mg; folic acid, 56.75 mg; choline, 31,780 mg, biotin, 3.632 mg; Mn, 40,000 mg; Zn, 40,000 mg; Fe, 20,000 mg; Cu, 4,500 mg; I, 500 mg; and Se, 60 mg.

Table 4.2 Composition of experiment 2 diet[1]

Ingredient, %	
Corn	54.01
Soybean meal	30.10
Wheat bran	6.00
Soy oil	5.80
L-Threonine	0.30
L-Lysine	0.18
DL-Methionine	0.39
Monocal P, 21%	1.82
Limestone	0.75
Salt	0.40
Vitamin TM premix[2]	0.25
Total	100
Calculated Analysis	
ME, MJ/kg	12.36
CP, %	20.02
CF, %	8.43
Fiber, %	2.78
Lysine, %	1.25
Methionine, %	0.60

[1]Diet contained corn of particle size 600 µm.

[2]Supplied the following minimum supplements per kilogram of diet; vitamin A, 635,600 IU; vitamin D_3, 22,7000 ICU; vitamin E, 1,362 IU; menadione, 68.1 mg; riboflavin, 544.8 mg; thiamine, 90.8 mg; d-pantothenic acid, 544.8 mg; niacin 2.270 mg; vitamin B_6, 113.5 mg; folic acid, 56.75 mg; choline, 31,780 mg, biotin, 3.632 mg; Mn, 40,000 mg; Zn, 40,000 mg; Fe, 20,000 mg; Cu, 4,500 mg; I, 500 mg; and Se, 60 mg.

Table 4.3 Interaction between diets and method of crude protein analysis[1]

Diet[2]	Method[3]	Crude protein
SD	Laboratory	20.14[a]
SFD	Laboratory	20.69[a]
SFB	Laboratory	20.57[a]
SB	Laboratory	20.32[a]
SD	NIRS-ground	19.45[d]
SFD	NIRS-ground	20.05[bdac]
SFB	NIRS-ground	19.68[bdc]
SB	NIRS-ground	19.78[bdc]
SD	NIRS-unground	17.23[f]
SFD	NIRS-unground	19.50[dc]
SFB	NIRS-unground	19.62[dc]
SB	NIRS-unground	18.26[e]
SEM[4]		0.1391
P-value		<0.0001

[1]Treatments were arranged as a 3 x3 x 4 factorial design and contained 20% crude. Factors were particle size, method of analysis and diets. Means of each treatment were obtained from three replicates of each diet.
[2]Diet; SD: Soybean meal-DDGS, SFD: Soybean meal-Fish meal-DGGS, SFB: Soybean meal-Fish meal-Wheat bran, SB: Soybean meal-Wheat bran.
[3]Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (AOAC. 990.09), Ground and Unground: Foss DS2500 NIRS at wavelength between 400 and 2500 nm.
[4]SEM: Standard error of means of interaction.
[a-f]Means with different superscripts within a column are significantly different based on $P \leq 0.05$.

Table 4.4 Interaction between particle size and method of crude protein analysis[1]

Particle size	Method[2]	Crude protein
400	Laboratory	20.48[b]
600	Laboratory	20.42[ba]
800	Laboratory	20.38[a]
400	NIRS-ground	19.75[b]
600	NIRS-ground	19.73[b]
800	NIRS-ground	19.73[b]
400	NIRS-unground	18.41[c]
600	NIRS-unground	18.50[c]
800	NIRS-unground	19.06[c]
SEM[3]		0.1204
P-value		0.4266

[1]Treatments were arranged as a 3 x3 x 4 factorial design and contained 20% crude. Factors were particle size, method of analysis and diets. Means of each treatment were obtained from three replicates of each diet.

[2]Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (AOAC. 990.09), NIRS-ground and NIRS-unground: Foss DS2500 NIRS at wavelength between 400 and 2500 nm.

[3]SEM: Standard error of means of interaction.

[a-c]Means with different superscripts within a column are significantly different based on $P \leq 0.05$.

Table 4.5 Main effects of particle size, diets and method on crude protein analysis[1]

Particle size	Diet[2]	Method[3]	Crude protein
400			19.54
600			19.56
800			19.72
SEM[4]			0.0695
	SD		18.94[c]
	SFD		20.08[a]
	SFB		19.96[a]
	SB		19.45[b]
SEM[5]			0.0803
		Laboratory	20.43[a]
		NIRS-ground	19.74[b]
		NIRS-unground	18.65[c]
SEM[6]			0.0695
P-value			
Particle size			0.1241
Diet			<0.0001
Method			<0.0001

[1]Treatments were arranged as a 3 x3 x 4 factorial design and contained 20% crude. Factors were particle size, method of analysis and diets. Means of each treatment were obtained from three replicates of each diet.

[2]Diet: SD: Soybean meal-DDGS, SFD: Soybean meal-Fish meal-Wheat bran, SFB: Soybean meal-Fish meal-Wheat bran, SB: Soybean meal-Wheat bran

[3]Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (AOAC. 990.09); NIRS-ground and NIRS-unground: Foss DS2500 NIRS at wavelength between 400 and 2500 nm.

[4-6]SEM: Standard error of means of main effects.

[a-c]Means with different superscripts within a column are significantly different based on $P \leq 0.05$.

Table 4.6 Interaction between feed form and method on crude protein analysis[1]

Feed form	Method[2]	Crude protein
Mash	Laboratory	20.26[a]
Mash	NIRS-ground	19.93[ba]
Mash	NIRS-unground	18.81[b]
Pellet	Laboratory	20.32[ba]
Pellet	NIRS-ground	19.20[ba]
Pellet	NIRS-unground	16.88[c]
SEM[3]		0.2792
P-value		<0.0079

[1]Treatments were analyzed as a 3 × 2 factorial, with method (laboratory, NIRS-ground and NIRS-unground), and feed form (mash and pellet).
[2]Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (AOAC. 990.09); NIRS-ground and NIRS-unground: NIRS. Means of each treatment was obtained from four samples.
[3]SEM: Standard error of mean of interaction.
[a-c]Means within a column with different superscript are significantly based on $P \leq 0.05$.

Table 4.7 Main effect of feed form and method of crude protein analysis[1]

Feed form	Method[2]	Crude protein
Mash		19.69[a]
Pellet		18.80[b]
SEM[3]		0.1612
	Laboratory	20.29[a]
	NIRS-ground	19.60[a]
	NIRS-unground	17.84[b]
SEM[4]		0.1974
P-value		
Feed form		0.0010
Method		<0.0001

[1]Treatments were analyzed as a 3 × 2 factorial, with method (laboratory, NIRS-ground and NIRS-unground), and feed form (mash and pellet).
[2]Laboratory: LECO Nitrogen Analyzer based on Dumas Combustion Method (AOAC. 990.09); NIRS-ground and NIRS-unground: NIRS. Means of each treatment was obtained from four samples.
[3-4]SEM: Standard error of mean of interaction.
[ab]Means within a column with different superscript are significantly based on $P \leq 0.05$.

Chapter 5 - Summary of Findings

The first study compared the effect of FM, SBM and PBM on broiler performance and total feed cost per kg of gain. Significant differences ($P \leq 0.05$) were found between overall BW and ADFI for all birds fed the SBM and FM diets. Birds fed the SBM and PBM were heavier compared to birds fed fish meal. Replacing FM with SBM and PBM protein in broiler diets reduced the total cost of feed/kg of gain.

The second study investigated the effect of storage time and trace minerals on the stability of vitamins stored at high temperature and relative humidity and their subsequent effect on broiler performance by mixing vitamins and trace minerals. Losses in vitamin activity were greater in VTMP than VP. Feeding broilers with reduced vitamin concentrations from storage did not significantly ($P \geq 0.05$) affect growth performance, bone strength and ash. The results from this study indicated that storing VTMP in hot and humid environments for 90 days increased loss of vitamin activity but did not negatively affect broiler growth performance, bone strength and ash when birds were supplied with NRC recommended levels.

The third study determined the effect of particle size, diet, methods of analysis (laboratory, NIRS-unground and NIRS-ground) and feed form (mash and pellet) on the CP predictability of the NIRS while using standard calibrations installed with the NIRS. Experiment 1 evaluated the effect of particle size, diets and methods of analysis (laboratory, NIRS-ground and NIRS-unground) on CP predictability with the NIRS. Interactions ($P \leq 0.05$) were observed in diet and method and particle size and method. This study demonstrated that once samples were ground, particle sizes had no effect on CP determination. Results of NIRS-ground samples were 0.7% lower in CP than laboratory results as compared to 1.8% in CP for NIRS-unground samples. Experiment 2 demonstrated the effect of feed form and methods of analysis on CP

predictability by comparing mash and pellets containing 600 µm corn particle size and 20% crude protein analyzed with the NIRS as either ground or unground and laboratory results. There was a feed form and method interaction ($P \leq 0.05$). Feed form and method significantly ($P \leq 0.05$) affected the prediction of CP content of unground samples. However, grinding samples yielded similar results for both the NIRS and laboratory analysis. Results from this study suggested that for better prediction of CP of mash and pellets, samples should be ground prior to analysis when using the standard calibrations installed on the NIRS.